Eastern Canadian Arctic killer whale demographics, population structure, and ecology

by

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Abstract

Characterizing the structure, dynamics, and ecology of predator populations is essential for understanding their role in ecosystems and for predicting their potential impact on prey. Killer whales (*Orcinus orca*) are a genetically and ecologically diverse marine predator with ecosystem-level influence. Globally, they occupy a broad ecological niche, but are often locally specialized and categorized as ecotypes or morphotypes. In the eastern Canadian Arctic, their seasonal presence is apparently expanding and is predicted to negatively impact Arctic-endemic prey, but certain aspects of their demographics have yet to be determined. Further, it is unknown whether genetically distinct populations demonstrate differences in ecology. In this thesis, I use physical and molecular markers to address these questions, investigating the demographics and relative ecology of killer whales in the eastern Canadian Arctic and Northwest Atlantic. First, I used photographic and genetic-identification to assess the abundance and population trend. Mark recapture analysis of photo-identification data estimated an abundance of 217 individuals and a growing population, but genetic-identification data was insufficient to produce reliable estimates. Epigenetic aging suggested a population age structure skewed to juveniles, while the proportion of calves and young juveniles based on group composition in photographs was comparable to other stable or growing populations. Second, I used whole genome sequences and compound-specific stable isotope analysis of amino acids to evaluate genetic and ecological differentiation between killer whale populations in the Northwest Atlantic. Analysis of genetic population structure confirmed two previously identified, genetically-isolated populations in Eastern Canada and Greenland. $\delta^{13}C$ and $\delta^{15}N$ of amino acids, used to infer relative distribution and diet, respectively, revealed moderate differences in source carbon values and a considerable difference in trophic level between the two populations. Taken together the stable isotope data show that the two genetic populations differ in ecology, but further research is needed before classification as ecotypes or morphotypes. This work contributes to our knowledge of killer whale demographics and ecology in the eastern Canadian Arctic, with implications for the management and conservation of this population as they expand in the Arctic.

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1. General Introduction

Warming temperatures have led to species range shifts and poleward expansions, which are altering marine community structure and species interactions (Sorte et al. 2010; HilleRisLambers et al. 2013; Vergés et al. 2016; Donelson et al. 2019). Despite the predominately negative consequences of these climate-related changes for many Arctic-endemic species, some marine species stand to benefit in the short term from greater spatial and temporal access to resource-rich regions seasonally limited by sea ice (Yurkowski et al. 2017; Descamps and Strøm 2021; Dupont et al. 2024). One such species are killer whales (*Orcinus orca*), marine apex predators that have seasonally visited the eastern Canadian Arctic since at least the mid-1800s, but appear to have expanded their presence over the last several decades as the ice-free season has lengthened (Reeves and Mitchell 1988; Higdon and Ferguson 2009; Higdon et al. 2014).

Killer whales exhibit remarkable genetic and ecological variation among populations throughout their global distribution, and are a notable species for the study of ecological divergence and sympatric speciation (Riesch et al. 2012; de Bruyn et al. 2013; Morin et al. 2024). They exert top-down effects on ecosystems through direct predation (Lima 2008; Estes et al. 1998; Springer et al. 2003) and by influencing prey behaviour, such as foraging and movement patterns (Peckarsky et al. 2008; Sironi et al. 2008). A greater killer whale presence in the eastern Canadian Arctic could have a substantial impact on the ecosystem through both consumptive and non-consumptive effects (Ferguson et al. 2012b; Breed et al. 2017; Westdal et al. 2017; Matthews et al. 2020a; Lefort et al. 2020a). Inuit communities in the North, who face food insecurity and who are expected to be disproportionately affected by further climate-related changes, rely upon endemic Arctic whales for subsistence and are concerned about the potential impact of increased killer whale predation on the populations of beluga (*Delphinapterus leucas*), narwhal (*Monodon monoceros*), and bowhead whales (*Balaena mysticetus*; Westdal et al. 2013; Ford et al. 2021; Huntington et al. 2022). As killer whales have expanded their presence in the region, a better understanding of their abundance and ecology has emerged as a key research need, not only to inform management and identify conservation needs, but to understand how they fit within the highly diverse mosaic of global killer whale populations (Higdon et al. 2012; Jourdain et al. 2019; Lefort et al. 2020b).

Collaboration with Inuit communities and dedicated field research conducted over the last fifteen years has substantially improved our understanding of the demographics, population structure, diet, and distribution of killer whales in the eastern Canadian Arctic. The number of killer whales seasonally resident around Northern Baffin Island is estimated to be 163 ± 27 individuals, based on mark-recapture analysis of 63 individuals photo-identified between 2009 and 2018 (Lefort et al. 2020a). The photo-identification catalogue was later updated to document new and re-sighted individuals photographed in 2019-2020 and to include individuals in Cumberland Sound, since whales were re-sighted between the two locations (Kucheravy et al. 2023). Analysis of whole genome sequences identified two genetically distinct populations in the eastern Canadian Arctic and surrounding North Atlantic waters, both with concerningly low genetic diversity (Garroway et al. 2024). Direct observations and dietary biomarkers revealed that these killer whales are primarily, if not solely, generalist mammal-eaters during their Arctic residency and likely elsewhere in their range (Ferguson et al. 2012a; Higdon et al. 2012; Matthews et al. 2021a, 2024). Finally, these killer whales are capable of rapid long-distance movements and appear to occupy a broad, and potentially different, North Atlantic range, before converging seasonally in the Arctic (Matthews et al. 2011, 2020b, 2021b, 2024).

Despite this progress, several relevant questions remain unanswered. An increase in reported sightings over the latter half of the 20th century alludes to a greater number of killer whales seasonally present in the region (Higdon and Ferguson 2009). However, further information on the demographic structure and population dynamics is required to discern whether this increase in sightings is a result of a range expansion, a growing population, other confounding factors (e.g. ease of reporting), or a combination of these. Further, while we have identified two reproductively isolated populations that seasonally exploit Arctic waters in the western North Atlantic, details of ecological differentiation between the two are unclear.

This research aims to address these knowledge gaps by using both physical and molecular markers from archived photographs and biopsy samples to build upon the aforementioned studies. The objective of my second chapter is to use photo-identification and capture-mark-recapture models to estimate the abundance and population growth rate of killer whales in the eastern Canadian Arctic. In addition, I use genetic sexing and epigenetic aging of DNA extracted from skin biopsies to approximate the sex- and age-class structure, respectively, supplementing the genetic methods with analysis of group composition in photographs. In the

third chapter, my objective is to use whole genome sequences and compound-specific stable isotope analysis of amino acids from skin biopsies to evaluate whether the distinct genetic populations also diverge in their distribution and diet. This work will further our knoweldge of killer whale population dynamics, structure, and ecology in the eastern Canadian Arctic and western North Atlantic, and contribute the management and conservation of these populations.

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2. Eastern Canadian Arctic Killer Whale Demographics revealed using photo-identification, genetics, and epigenetics

Abstract

Understanding the demographics of predator populations is critical to predict their regulatory impact on prey populations. Killer whale (*Orcinus orca*) sightings in the Canadian Arctic have increased as sea ice has declined, and increased predation pressure may affect the abundance and behaviour of marine mammal prey species. However, the population demographics (sex and age structure, abundance, and growth rate) of Eastern Canadian Arctic killer whales remain unclear. Here, I investigate population sex- and age-class structure using skin biopsies sampled from 51 Arctic killer whales. I determined sex genetically (49% males, 51% females) and estimated the age of individuals using epigenetic aging based on DNA methylation patterns (mean age=9.7 years). For individuals with tooth samples available (n=3), epigenetic age estimates were within 3 years of age estimated from counts of dentine growth layer groups. To account for calves, an age class not represented in biopsy samples, I analyzed group composition observed in a 14-year photographic database, in which calves constituted approximately 10-20% of observed groups. I conducted mark-recapture analysis using a Bayesian POPAN Jolly-Seber model of photo-identified (2009-2023; n=101) and genetically identified (2013-2021; n=46) individuals to estimate population abundance (215±29.4 and 93±19.8, respectively) and the realized population growth rate (λ; 1.02 and 1.32, respectively). The positive estimated growth rate from photo-ID is within the theoretical maximum for cetaceans (1.04) and aligns with the apparent increase from sightings reports, but the genetic-ID growth rate estimate is unrealistically high. The estimated number of killer whales in this population could have substantial consumptive and non-consumptive impacts on Arctic-endemic prey, including beluga and narwhal, which are of socioeconomic and cultural significance to Inuit. However, concerns about the impact of killer whale predation on Arctic prey should be tempered with efforts to conserve this relatively small killer whale population.

Introduction

Invading or expanding predator populations are likely to disrupt ecosystems through predation and competition (Laidre et al. 2008; Thomsen et al. 2014). In the Canadian Arctic Archipelago, sea ice is declining in thickness and extent throughout the year and the ice-free

season continues to lengthen (Stroeve and Notz 2018; Box et al. 2019). With warming temperatures, boreal marine species have shifted their range towards the poles and are altering community structure and species interactions (Sorte et al. 2010; Fossheim et al. 2015; Vergés et al. 2016; Mueter et al. 2021). For some marine predators, range expansions into previously restricted regions could result in increased access to prey, increasing predation pressure on endemic prey and potentially driving further predator population growth and expansion in the area (Carroll et al. 2024). Therefore, knowledge of the size and growth rate of predator populations is necessary to predict how changing predator dynamics might influence ecosystems.

Killer whales (*Orcinus orca*) have been observed in the eastern Canadian Arctic during the ice-free season for several centuries (Reeves and Mitchell 1988; Higdon and Ferguson 2009). They appear to be ice-avoidant in this region, arriving in the Arctic once the sea ice has cleared and departing in the fall as the sea ice forms (Higdon 2007; Matthews et al. 2011; Higdon et al. 2014). Annual sightings reports and Inuit knowledge provided during structured interviews have suggested that in recent years, killer whale presence in the eastern Canadian Arctic has increased as the ice-free season has lengthened (Higdon and Ferguson 2009; Higdon et al. 2012, 2014). The number of killer whales seasonally resident around northern Baffin Island, an apparent high-use region, was estimated to be 163 ± 27 based on mark-recapture analysis of 63 individuals photo-identified between 2009 and 2018 (Lefort et al. 2020). However, certain aspects about the population demographics, including the age-class structure and population growth rate, remain unanswered. Details on the population demographic structure could help to distinguish whether the apparent increase of killer whale in the eastern Canadian Arctic is due to a range shift, a growing population, or to a higher number of sightings reports reflecting increased awareness and ease of reporting.

As a marine apex predator, killer whales can influence ecosystems through top-down direct (consumptive) and indirect (non-consumptive) effects (Estes et al. 1998; Wirsing et al. 2008). Direct observations and dietary biomarkers indicate killer whales in the eastern Canadian Arctic are mammal-eating, with primary prey species including beluga (*Delphinapterus leucas*), narwhal (*Monodon monoceros*), bowhead whales (*Balaena mysticetus*), and several phocid species (Ferguson et al. 2012a; Higdon et al. 2012; Matthews et al. 2021, 2024). Killer whales also exert indirect effects on prey by influencing prey behaviour, for example by disrupting their habitat use and movement patterns in ways that may be energetically costly (Westdal et al. 2013;

Breed et al. 2017; Matthews et al. 2020). Between consumptive and non-consumptive effects, an increased killer whale presence could significantly impact the abundance, behaviour, and potentially distribution of marine mammal prey (Ferguson et al. 2012b; Matthews et al. 2020; Lefort et al. 2020).

Previous studies have shown even relatively small killer whale populations can have cascading regulatory impacts on large numbers of prey (Estes et al. 1998; Springer et al. 2003; but see DeMaster et al. 2006; Wade et al. 2007). Understanding the demographics of this killer whale population is therefore necessary to predict their potential impact on Arctic ecosystems, particularly endemic marine mammal prey populations. The objective of this research is to evaluate the abundance, population growth rate, and age-class structure of killer whales seasonally resident in the eastern Canadian Arctic. I predict that the population of killer whales in the eastern Canadian Arctic is growing, as a response to the lengthening open-water season increasing their access to prey in Arctic waters. I conduct mark-recapture analyses of photo-identified and genetically-identified individuals to estimate the abundance and realized population growth rate. I also use genetic sexing and epigenetic aging to construct a sex- and age-class structure, and complement the genetic age structure by analyzing photographs from group encounters to estimate the proportion of calves per group. This information will contribute to our understanding of this killer whale population and the dynamics of their Arctic expansion.

Methods

Sample collection and processing

Image collection – Photographs of killer whales in the eastern Canadian Arctic have been collected since 2009 by researchers, wildlife photographers, ecotourists and Inuit community members (Figure 2.1). During dedicated boat-based surveys, groups of whales were approached by boat and attempts were made to photograph the eye patch, dorsal fin, and saddle patch of all individuals, as well as group composition and interactions. Photographs were taken using a Canon EOS-1DX DSLR camera with a 24-70mm lens or a 70-200mm image stabilizing telephoto lens. Additional photographs and videos contributed by independent parties were taken opportunistically. From the video footage, I extracted still images of individual whales in which saddle patch markings were visible and images in which most or all of the observed group was visible.

Tissue sample collection – Tissue samples including skin and blubber were collected from free-ranging killer whales using a biopsy dart near Mittimatalik (Pond Inlet) and Pangnirtung, NU (Figure 2.1) in 2013 and 2018-2020. When a group of killer whales was located, they were slowly approached by boat and biopsied using a crossbow or CO_2 gun equipped with a sterile steel biopsy tip. Calves were avoided. Biopsies were frozen immediately in a liquid N2 cryoshipper or kept on ice and frozen within 8 hours. Samples were shipped to the Freshwater Institute in Winnipeg, Manitoba where they were kept in a -80°C freezer.

DNA extraction and whole genome sequencing – DNA was extracted for whole genome sequencing to identify individuals and determine their sex, and for epigenetic aging to estimate their ages from DNA methylation rates. Skin samples were prepared for DNA extraction by lysing the tissue using Buffer ATL, Proteinase K, and DTT. Total genomic DNA was extracted from skin using Qiagen DNeasy blood and tissue kits (Valencia, CA, USA), and DNA concentration was measured on a Nanodrop 8000 Spectrophotometer. DNA for was sent to the Centre for Applied Genomics at the SickKids Hospital in Toronto, ON for whole genome sequencing.

I processed sequence reads for analysis following the protocol described by Foote et al. (2016; 2019) and Garroway et al. (2024), described briefly here. I trimmed reads and merged lanes using Trimmomatic v0.35 (Bolger et al. 2014), then mapped and indexed reads to a chromosome-level reference genome (accession #GCA_000331955.1, Foote et al. 2022) using BWA 0.7.17 (Li and Durbin 2009) and Samtools 1.9 (Li 2011). Single nucleotide polymorphisms (SNPs) are genetic variants that can be used to identify individuals and to assess genetic variation among groups. I called genetic variants from the processed genomes using Platypus 0.8.1 (Rimmer et al. 2014) and filtered SNPs for quality using BEDtools v2.27.1 (Quinlan and Hall 2010), Vcftools v0.1.17 (Danecek et al. 2011), GATK v4.1.9 (McKenna et al. 2010).

DNA methylation measurement – Methylation was measured for 39 samples and nine known duplicate samples at University of California Los Angeles. I processed an additional 54 samples and one known duplicate sample at the University of Manitoba. Additional genetic duplicates were identified during genetic identification, described below. For the samples processed at the University of Manitoba, I converted DNA samples using bisulfite conversion (EZ DNA Methylation – Gold Kit, Zymo Research, Irvine, CA) to distinguish between

methylated and unmethylated cytosines (Clark et al. 2006). I measured methylation from converted DNA using eight mammalian methylation array chips (HorvathMammalMethylChip40; Arneson et al. 2022) following the methods described for Illumina Infinium methylation assay reference guide (Illumina 2020). The mammalian methylation array targets CpG sites, which are sites in the DNA sequence where cytosines are followed immediately by a guanine, specifically those that are highly conserved across the mammalian genome (Goldberg et al. 2007; Arneson et al. 2022). The array maps to 30,467 CpG sites in the killer whale genome (Parsons et al. 2023). Methylation levels for each sample on the array were measured using the Ilumina iScan system (Illumina, San Diego, CA), which generates red and green channel IDAT (Illumina BeadArray Data) files for each sample.

Analysis

Photo identification, genetic identification, and genetic sex – Adult killer whales can be uniquely identified by nicks, scratches, scars, and pigmentation patterns on their dorsal fin and saddle patch, as well as the shape and size of the dorsal fin and large scars visible on other parts of the body (Bigg 1982). I examined photographs and stills from video footage collected during encounters for identifiable individuals, using the right side of the body for identification, and recorded the number of new individuals identified and the number of individuals re-sighted in each year (Kucheravy et al. 2023).

To genetically identify re-sampled individuals, I calculated kinship coefficients ($\hat{\pi}$) among genomes using plink v.1.90 (Meyer 2018) and the package PlinkQC (Meyer 2021) in R (R Core Team 2024), which evaluates relatedness based on the proportion of alleles shared at genotyped SNPs. Sample pairs with $\hat{\pi} > 0.9$ were considered to be the same individual. I determined sex from whole genome sequences using the DifCover pipeline, by comparing read coverage on the X chromosome between two samples using a male genome as reference (Smith et al. 2018).

Population size and growth rate – To estimate population size and growth rate, I used the superpopulation parameterization of the Jolly-Seber model (Schwarz and Arnason 1996) in a Bayesian state-space framework, as described by Kéry and Schaub (2012; translated for JAGS

by Gimenez 2020). This parameterization estimates the superpopulation N_{super}, which is the number of individuals available to enter the study and be sampled during the study period, or in other words the total number of individuals alive over the course of the study period. I used constant survival probability and time-dependent capture and entry probabilities. To estimate the realized average population growth rate (λ, the observed growth rate of the population calculated from successive population sizes; Cooch and White 2024) over the study period, I calculated the geometric mean of the annual population growth rate ($\lambda_t = N_{t+1}/N_t$) from the derived annual population sizes (N_t) (Feng et al. 2019; Cooch and White 2024).

I constructed a photo-identification (photo-ID) and a genetic-identification (genetic-ID) capture history, and augmented each capture history by 200 pseudo-individuals (individuals with zero captures; Kéry and Schaub 2012; Royle and Dorazio 2012). I implemented a Gibbs sampling algorithm in JAGS (Plummer 2003) using the package R2jags (Su and Yajima 2024) to obtain posterior estimates for model parameters. For both models, I ran three MCMC sampling chains of 50,000 draws with a 10,000 draw burn-in period. I used uninformative priors U(0, 1) for survival (ϕ), capture (p), and inclusion (ψ) probabilities, and a Dirichlet prior Dir(1) for entry (b) probability. To evaluate model fit, I checked convergence for each parameter using the Gelman-Rubin diagnostic and by visually assessing parameter trace plots and density plots.

Epigenetic aging –DNA methylation of cytosines is a common epigenetic modification (Bird 1986; Singal and Ginder 1999; Moore et al. 2013), and changes in DNA methylation occur throughout an individual's lifetime (Wilson et al. 1987). Overall, DNA methylation decreases with age, a pattern that allows prediction of age from methylation levels (Bollati et al. 2009; Hannum et al. 2013). Epigenetic aging measures DNA methylation patterns at specific CpG sites to estimate the age of individuals using species- or taxon-specific "epigenetic clocks" (Bocklandt et al. 2011; Simpson and Chandra 2021). As a molecular biomarker of age using DNA extracted from tissue samples, it is currently one of the only methods of aging free-ranging mammals when the time of birth is unknown (Jarman et al. 2015). To epigenetically age individuals from the methylation data, I filtered out samples that failed to read in the iScan machine and did not contain both a red and green channel IDAT file (n = 1). I used the package minfi (Aryee et al. 2014) to normalize beta values, which represent the percent methylation at each CpG site. To check sample quality, I plotted the median intensity of methylated sites against unmethylated

sites, density plots of beta values, and a control strip plot showing the intensity of red and green channels. I removed samples that appeared as outliers across the three plots (indicating poor quality; n = 3), and re-normalized the beta values with outliers removed. I applied batch corrections to the beta values using the package sva (Leek et al. 2012) to account for technical variation in signal intensity, correcting for samples processed in different laboratories, located on different chips in the array, and located on different rows on the chip.

I used the killer whale skin clock developed by Parsons et al. (2023) to estimate epigenetic age (DNAm) of biopsied individuals, which calculates the DNAm age from normalized methylation data using a weighted linear combination of 50 CpG sites. For samples that were later determined to be genetic duplicates, I used the age estimated from the first sample collected. Since samples were collected across several years, I calculated an adjusted age by adding the difference between 2020 and the sampling year (assuming 100% survival) to the estimated age to compare individual ages and age structure in a single year.

There are no known age individuals in the epigenetic aging sample. Further, methylation rates can be influenced by factors such as environmental stressors (Hala et al. 2014; Lamka et al. 2022), so using a clock developed using another killer whale population could introduce bias into estimates. To assess the accuracy of the epigenetic ages, I compared the DNAm age to age estimated from dentine growth layer groups (Myrick et al. 1988) for individuals from Hudson Bay with tooth samples available (n = 2, with one sample measured in duplicate for methylation; Matthews and Ferguson 2014; Matthews et al. 2019). To evaluate the precision of the epigenetic ages, I compared duplicate ages estimated from the same sample (n = 9), for the same individual sampled twice in the same year (n = 13), and for the same individual sampled in different years (n = 3). Although biopsies collected outside the sampling area were included on the methylation array (e.g., the two individuals with tooth samples available were found stranded in Hudson Bay) and for the quality control process, only individuals sampled near Mittimatalik and Pangnirtung were included when evaluating population demographics (n = 51 after removal of duplicates and low-quality samples).

Proportion of calves and young juveniles – Calves and young juveniles are not biopsied, and are therefore are not represented in the epigenetic age dataset. To estimate the proportion of young individuals, I analyzed boat-based photographs or drone imagery from 18 group

encounters between 2009 and 2023. I identified calves and young juveniles based on dorsal fin and body size relative to adults and by the colour of the saddle patch, which is darker grey compared to older individuals. I considered an individual “young” if the dorsal fin was approximately half the size or less of adult females in the group (Supp. Figure 2.1). I identified individual calves and young juveniles when possible by unique markings, distinct dorsal fin shape, or if the individual was consistently photographed with the same adult. I estimated the proportion of young whales in each encountered group based on the observed number of calves and young juveniles to the observed number of older juveniles and adults.

Results

Population size and growth rate - From the photographic catalogue, I used 101 uniquely identified individuals and a total of 29 recaptures between 2009-2023 (Figure 2.2a). From the whole genome sequences, I identified 45 unique individuals and a total of 10 recaptures between 2013-2021 (Figure 2.2b). The posterior distributions estimated a superpopulation abundance (N_{Super}) of 215 and 93 and a realized population growth rate (λ) of 1.02 and 1.32 for the photo-ID model and the genetic-ID model, respectively (Table 2.1). Gelman-Rubin diagnostics were <1.07 (values <1.1 indicate convergence between chains) for all parameters in both models, and visual assessment of trace plots suggested chain convergence (Supp. Figure 2.2).

Age-class structure – The sampled group comprised 49% males (n = 25) and 51% females (n = 26). Epigenetic age estimates ranged from 2.8 to 23.3 years, with a mean and median epigenetic age of 9.7 and 9.4, respectively (Figure 2.3). Epigenetic age estimates were within 3 years of age estimated from counts of annual dentine growth layer groups (Table 2.2a), while the absolute mean (± SD) and median difference in epigenetic age estimated for genetic duplicates was 1.73 (± 1.53) and 1.12, respectively (range = -5.96, 4.15; Table 2.2b, Supp. Figure 2.3). From photographs, the number of calves and young juveniles observed per group varied between 0-4 (n = 18) constituting 0-26% (mean = 17.8%) of the observed group total (Table 2.3).

Discussion

The photo ID-based mark-recapture abundance estimate of 215 individuals is greater, though comparable, to the previous estimate of 163 for Northern Baffin Island (Lefort et al. 2020). In this study, the sampling region for photographs was extended to include Cumberland Sound, since genetic analyses suggest that although one whale biopsied in Cumberland Sound is part of a different genetic group (Garroway et al. 2024), the majority cluster genetically with individuals biopsied around Northern Baffin Island (C. Kucheravy and E. de Greef; see chapter 3). Further, individuals in both the photo-ID and genetic-ID datasets have been re-captured between Cumberland Sound and northern Baffin Island (Kucheravy et al. 2023, this thesis). Between the addition of Cumberland Sound individuals photographed in 2011 and new individuals photographed from 2019-2023 in all sampling regions, 38 newly identified individuals were included in the photo-ID catalogue since the previous estimate. The estimated realized population growth rate of 1.017 aligns with the increase in sightings and reports from Inuit communities indicating that the seasonal killer whale population in the eastern Canadian Arctic is growing. A growth rate of approximately 2% per year is comparable to rates observed or estimated in other killer whale populations globally (1.2-4.1%, estimated from population matrices, change in population abundance from annual census, or mark-recapture modelling; Brault and Caswell 1993; Olesiuk et al. 2005; Matkin et al. 2014; Towers 2019; Jordaan et al. 2020; DFO 2022).

The genetic-ID model abundance and growth rate estimates of 93 and 1.32 are less plausible than the results from the photo-ID model. First, an abundance of 93 ± 20 is approximately equal to the 101 identified individuals used in the photo-ID model. Given the low frequency of sightings and the difficulty obtaining high quality photographs of every individual in a group, it is unlikely that we have identified every whale in the population. Further, an average population growth rate of 32% per year is biologically and ecologically unrealistic. While annual growth rates up to 13% have been observed in some marine mammals such as phocids, otaarids and mysticetes, the theoretical maximum intrinsic population growth rate for odontocetes is generally considered to be 1.04 due to slow life history characteristics and low reproductive rates (Reilly and Barlow 1986; Best 1993; Wade 1998, 2018). As such, immigration rates exceeding 20% per year on average would be required to achieve this degree of population growth. Biopsies are more difficult to obtain than photographs during an encounter due to the

proximity required to sample, the need to retrieve the sampling arrow after a successful shot, and the need for trained personnel to biopsy. We sampled only 45 unique individuals and 10 total recaptures over 8 sampling years, with a 4-year gap from 2014-2017 where no biopsy samples were collected. The precision of mark-recapture estimates decreases with decreasing capture probability, and in this case the capture and re-capture rates for genetic data may be insufficient to estimate population size and, even more so, demographic rates (Otis et al. 1978; Pollock et al. 1990).

Although the superpopulation Jolly-Seber model estimates the probability of entry, it cannot distinguish between entry due to recruitment or to immigration (Kéry and Schaub 2012). Therefore, while the capture-recapture data suggests a growing population, the relative contribution of births and immigration are difficult to discern. The social behaviour of killer whale varies among populations, and social groups experience different rates of immigration. For example, the North Pacific Resident populations are generally considered closed to immigration, with only negligible levels of immigration measured in these populations (Bigg et al. 1990; Olesiuk et al. 2005; Pilot et al. 2010). Transient killer whales, on the other hand, disperse from natal groups to associate with others, and immigration contributed to rapid population growth of coastal Transients between 1975 and 1990 (Ford et al. 2007). The social organization of killer whales in the eastern Canadian Arctic, and their tolerance of permanent or temporary immigration, is currently unknown. Further, the Canadian Arctic Archipelago may previously have been at the population's range edge and used by only a portion of the population. With the extension of the open-water season, it is possible that the whole population is shifting its range and a greater proportion of the population is now exploiting Arctic resources. The POPAN formulation of the Jolly-Seber model postulates the existence of a source "superpopulation" from which individuals enter into the sampled population through birth or immigration (Schwarz and Arnason 1996). It was used here to try and account for the possibility of a source population in the North Atlantic, but assumes that all individuals enter by the end of the study (Kéry and Schaub 2012). Estimates may still be skewed by a large-scale population range shift. Further data on the social structure and demographic rates of this population, such as fecundity, as well as distribution is needed to determine the contribution of immigration to the observed population growth.

The epigenetic aging results suggest that the sampled population is skewed towards juveniles and subadults (individuals <13 years of age), comparable to other killer whale populations with described age structures. When ages are adjusted to 2020, representing a single-year snapshot of the age structure (assuming all sampled individuals survived), 51% of the group is under 13 years of age, similar to the growing Northern and Southern Resident populations between 1973-1987 (50% juveniles; Olesiuk et al. 2005) and the Alaskan Residents between 1984-2004 (49% juveniles; Matkin et al. 2014). The age structure estimated here, however, excludes calves, while the age structures of the Resident populations include calves. The age distribution of growing versus stable killer whale populations are quite similar (Olesiuk et al. 2005), and since this sample represents only about one quarter of the estimated abundance collected over several sampling years it is difficult to draw conclusions about the population trend from the age structure. Notably, there are no calves and few older individuals in the epigenetic age distribution.

When the ages are not adjusted, however, 81% of the group is under 13 years of age, much higher than expected. In both the adjusted and unadjusted age distributions, the low proportion of epigenetically aged individuals greater than 20 years old and the absence of any post-reproductive females in the sample may be due to the clock underestimating chronological age, field sampling bias, or chance. Epigenetic clocks, including those developed for odontocetes, generally perform best for mid-range ages and tend to underestimate the age of older individuals (El Khoury et al. 2019; Bors et al. 2021; Parsons et al. 2023). However, the killer whale clock was developed with individual ages ranging from 0-79 years, so ages 20-30 would be considered mid-range for this clock. For the individuals that were biopsied in both 2013 and 2020, the epigenetic age estimates from the 2020 biopsies were lower than the 2013 epigenetic ages plus the difference in years, which also occurred with duplicated individuals in the sample used to build the killer whale clock (Parsons et al. 2023), and possibly indicating age underestimation of older individuals. On the other hand, this clock accurately estimated the age of a 29-year-old killer whale from Naujaat processed on the same array, making it difficult to assess the accuracy and precision of epigenetic age estimates for this group. Epigenetic aging is a relatively new method for aging marine mammals, and further use and refinement of this method will help to ensure reliable age estimates.

Killer whales demonstrate a range of behaviours during encounters that could influence sample collection. During encounters, some groups of killer whales approach the boat and swim alongside the vessel, while other groups avoided the vessel and are more difficult to obtain biopsy samples. Within groups, individuals may be more or less likely to approach the boat based on sex, age, or other individual variables (Williams et al. 2009; Holt et al. 2021). If the group is near to the boat all individuals are generally within equal sampling probability, but it is possible that the aforementioned factors may contribute to some sampling bias. However, as mentioned previously, this sample represents approximately one quarter of the estimated abundance. Older adults constitute a smaller proportion of killer whale populations (e.g. Olesiuk et al. 2005), and by chance we may not have sampled many older adults, skewing the epigenetic age sample towards juveniles.

As mentioned previously, calves are not biopsied during encounters, necessitating the photographic analysis of group composition. I estimated a proportion of calves and young juveniles between 0-26% from photographs. This proportion is reasonable compared to other studied populations, in which average fecundity rates vary around 0.15-0.24 calves per year (Olesiuk et al. 2005; Matkin et al. 2014; Kuningas et al. 2014; Robeck et al. 2015). However, while photographic analysis is useful when other age data is unavailable, estimating group composition from photographs can be challenging. Precise age estimation is not possible, so the calves and young juveniles identified likely included individuals within the first few years of life and may overlap marginally with the epigenetic age distribution. It is also more difficult to discern individual calves, since they typically bear fewer unique markings than adults. Finally, despite efforts to photograph every individual in the group and of the whole group, we cannot be certain of the total number of individuals in the group.

The estimated abundance of killer whales in this population could have both consumptive and non-consumptive impacts on Arctic-endemic marine mammal prey (Ferguson et al. 2012b; Higdon et al. 2012; Lefort et al. 2020). For example, energetics modelling suggested that killer whales could consume significant numbers of narwhal during their seasonal Arctic residency (Lefort et al. 2020), while predator-prey modelling of killer whales in Hudson Bay suggested they could consume 0.275% of the total number of marine mammal prey per year (Ferguson et al. 2012a). Persistent behavioural changes by prey species in response to killer whales are well known by Inuit communities and are documented in telemetry studies. Narwhal, beluga,

bowhead whales, and seals seek refuge in shallow, nearshore waters or near sea ice when killer whales are present in the area (Ferguson et al. 2012b; Breed et al. 2017; Matthews et al. 2020), and groups of narwhal and beluga demonstrated altered behaviour during and after killer whale attacks (Laidre et al. 2006; Westdal et al. 2017). Inuit communities in Nunavut are concerned about the impact of killer whale predation on the populations of narwhal, beluga, and bowhead whales, since these species are of socioeconomic and cultural significance to Inuit. Many Inuit hunters have negative or conflicting feelings towards killer whales, since they consume or drive away prey and thus are viewed as competition for food resources (Westdal et al. 2013). As such, an expanding killer whale presence in the Arctic may lead to increased conflicts with humans (Westdal et al. 2013; Jourdain et al. 2019).

However, although these results suggest that the population is growing, the estimated population abundance remains relatively low. While currently classified as "Special Concern" by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC), an abundance of 215 individuals is smaller than the Northern Resident (approximately 330 individuals) and Offshore (approximately 300 individuals) killer whale populations in the northeast Pacific, both of which are considered "Threatened" (Ford et al. 2014; Towers et al. 2022; COSEWIC 2023). Killer whales in the eastern Canadian Arctic and Greenland have low effective population sizes, a measure of genetic drift wherein low effective population sizes imply a more rapid loss of genetic diversity, suggesting they may have difficulty adapting to future environmental changes (Waples 2022; Garroway et al. 2024). This population also faces several other threats, including increasing ship traffic and contaminant exposure, and has historically faced harvest pressure in Greenland (Pizzolato et al. 2016; Remili et al. 2023; Desforges et al. 2024; NAMMCO 2024; Garroway et al. 2024; Tennessen et al. 2024). Therefore, concerns about the impact of killer whale predation on Arctic-endemic species should be tempered with efforts to conserve this relatively small killer whale population.

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Figures

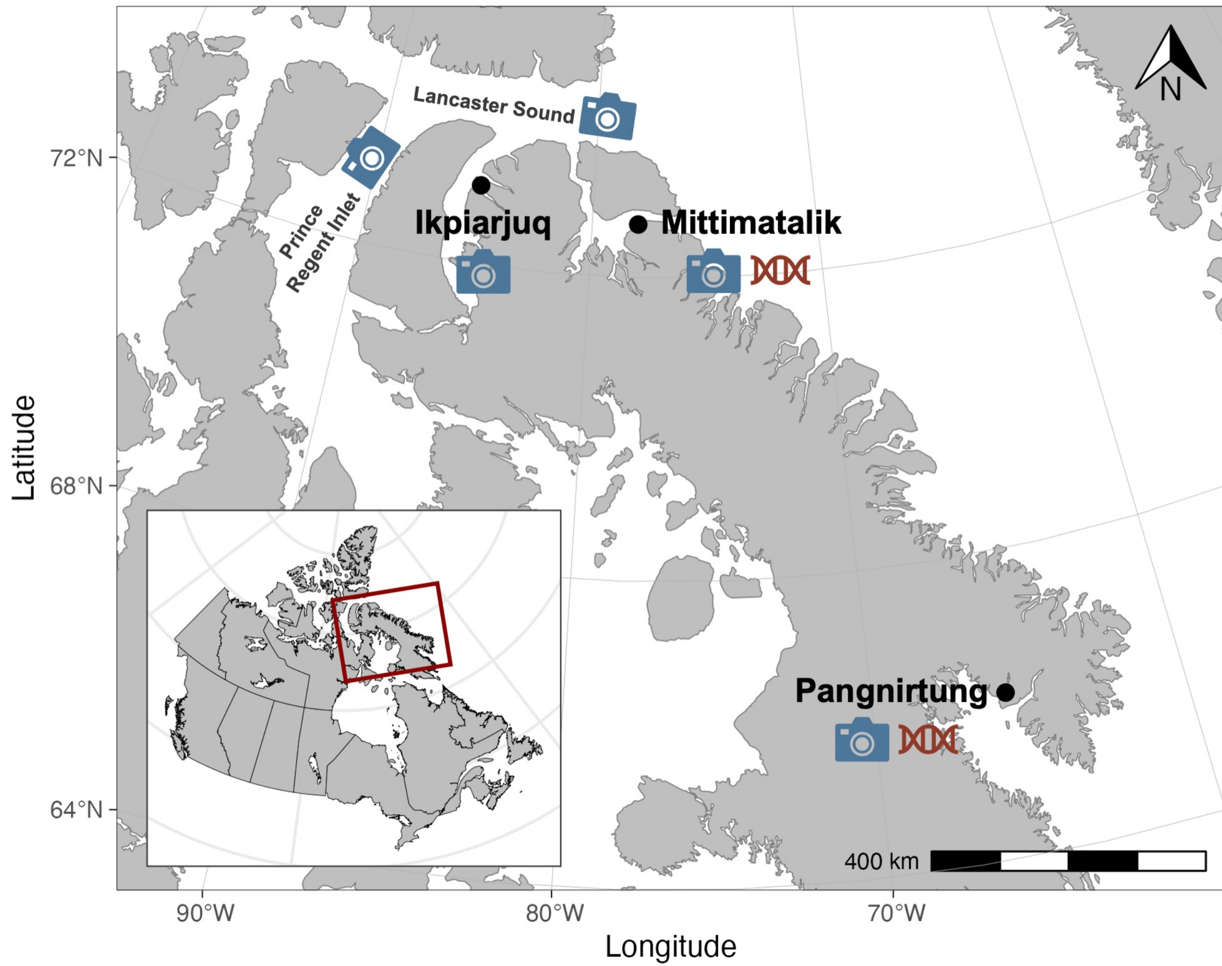


Figure 2.1. Map of study area. Primary photographic and biopsy sampling locations, indicated by camera icons and DNA icons, respectively, included the water bodies around Mittimatalik (Pond Inlet; Eclipse Sound, Pond Inlet, Milne Inlet, Tremblay Sound, and Navy Board Inlet), Ikpiarjuq (Arctic Bay; Admiralty Inlet), and Pangnirtung (Cumberland Sound). Photographs were also collected in Lancaster Sound and Prince Regent Inlet.

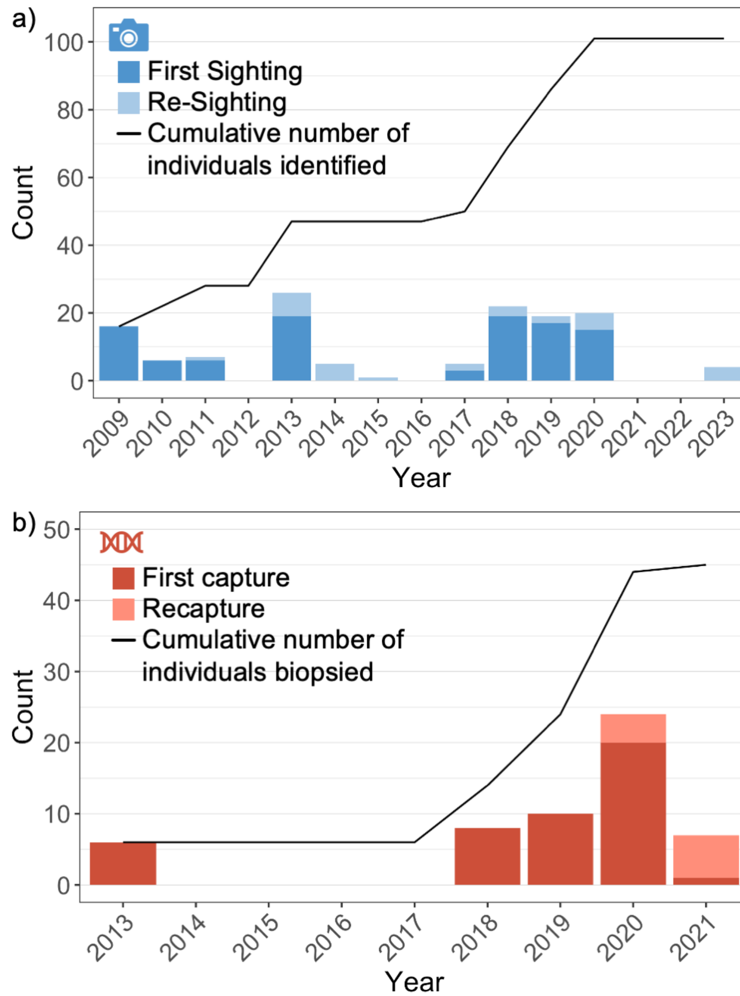


Figure 2.2. Summary of killer whale (a) photo-identification and (b) genetic capture histories near Mittimatalik, Ikpiarjuq, and Pangnirtung, Nunavut.

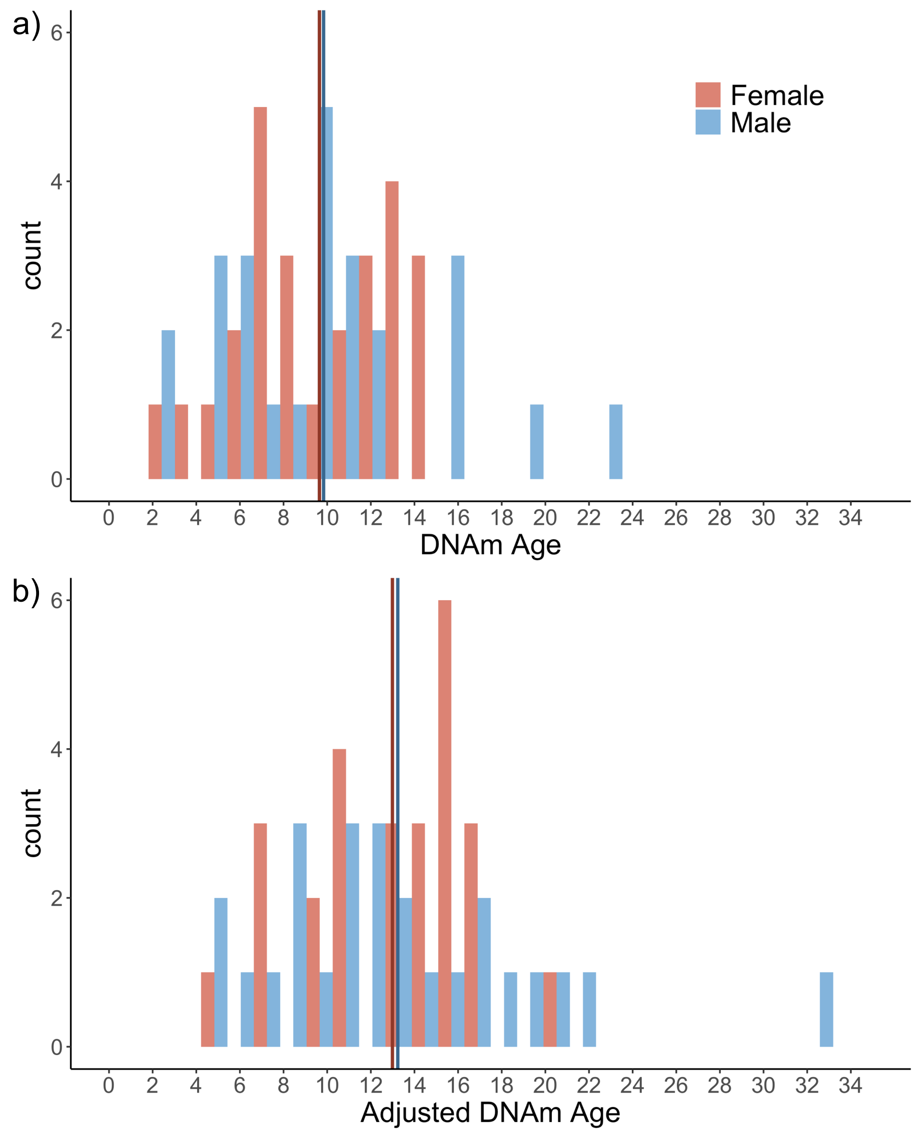


Figure 2.3. Epigenetic (DNAm) age distribution of Eastern Canadian Arctic killer whales from biopsy samples collected between 2013-2020. (a) DNAm age at year of capture, (b) DNAm ages adjusted to 2020 for comparability (adjusted DNAm age = epigenetic age + (2020 – year of capture)). Solid lines indicate mean ages for males and females.

Tables

Table 2.1. Posterior estimates for the mean (± standard deviation and 95% Bayesian credible interval) superpopulation size (N_{super}) and geometric mean realized population growth rate (l) of Eastern Canadian Arctic killer whales using a Bayesian superpopulation Jolly-Seber model for each photographic (n = 101) and genetic (n = 46) capture types.

Sample Type	Sampling Period	Estimated population size ± SD (95% B.C.I.)	Estimated population growth rate ± SD (95% B.C.I.)
Photo-ID	2009-2023	214.6 ± 29.4 (166.0 - 282.0)	1.017 ± 0.041 (0.93 - 1.10)
Genetic-ID	2013-2021	93.0 ± 19.8 (64.0 - 139.0)	1.323 ± 0.070 (1.17 - 1.43)

Table 2.2. (a) Accuracy of epigenetic ages assessed by comparing epigenetic age to age estimated from dentine growth layer groups (GLGs) for two individuals, one aged in duplicate. (b) Precision of epigenetic ages evaluated by calculating the mean, median, and standard deviation of the absolute difference in epigenetic ages estimated multiple times for the same individual from the same sample, from different samples collected in the same year, and for different samples collected in different years. Duplicates from different samples determined using whole genome sequences.

a)

Sample ID	Age from dentine GLGs	Epigenetic Age	Difference
ARRB-xx-1291	28	29.25	1.25
ARSQ-xx-1397 (1)	6	3.10	-2.90
ARSQ-xx-1397 (2)	6	5.46	-0.54

b)

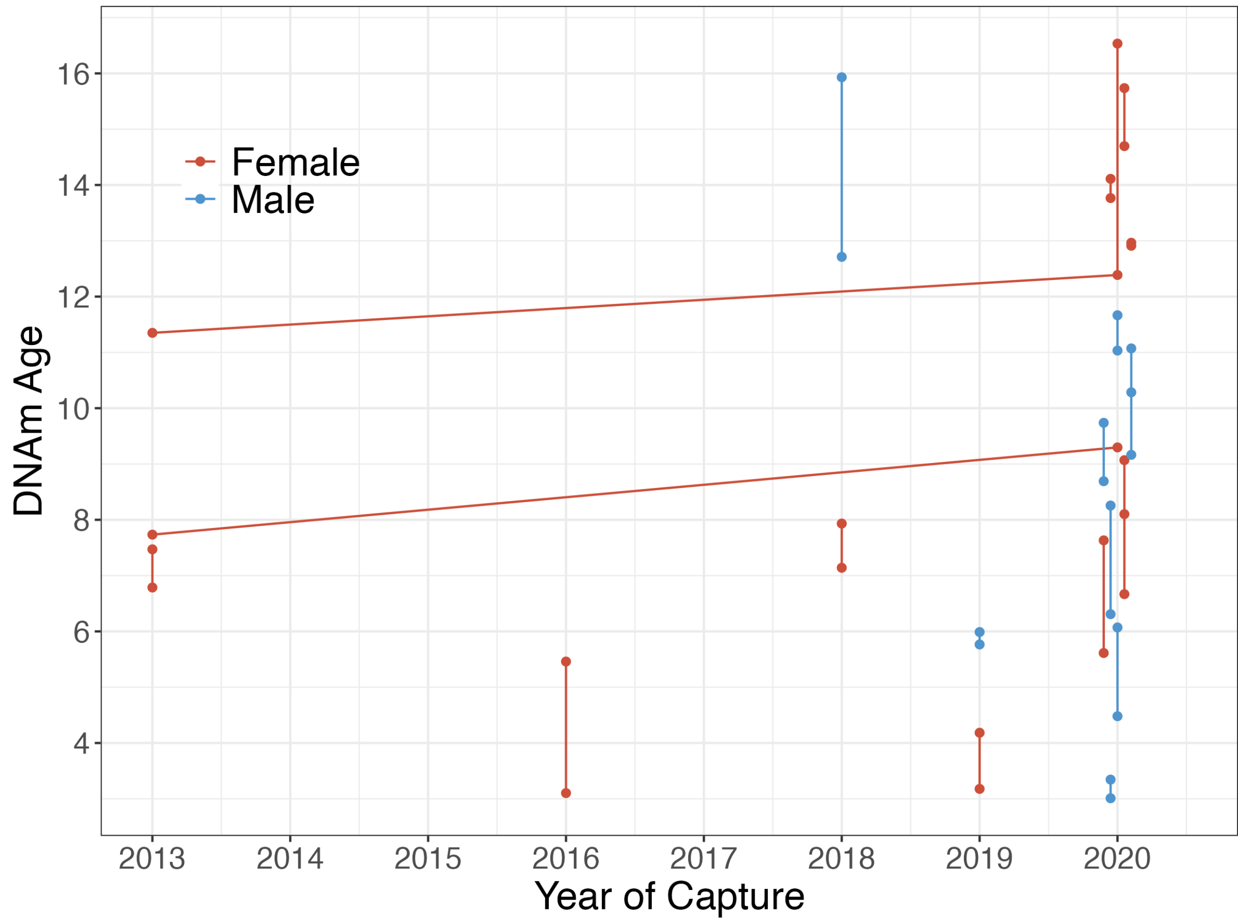
	Same sample	Different sample, same year	Different sample, different year
Range	-1.12, 2.36	-2.40, 4.15	-5.96, -1.82
Abs. median	0.97	1.59	5.44
Abs. mean	0.87	1.71	4.41
Abs. st. dev.	0.67	1.09	2.26

Table 2.3. The number of adult males (M), adult females (F) and older juveniles (J), and calves (C) and young juveniles (J), and the proportion of calves and young juveniles to the total group size observed in killer whales groups encountered at different locations during the ice-free season in the eastern Canadian Arctic.

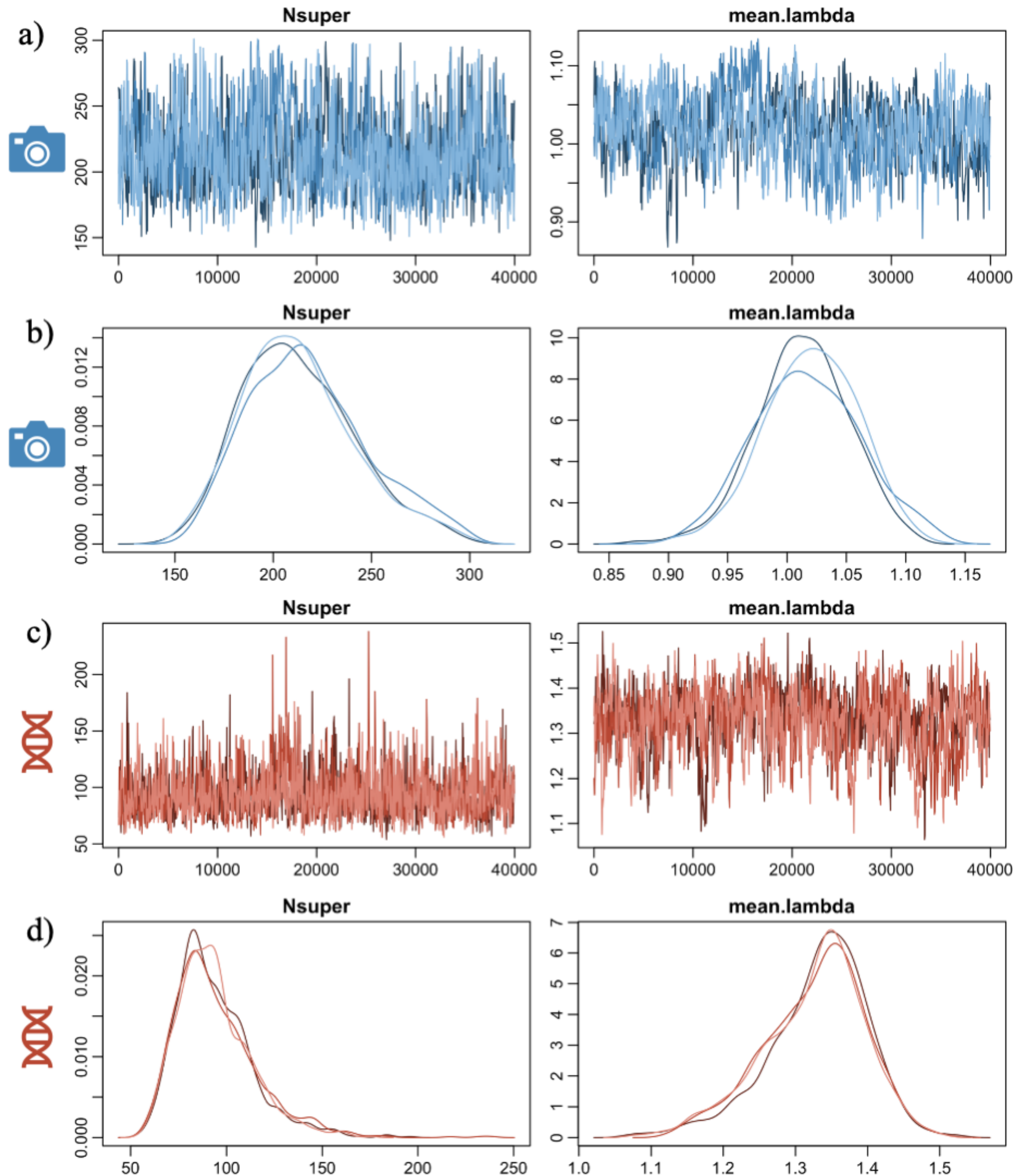
Date (yyyy-mm-dd)	Location	Photographer	Group Size	# Adult M	# Adult F/ J	# C/ Young J	Proportion C/ Young J
2009-08-12	Admiralty Inlet	S. Petersen	10	2	6	2	0.20
2009-08-14	Admiralty Inlet	S. Petersen, G. Freund	18	2	14	2	0.11
2010-08-12	Cumberland Sound	D. Kilabuk	9	3	5	1	0.11
2010-08-30	Admiralty Inlet	G. Freund	7	3	4	0	0.00
2013-08-04	Lancaster Sound	J. Putman	9	1	6	2	0.22
2013-08-11	Eclipse Sound	G. Freund	22	5	14	3	0.14
2013-09-17	Eclipse Sound	Wild Canada	9	1	7	1	0.11
2015-08-23	Admiralty Inlet	G. Freund	12	2	7	2	0.17
2017-08-02	Eclipse Sound	M. Bakken	9	1	6	2	0.22
2018-08-12	Eclipse Sound	C. Matthews, Y. Watanabe	15	2-3	9-10	3	0.20
2018-09-11	Eclipse Sound	K. Lefort, M. Ghazal	16	3	10	3	0.18
2019-08-17, 18, 19, 21	Eclipse Sound	M. Ghazal	14	2	8	3	0.21
2019-08-29 2019-09-01	Eclipse Sound	M. Ghazal	17	3	10	4	0.24
2020-08-18	Eclipse Sound	E. Inuarak	13	2	8	3	0.23
2020-08-25, 26	Cumberland Sound	R. Kilabuk	17	3	11	3	0.18
2020-08-26, 27	Eclipse Sound	E. Inuarak	16	3	10	3	0.19
2023-08-29	Admiralty Inlet	Silverback Films	9	2	5	2	0.22
2023-09-02	Admiralty Inlet	Silverback Films	15	1	10	4	0.27

Supplemental Material

Supplementary Figure 2.1. Examples of killer whale calves relative to adults in the photographic database, used to estimate the proportion of calves per group.



Supplementary Figure 2.2. Difference in epigenetic (DNAm) age estimates for individuals re-sampled in the same or different sampling year. Re-sampled individuals, connected with a solid line, were determined using whole genome sequences. Samples in 2020 were jittered to avoid overlap of duplicated individuals; location along x-axis does not indicate timeline.



Supplementary Figure 2.3. Diagnostic trace plots (a, c) and density plots (b, d) from three model chains for posterior estimates of N_{Super} and mean lambda, generated from a Bayesian POPAN Jolly-Seber models of killer whales photo-identified (n = 101, 2009-2023) and genetically-identified (n = 46, 2013-2021) in the eastern Canadian Arctic.

3. Genetically distinct Killer whale populations in the Northwest Atlantic differ in diet and distribution

Abstract

Killer whales are an apex marine predator that exhibit substantial genetic and ecological variation across their global distribution. Ecological divergence between neighboring or sympatric populations is theorized to be driven by diet and foraging specialization, leading to reproductive isolation. In some regions these populations are classified as ecotypes, morphotypes, or by their dietary specialization. In eastern Canada and Greenland, two seasonally-resident, genetically distinct populations, ECAG1 and ECAG2, have been identified, but it is not known whether these populations are ecologically distinct. Here, I use whole genome sequences (WGS) and compound-specific stable isotope analysis (CSIA) of amino acids (AA) from killer whale skin samples collected across the Northwest Atlantic from 2002-2022 to investigate the genetic population structure, geographic distribution, and dietary ecology of these populations. Analysis of WGS confirmed the two previously identified genetic groups, but also revealed genetic substructure within ECAG1 between the eastern Canadian Arctic and Atlantic Canada. Multivariate analysis of essential AA $\delta^{13}C$ values, which reflect source carbon values and are thus a proxy for spatial distribution, suggested some differences in distribution between the two populations. $\delta^{15}N$ of trophic and source AAs, used to infer diet, indicated a trophic difference between ECAG1 and ECAG2 approximately equivalent to one trophic step, likely resulting from a solely marine-mammal diet for the former and a diet mixed with fish for the latter. For seven ECAG1 whales that were re-sampled in different years, considerable differences in $\delta^{13}C$ and $\delta^{15}N$ AA values between sampling occasions imply a generalist diet and inter-annual variation in distribution. Overall, these results reveal ecological differences between the two genetic populations, improving our understanding of the complex global population structure of killer whales for both management and research. However, further information on social structure, acoustic behaviour, and morphology is required before categorizing them as ecotypes.

Introduction

Populations of species that are geographically widespread can become adapted to different environmental variables or ecological niches within their local habitat (Futuyma and

Moreno 1988). Ecological specialization and behavioural divergence among groups, including those that are sympatric, can lead to social and reproductive isolation, and subsequently to phenotypic and genetic differentiation (Dieckmann and Doebeli 1999; McKinnon et al. 2004; Funk et al. 2006). These groups have been termed 'ecotypes', defined as populations within a species with heritable adaptations to specific ecological conditions (Turesson 1922; de Bruyn et al. 2013; Le Moan et al. 2016).

Killer whales (*Orcinus orca*) are globally distributed and are often classified into ecotypes that exhibit significant ecological, morphological, and genetic differences (Forney and Wade 2007; Ford 2009; Foote et al. 2009; Morin et al. 2010). Several killer whale ecotypes have been identified, with the best-studied being those in the eastern North Pacific (Ford 2009; de Bruyn et al. 2013). These neighboring or sympatric ecotypes differ in morphology and coloration, diet and foraging behaviour, social structure and acoustic behaviour, and movement patterns, often showing limited or no gene flow despite their physical proximity (Hoelzel 1998; LeDuc et al. 2008; Pilot et al. 2010; Morin et al. 2010; Riesch et al. 2012; de Bruyn et al. 2013). Killer whales are currently considered a single global species, though there is ongoing debate that certain ecotypes should be classified as separate species (Morin et al. 2010, 2024; Riesch et al. 2012; Foote and Morin 2015). In other regions, killer whale groups that differ in some categories but do not yet meet the criteria for ecotype designation are classified as morphotypes (e.g., Antarctic Type A, B, C, and D; Pitman and Ensor 2003; Pitman and Durban 2012; de Bruyn et al. 2013) or simply by their dietary preferences (e.g. Norwegian seal- and fish-eating killer whales; Jourdain et al. 2020).

Ecological specialization in diet and foraging strategy is considered the primary driver of ecotypic divergence in killer whales, perpetuated by social organization and learning within groups (Hoelzel et al. 2007; Foote et al. 2009, Pilot et al. 2010; de Bruyn et al. 2013, Foote et al. 2016). As a species, killer whales are generalist predators, though many populations specialize on either fish or mammals. For example, in the eastern North Pacific, the fish-eating "Resident" killer whales feed almost exclusively on salmonids, while the neighbouring mammal-eating "Transient" population preys upon seals and cetaceans (Ford et al. 1998). Further specialization can occur within mammal- or fish-eating populations. In Antarctic waters, sympatric populations Type A and Type B are both mammal-eating, but Type A whales specialize on Antarctic minke whales (*Balaenoptera bonaerensis*), while Type B whales mainly hunt seals (Pitman and Ensor

2003; Pitman and Durban 2012). Some populations, however, have a more generalist diet, feeding on both marine mammals and fish, or a variety of other prey such as sharks, squid, sea turtles, and occasionally seabirds (Pitman and Ensor 2003; Ford 2009; Foote et al. 2010; Best et al. 2010).

In the eastern Canadian Arctic, killer whales are seasonally resident during the ice-free summer months (Higdon et al. 2012). During this time they are mammal-eating, consuming endemic Arctic whale (beluga *Delphinapterus leucas*, narwhal *Monodon monoceros*, and bowhead whales *Balaena mysticetus*) and seal (ringed *Pusa hispida*, harp *Pagophilus groenlandicus*, hooded *Crystophora cristata*, and bearded *Erignathus barbatus*) species (Ferguson et al. 2012; Higdon et al. 2012). While their winter distribution remains largely unknown, oxygen isotopes suggest a broad North Atlantic range while telemetry analyses and the presence of temperate epizoic barnacles on dorsal fins indicate that at least some individuals reside in warmer mid-Atlantic waters for parts of the year (Matthews et al. 2011, 2020a, 2021b, 2024). Dietary biomarkers, specifically bulk stable isotope analysis (SIA) and compound-specific stable isotope analysis of amino acids (CSIA-AA), support that most individuals maintain a generalist marine mammal diet in the weeks to months preceding their Arctic residency (Matthews et al. 2021a, 2024). However, a few individuals show stable isotope ratios and tooth wear consistent with fish-eating ecotypes in the eastern North Pacific, suggesting some variability in foraging behaviour (Matthews et al. 2021a, 2024). Individuals photo-identified in the eastern Canadian Arctic have also been sighted in Baffin Bay near West Greenland, where killer whales are reported to prey primarily on seals, as well as various fish and whale species (Heide-Jørgensen 1988; Foote et al. 2009; Nielsen 2011; Kucheravy et al. 2023; Remili et al. 2023).

Stable isotope measurements of specific compounds can be more informative than those of bulk tissues when combined with knowledge of their biochemistry. The carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope ratios of individual amino acids (AAs) reflect unique metabolic pathways by which they are formed and broken down, and CSIA-AA can therefore reveal information obscured by SIA of bulk proteins or tissues. Essential amino acids (AA_{ESS}), for example, can be synthesized by primary producers but not by consumers, and therefore source $\delta^{13}C_{AA}$ values are largely conserved in consumer tissues through the food web and can be used to infer spatial distribution (Hare et al. 1991; Larsen et al. 2013; McMahon et al. 2016; Whiteman

et al. 2019). For nitrogen isotopes, AAs are categorized into “trophic” AAs that undergo deamination and transamination reactions and change with each trophic level, and “source” AAs that do not undergo the same reactions and thus remain approximately at the baseline $\delta^{15}N$ values of the food web (McClelland and Montoya 2002; Popp et al. 2007; Chikaraishi et al. 2007, 2009). These categorizations provide an advantage over bulk SIA, since the isotopic composition of trophic AAs can be calibrated against source AAs from the consumer tissue sample, rather than prey samples (McMahon and McCarthy 2016). The relative difference in $\delta^{15}N$ between trophic-source AA pairings, such as glutamic acid and phenylalanine ($\delta^{15}N_{Glx\text{-}Phe}$), is traditionally expected to increase with each trophic step (Nielsen et al. 2015), but decreases with trophic position in killer whales due to some unknown mechanism (Matthews et al. 2021a, 2024; see all Matthews et al. 2020).

Genomic analyses have identified two genetically distinct populations in the eastern Canadian Arctic and Greenland; Eastern Canadian Arctic and Greenland 1 (ECAG1) and Eastern Canadian Arctic and Greenland 2 (ECAG2). The former includes individuals sampled in the Canadian High Arctic and Newfoundland, while the latter includes individuals sampled in the Canadian Low Arctic and East Greenland (Garroway et al. 2024). It is likely that the ranges of these genetic populations overlap, but it is currently unknown whether they are ecologically distinct. In this study, I expand genomic (Garroway et al. 2024) and compound-specific stable isotope (Matthews et al. 2021a, 2024) datasets with additional samples to investigate the relative diet and spatial ecology of genetically-identified populations, and the possibility of candidate ecotypes in this region. I hypothesize that genetically isolated populations in the Northwest Atlantic will differ in their foraging behaviour and spatial distribution, comparable to the ecotypes in the eastern North Pacific. My first objective is to use whole genome sequences to assess the genetic population structure of killer whales sampled across the Northwest Atlantic (see Garroway et al. 2024). I then examine whether genetic variation corresponds to ecological variation, using $\delta^{13}C$ values of AA_{ESS} and $\delta^{15}N$ values of AA_{Source} to assess spatial structure of genetic groups, and $\delta^{15}N$ of $AA_{Trophic\text{-}Source}$ and $AA_{Trophic}$ to investigate relative trophic-level dietary differences among genetic groups. Finally, I compare $\delta^{13}C$ AA_{ESS}, $\delta^{15}N$ $AA_{Trophic\text{-}Source}$, and $\delta^{15}N$ $AA_{Trophic}$ values of individuals that were re-sampled across different years to examine individual inter-annual differences in diet and distribution. This work will help to align our knowledge of killer whale genetic and ecological divergence in the western North Atlantic, and

contribute to our understanding of the variation of these populations in the context of global killer whale diversity.

Methods

Sample Collection

Skin biopsies were collected between 2013 and 2021 from free-ranging killer whales during the ice-free season near Mittimatalik (Pond Inlet), Pangnirtung, St. Pierre et Miquelon (a French territory located southwest of Newfoundland), and the east coast of Newfoundland (Figure 3.1). We approached groups of killer whales by boat and sampled juvenile and adult individuals using a crossbow or a CO_2 rifle equipped with a sterile, tubular, steel biopsy tip. Biopsies were collected and immediately frozen in liquid N_2 cryoshippers. We also received skin samples collected from harvested or stranded killer whales in East (Tasiilaq, 2012, Ittoqqortoormiit, 2021) and West (Nuuk, 2021) Greenland and eastern Newfoundland (2002, Figure 3.1). All samples (see Supp. Table 1 for sample list) were shipped to the Freshwater Institute in Winnipeg, Manitoba, where they were kept in a -80°C freezer.

DNA extraction and whole genome sequencing

Subsamples of skin (n = 89) were lysed using Buffer ATL, Proteinase K, and DTT before total genomic DNA was extracted using Qiagen DNeasy blood and tissue kits (Valencia, CA, USA). DNA concentration was measured using a Nanodrop 8000 Spectrophotometer. Whole genomes were sequenced by the Centre for Applied Genomics at the SickKids Hospital in Toronto, ON. The protocol by Foote et al. (2016, 2019) was followed to prepare sequence reads for analysis, described in detail by Garroway et al. (2024) and briefly here. To align reads to their corresponding sequence in a known genome for analysis, sequence reads were trimmed and merged using Trimmomatic v0.35 (Bolger et al. 2014), then mapped and indexed to a chromosome-level reference genome (accession #GCA_000331955.1, Foote et al. 2022) using BWA 0.7.17 (Li and Durbin 2009) and Samtools 1.9 (Li 2011). I removed duplicate reads, added read groups, and filtered the genomes to keep reads mapped in the proper pair using Picard 2.20.6 (Broad Institute 2019) and Samtools 1.9 (Li 2011), which ensures reads are not repeated and are mapped within an appropriate distance and oriented correctly to one another. To avoid imbalances in read coverage between genomes, those exceeding 19x modal coverage were

downsampled to 19x using GATK 4.1.2.0 (McKenna et al. 2010). Genetic variants including single nucleotide polymorphisms (SNPs), insertions, and deletions were called from the processed genomes using Platypus 0.8.1 (Rimmer et al. 2014). SNPs are the most common genetic variant, used to analyze individual- and population-level genetic variation. To prepare genomes for analysis, non-SNP variants were excluded and SNPs were quality-filtered to remove low-quality sites (quality <50), sites with high missingness (>0.25), small scaffolds (<100kb in length), and sex-linked SNPs using Vcftools v0.1.17 (Danecek et al. 2011) and GATK. Finally, sites out of Hardy-Weinberg equilibrium and minor allele frequency < 0.05 were removed, and sites were pruned for linkage disequilibrium (LD $r^2 > 0.8$).

Compound-specific stable isotope analysis (CSIA-AA)

Skin subsamples (n = 81) were finely diced, freeze-dried for 48 hours, and homogenized using a mortar and pestle. Lipids were extracted by adding 1.9 ml 2:1 Chloroform: Methanol to each sample, vortexing, and placing the samples in a 30°C water bath for 24 hours. Solvents were removed from the vial and the process was repeated before drying the samples for 24 hours. Samples were sent to the Stable Isotope Facility at the University of California Davis for compound-specific stable isotope measurement, following the protocol described in detail by Matthews et al. (2024). Briefly, samples were hydrolysed in HCl under a N_2 headspace before $\delta^{15}N$ and $\delta^{13}C$ measurement in either AAs derivatized as N-acetyl methyl esters ($\delta^{15}N$ of all samples and $\delta^{13}C$ of 2018, 2019, 2020, and 2021 samples; Corr et al. 2007) or AAs derivatized using methoxycarbonylation ($\delta^{13}C$ of 2009 and 2013 samples; Walsh et al. 2014). AA derivatives were separated on chromatography columns and measured using GC-C-IRMS on a gas chromatograph coupled to an isotope ratio mass spectrometer. $\delta^{15}N$ and $\delta^{13}C$ were measured in 12 AAs: alanine (Ala), aspartic acid + asparagine (Asx), glutamic acid + glutamine (Glx), glycine (Gly), isoleucine (Ile), leucine (Leu), lysine (Lys), methionine (Met), phenylalanine (Phe), proline (Pro), valine (Val) and threonine (Thr). Calibration, scale-normalization, and quality control followed the protocol described by Yarnes and Herszage (2017). Laboratory reference materials were measured in replicate every five samples, and all samples were analyzed in duplicate or triplicate.

Analysis

Genetic identification, sex, and population structure – I used the DifCover pipeline (Smith et al. 2018), which compares read coverage on the X chromosome between two samples using a male genome as reference, to determine sex from whole genome sequences. For seven samples that were not whole genome sequenced but that were used in the CSIA-AA analysis, sex was determined using microsatellites by amplifying the ZFX/ZFY region on the sex chromosomes of males and females (Bérubé and Palsbøll 1996). I estimated kinship coefficients ($\hat{\pi}$) using plink v.1.90 (Purcell et al. 2007) and the package PlinkQC (Meyer 2021) to identify re-sampled individuals from whole genome sequences. In pair-wise comparisons of genomes, kinship coefficients calculate the proportion of alleles shared at genotyped SNPs to evaluate relatedness. After removing one of each duplicate or close-kin pair (n = 62 remained after removals; Supp.Table 1), I used the R package pcadapt v4.3.5 (Privé et al. 2020) to assess killer whale population structure using principal component analysis (PCA). Finally, I estimated genetic differentiation between the groups identified in the PCA by calculating the fixation index (F_{ST}), which relates the genetic variation within a population to the total genetic variation among populations, using the R package StAMPP v1.6.3 (Pembleton et al. 2013). F_{ST} ranges from 0 to 1 with F_{ST} values between 0.15-0.30 considered high, indicating little to no gene flow between populations (Waples 1998; Holsinger and Weir 2009; Pilot et al. 2010). I used the groups identified here for the subsequent CSIA-AA.

$\delta^{15}N$ and $\delta^{13}C$ CSIA-AA – I considered five δ^{13}C AA_{ESS} (Ile, Leu, Met, Phe, and Val) for analysis (Supp.Table 2; McMahon et al. 2010; Matthews et al. 2024). I used a PCA, which maximizes the variance of a dataset, to visualize the overall variance of δ^{13}C AA_{ESS} values. I used linear discriminant analysis (R package MASS; Venables and Ripley 2002), which maximizes the separability among labelled groups, to evaluate whether genetic groups differ based on δ^{13}C values of AA_{ESS}. $\delta^{13}C_{AA}$ values were scaled and centered to standardize variance among the five AA_{ESS}. To further test whether the AA_{ESS} individually differ among the genetic clusters, I used Bayesian multivariate linear regression in the R package brms (Bürkner 2017), which implements the Hamiltonian Monte Carlo sampling algorithm through the Stan programming language (Carpenter et al. 2017).

I used Bayesian univariate linear regression in brms to determine whether $\delta^{15}N_{Glx\text{-}Phe}$, $\delta^{15}N_{Thr}$, and $\delta^{15}N_{Thr\text{-}Phe}$ (Supp.Table 2) differ between genetic groups. $\delta^{15}N_{Glx\text{-}Phe}$ is the most common trophic-source pairing used to evaluate consumer trophic level (McMahon and McCarthy 2016). Thr is less commonly used as a trophic AA, but $\delta^{15}N_{Thr}$ and $\delta^{15}N_{Thr\text{-}Phe}$ are positively correlated with $\delta^{15}N_{Glx\text{-}Phe}$ in killer whale tissues and are also consistent with known trophic differences in fish- and mammal-eating killer whales (Matthews et al. 2024). As an additional proxy of spatial distribution, and since $\delta^{15}N_{Phe}$ is used as the source AA to calibrate $\delta^{15}N_{Glx}$ and $\delta^{15}N_{Thr}$ but can increase with trophic level (Matthews et al. 2020b, 2024; Troina et al. 2021), I tested whether $\delta^{15}N_{Phe}$ and $\delta^{15}N_{Lys}$ (another source AA; McMahon et al. 2015) differed between genetic groups. Finally, to assess potential confounding factors I tested whether $\delta^{15}N_{Glx\text{-}Phe}$, $\delta^{15}N_{Thr}$, and $\delta^{15}N_{Thr\text{-}Phe}$ differed with sex or Julian day when modelled with genetic group. Genetic duplicates were removed for the above analyses, with samples from the duplicate pair selected for removal to maintain representation from all sampling groups (Supp.Table 1).

All models were run with default uninformative priors and three parallel chains. I used 50,000 draws with a burn-in period of 20,000 draws to improve model performance and to compensate for group sample size differences. To evaluate model chain convergence, I visually assessed parameter trace plots and density plots and ensured the Gelman-Rubin statistic, which compares the variance within chains to the variance between chains, was below 1.1 (indicating convergence; Gelman and Rubin 1992). To evaluate model fit, I performed graphical posterior predictive checks (Gabry et al. 2019), plotting (1) the distribution of y to distributions of multiple simulated y datasets for each group, (2) the mean of y to the distribution of mean y from multiple simulated y datasets for each group, and (3) a scatterplot of the mean and standard deviation of y with the mean and standard deviation of multiple simulated y datasets.

To investigate individual inter-annual differences in diet for seven individuals that were re-sampled in different years, I re-ran the PCA with between-year duplicates included to compare $\delta^{13}C$ AA_{ESS} values. I also calculated the mean difference in $\delta^{15}N_{Glx\text{-}Phe}$, $\delta^{15}N_{Thr}$, and $\delta^{15}N_{Thr\text{-}Phe}$ between sampling occasions and plotted change in values across years. All analyses in R were performed in v4.4.1 (R Core Team 2024).

Results

Genetic population structure

The PCA of SNP data shows grouping consistent with Garroway et al. (2024), with a large group of individuals clustered to one end of the PC1 axis (explaining 14.34% of the variation in the data) corresponding to ECAG1, and a smaller group of individuals clustered to the opposite end corresponding to ECAG2 (Figure 3.2). Within the ECAG1 cluster, the individuals sampled in Ittoqqortoormiit, East Greenland, and those sampled in St. Pierre et Miquelon and Newfoundland both separated out from the individuals sampled in Mittimatalik and Pangnirtung. The ECAG2 cluster includes individuals sampled in Tasiilaq (East Greenland), Nuuk (West Greenland), Naujaat, and one individual sampled in Pangnirtung in 2013. The two clusters were highly differentiated (F_{ST} = 0.225, 95% C.I. = 0.225-0.226), also consistent with Garroway et al. (2024).

$\delta^{13}C$ CSIA-AA

PCA scores for killer whale skin $\delta^{13}C$ AA_{ESS} values of the two genetic groups overlapped on both PC1, which explained 45.53% of the variation and was driven primarily by the positive loadings of Ile, Leu, Met, and Phe, and PC2, which explained 24.72% of the variation and was driven by the positive loading of Val (Figure 3.3A). Some separation occurred along the PC2 axis, however, with ECAG1 and ECAG2 clustered towards positive and negative scores, respectively (Figure 3.3A). When the PCA was re-run to include genetic duplicates sampled in different years, an individual's $\delta^{13}C$ values in a given sampling year grouped more closely to the other individuals sampled in the same location and year, rather than the same individual's $\delta^{13}C$ values from the previous sample, varying across both principal components (Figure 3.3B, C).

The LDA correctly classified 64 of 65 samples (98.5%). The variation between groups was primarily driven by Leu and Phe (coefficients of LD1 = -1.63 and 1.67, respectively), followed by Ile (-0.22), Val (0.17), and Met (0.04; Figure 3.3D). In the Bayesian multivariate regression models, $\delta^{13}C$ values of essential AAs Ile, Leu, Phe, and Val differed between the two genetic groups, while Met did not (Table 1). When modelled as co-variates with genetic group, $\delta^{13}C$ AA_{ESS} did not differ between sexes or with Julian day (Supp.Table 3.3, Supp.Figure 3.1). For all models, the Gelman-Rubin diagnostic for all parameters was <1.01 and visual inspection of parameter trace plots and density plots indicated model convergence (Supp.Figure 3.3).

$\delta^{15}N$ CSIA-AA

ECAG1 had lower $\delta^{15}N_{Glx-Phe}$, $\delta^{15}N_{Thr}$, and $\delta^{15}N_{Thr-Phe}$ than ECAG2 (Figure 3.4A, B, C; Table 1). $\delta^{15}N_{Phe}$ and $\delta^{15}N_{Lys}$ did not differ between the two genetic groups (Table 1, Figure 3.4D, E). As co-variates, $^{15}N_{Glx-Phe}$, $\delta^{15}N_{Thr}$, and $\delta^{15}N_{Thr-Phe}$ did not differ between sexes or with Julian day (Supp. Table 3.3, Supp. Figure 3.1). The Gelman-Rubin diagnostic (<1.01) and parameter trace plots and density plots indicated model convergence for all models (Supp. Figure 3.4).

When comparing individuals re-sampled in different years, $\delta^{15}N_{Glx-Phe}$ decreased for all individuals between the first and second sampling occasion, on average by 1.15‰ (min = -1.85‰, max = -0.48‰; Figure 3.5A). For $\delta^{15}N_{Thr}$ and $\delta^{15}N_{Thr-Phe}$, some individuals increased between the first and second sampling occasions while others decreased, with $\delta^{15}N_{Thr}$ increasing on average by 0.82‰ (min = -2.61‰, max = 3.37‰, absolute mean difference = 2.21‰; Figure 5B), and $\delta^{15}N_{Thr-Phe}$ decreasing on average by 0.09‰ (min = -2.87‰, max = 2.66‰, absolute mean difference = 1.63‰; Figure 3.5C).

Discussion

Killer whale populations worldwide show genetic and ecological divergence – in some regions to the point of speciation – but this variation had not previously been described for the northwest Atlantic. The genetic analysis and ecological characterization through stable isotope proxies presented here provide evidence that two genetically distinct populations in the Northwest Atlantic and Greenland are feeding at different trophic levels, and may be occupying sympatric or partially overlapping distributions. As such, these two populations could constitute candidate ecotypes.

As expected, the main patterns of genomic population structure are consistent with Garroway et al. (2024), with the PCA showing two main clusters that correlate with ECAG1 and ECAG2. The high F_{ST} indicates substantial genetic differentiation between populations, comparable to the difference between ecotypes in the North Pacific (pairwise F_{ST} for 8 North Pacific populations = 0.04-0.24, Hoelzel et al. 2007; Pilot et al. 2010). However, in Garroway et al. (2024) only one sample was available from Pangnirtung (ARPG-2013-01) which clustered with ECAG2. While that sample remains with ECAG2 here, all new samples collected from Pangnirtung are part of ECAG1. This indicates that individuals regularly returning to

Cumberland Sound (Pangnirtung) and Eclipse Sound (Mittimatalik) are part of the same genetic population, consistent with re-sightings of photo-identified individuals between Cumberland Sound and Eclipse Sound and that groups of killer whales generally appear to follow receding sea ice northward along the east coast of Baffin Island in spring (Lefort et al. 2020; Kucheravy et al. 2023).

Contrary to predictions that, due to their proximity, the two killer whales hunted in Ittoqqortoormiit, East Greenland would be part of ECAG2 with the whales sampled in Tasiilaq, East Greenland, the Ittoqqortoormiit whales clustered more closely to ECAG1. Similarly, since whales photo-identified in Mittimatalik were previously photographed in Baffin Bay between Baffin Island and West Greenland I predicted the whale hunted in Nuuk, West Greenland would cluster with ECAG1, but it grouped instead with ECAG2. Killer whales are highly mobile and capable of travelling large distances in relatively short periods (e.g. Matthews et al. 2011), and both populations may be travelling throughout East and West Greenland. This range would be consistent with the geographic distribution inferred from oxygen isotopes from the teeth of different killer whales sampled in the eastern Canadian Arctic and Northwest Atlantic (Matthews et al. 2021b). However, there also appears to be genetic substructure within ECAG1 across the sampling area. The individuals from Ittoqqortoormiit separate from the larger group of ECAG1 individuals sampled in Mittimatalik and Pangnirtung along the PC1 axis, and towards the end of the PC2 axis. Similarly, the samples collected in Newfoundland and St. Pierre et Miquelon separate out in a small subgroup between the Ittoqqortoormiit and the Mittimatalik/Pangnirtung group. Further sampling in Newfoundland, Greenland, and Hudson Bay will help to clarify the degree of genetic differentiation both between and within populations. Despite these minor differences population structure was largely consistent with Garroway et al. (2024), so I proceeded with exploring ecological differences between ECAG1 and ECAG2.

The $\delta^{13}C$ AA_{ESS} results from the three complementary multivariate methods, and the $\delta^{15}N$ values of source AAs Phe and Lys, suggest a moderate degree of sympatry between the two genetic populations. In the PCA, which reduces the dimensionality of multivariate data based on the total variation in the dataset, ECAG1 and ECAG2 overlapped completely along PC1 and only showed limited partitioning along the PC2 axis. The overlap of the two populations in the PCA implies an overlapping geographic distribution or similar source carbon values. Comparable $\delta^{15}N_{Phe}$ and $\delta^{15}N_{Lys}$ values, as source AAs that reflect regional baseline $\delta^{15}N_{AA}$,

between the two populations also point to similar distributions. Conversely, in the LDA, which reduces the dimensionality of multivariate data by finding linear features that maximize variance between labelled classes, ECAG1 and ECAG2 separate along LD1 with minimal overlap, suggesting a difference in source carbon when all five AA_{ESS} are considered. Finally, four of the five $\delta^{13}C$ AA_{ESS} (Ile, Leu, Phe, Val) differed between the two genetic populations in the Bayesian multivariate model, corroborating the difference observed in the LDA. The LDA was primarily driven by Leu, Phe, and Ile, while in the Bayesian model Val had the greatest posterior estimate followed by Leu, Phe, and Ile. The data were standardized for the LDA but not for the Bayesian model and Val had the greatest variance prior to standardization, explaining the large posterior estimate but the lesser influence on the LDA. Overall, while the PCA and $\delta^{15}N_{Phe}$ and $\delta^{15}N_{Lys}$ values do not show clear separation between the two genetic populations, the LDA and Bayesian model differentiate $\delta^{13}C$ AA_{ESS} values between ECAG1 and ECAG2. These apparently contrasting results are difficult to reconcile. One interpretation is that these two genetic populations occupy distinct, but possibly neighboring or partially sympatric geographic distributions in the Northwest Atlantic. This would be comparable to the distribution of ecotypes in the eastern North Pacific, that overlap in range but differ in movement patterns, which are generally associated with prey distribution (Ford and Ellis 2014). Alternatively, isoscapes of plankton bulk $\delta^{13}C$ and $\delta^{15}N$ show that primary producer carbon values can be broadly similar over vast oceanic regions in the mid- and North Atlantic (e.g., east of Newfoundland and south of Greenland; Graham et al. 2010; McMahon et al. 2013; Magozzi et al. 2017; Espinasse et al. 2022), potentially confounding results. Although marine isoscapes for compound-specific stable isotopes have not yet been characterized, it may be that the two populations do not overlap in distribution, but instead occupy different regions with similar or de-coupled source carbon and nitrogen profiles. Further, since $\delta^{13}C$ of AA_{ESS} can be used to identify consumer niches and resource partitioning in local marine ecosystems (Elliott Smith et al. 2018; Larsen et al. 2020; Stahl et al. 2023), it is possible, though probably less likely, that these populations have similar or overlapping distributions but are feeding on food webs characterized by different primary producers.

Considerable variation in $\delta^{13}C$ AA_{ESS} among groups of whales sampled in different locations and years indicates interannual variability in geographic distribution within ECAG1. For example, the groups sampled in Mittimatalik in 2013, 2018, and 2019 are distributed across

PC1 with little overlap in source carbon values, while the groups sampled in Pangnirtung in 2020 and 2021 cluster on opposite ends of PC2. The re-sampled individuals (e.g., one individual first sampled in Pond Inlet in 2013, ARPI-2013-01, and later sampled in Pangnirtung in 2020, KW-2020-PG-21) that are part of these different sampling groups show that these individuals, and potentially their social groups, have different distributions between sampling years. However, re-samples or photographic re-sightings of individuals do not necessarily mean that the composition of individuals within the group remains the same. By "group", I am referring to sampling group rather than to the social dynamic of the group ("social group"). The social structure of these killer whales has not yet been characterized and it is unknown whether individuals remain in the same social groups between years. Photo-identification and dentine oxygen isotopes suggest that some individuals maintain stable, long-term relationships, but that social group composition may not be consistent (Lefort et al. 2020, Matthews et al. 2021, Kucheravy et al. 2023). Social groups may exhibit fission-fusion dynamics similar to those observed in some Norwegian and sub-Antarctic populations (Tavares et al. 2017; Reisinger et al. 2017; Jordaan et al. 2021), potentially forming temporary or seasonal associations during their Arctic residency. While these re-samples and photo-ID (Lefort et al. 2020; Kucheravy et al. 2023) show that individuals and potentially similar social groups are regularly returning to the eastern Canadian Arctic during the open-water season, group composition of individual whales may differ between years. During the winter and spring, individuals and sampling groups may not consistently return to the same geographic region, potentially altering their movements in response to prey availability (Similä et al. 1996; Nichol and Shackleton 1996; Forney and Wade 2007; Nøttestad et al. 2014) or other environmental variables (Pitman and Ensor 2003; Higdon et al. 2012; Matthews et al. 2019). Interannual variation inferred from the individuals re-sampled here is consistent with variation in $\delta^{18}O$ in annual killer whale dentine growth layer groups, also interpreted by Matthews et al. (2021) to indicate individual variation in distribution between years. The inter-annual variation observed in ECAG1 was not as obvious in ECAG2, likely due to the limited sample size. However, the individual from Nuuk in 2021 was isolated from the other ECAG2 individuals along PC1, suggesting that there is also variation within the ECAG2 population that may become clearer with additional sampling.

The lower $\delta^{15}N_{Glx\text{-}Phe}$, $\delta^{15}N_{Thr}$, and $\delta^{15}N_{Thr\text{-}Phe}$ values of ECAG1 compared with ECAG2 indicate they were feeding at a higher trophic level (see Matthews et al. 2021a, 2024). Although

difficult to assess in terms of absolute dietary differences (see below), relative trophic differences indicated by differences in these metrics is consistent with reported differences in killer whale diet across the study area. Direct observations, reports from Inuit communities, bulk SIA, and CSIA-AA all indicate that killer whales sampled in the eastern Canadian Arctic are most likely exclusively consuming marine mammal prey during and prior to their Arctic residency, though consumption of some high-trophic level fish cannot be excluded (Ferguson et al. 2012; Higdon et al. 2012; Matthews et al. 2024). Killer whales in West Greenland primarily hunt cetaceans and seals, but fish and squid were also found in stomachs (Heide-Jørgensen 1988), while in East Greenland their diet appears to constitute mainly seals and fish from stomach content and fatty acid analysis (pers. comm. F. Ugarte; Foote et al. 2013; Remili et al. 2023). Overall, ECAG1 appears to consume predominately, or exclusively, marine mammals, while the diet of ECAG2 whales contains a large proportion of fish in addition to seals and some cetaceans, resulting in a lower trophic level.

Estimating absolute trophic differences from AA-specific $\delta^{15}N$, as with bulk $\delta^{15}N$, requires accurate trophic discrimination factors (TDF), which are the difference in stable isotope values between the consumer and its diet (Post 2002). The $TDF_{Glx\text{-}Phe}$ for lower trophic position marine consumers has been determined to be approximately 7.6‰ (Chikaraishi et al. 2007), but $TDF_{Glx\text{-}Phe}$ has since been shown to be highly variable in marine food webs (McMahon and McCarthy 2016), especially among top marine predators (Germain et al. 2013; Matthews et al. 2020b). Further, the relative difference in $\delta^{15}N$ values between trophic-source AA pairings decreases with higher trophic levels in killer whales, rather than increases as expected from studies of lower trophic position organisms (Matthews et al. 2021a, 2024). Because of these discrepancies in $TDF_{Glx\text{-}Phe}$ in killer whales, absolute trophic position was not calculated in this study. However, the difference in mean $\delta^{15}N_{Glx\text{-}Phe}$ values was approximately 2.4‰, comparable to the smaller trophic step calculated for marine mammal-eating killer whales using $\delta^{15}N_{Glx\text{-}Phe}$ values from dentine collagen (~2.2-2.9‰; Matthews et al. 2020). Further, the differences in mean $\delta^{15}N_{Thr}$ and $\delta^{15}N_{Thr\text{-}Phe}$ values between ECAG1 and ECAG2 killer whales were approximately 6.8‰ and 7.9‰, similar to $\delta^{15}N_{Thr}$ and $\delta^{15}N_{Thr\text{-}Phe}$ differences between killer whale ecotypes with known marine mammal and fish diets (Matthews et al. 2024), as well as the >6‰ difference in $\delta^{15}N_{Thr}$ with each trophic step (Hare et al. 1991; Styring et al. 2010; Bradley et al. 2015). Until species-specific and AA-specific TDFs are resolved for high trophic position

marine consumers, we must rely on inference of relative, rather than absolute, trophic differences. However, the three trophic $\delta^{15}N$ AA proxies used here all point towards a considerable trophic difference between ECAG1 and ECAG2.

The difference in AA-specific $\delta^{15}N$ values of re-sampled individuals in ECAG1 suggest significant inter-annual variability in diet. $\delta^{15}N_{Glx-Phe}$ values decreased between sampling occasions for all individuals, while $\delta^{15}N_{Thr}$ and $\delta^{15}N_{Thr-Phe}$ increased or decreased depending on the individual and sampling years. As discussed in the previous section, it is difficult to make conclusive statements about interannual differences given that TDFs for marine top predators remain unresolved. However, we can attempt to put differences into context based on previous work on killer whale trophic position. The mean decrease of 1.15‰ for $\delta^{15}N_{Glx-Phe}$ is approximately half a trophic step for marine mammal-eating killer whales (Matthews et al. 2020) and slightly less than half of the trophic steps calculated for other top predators (~4.3‰ for harbor seals *Phoca vitulina*, and ~3.5‰ for gentoo penguins *Pygoscelis papua*; Germain et al. 2013; McMahon et al. 2015). The absolute mean differences of 2.21‰ for $\delta^{15}N_{Thr}$ and 1.63‰ for $\delta^{15}N_{Thr-Phe}$ also convey a reasonably large change in diet between sampling occasions for individuals. In the years with higher $\delta^{15}N_{Glx-Phe}$, $\delta^{15}N_{Thr}$, and $\delta^{15}N_{Thr-Phe}$ values (and thus lower trophic positions), killer whales may have been eating more baleen whales, which typically sit at a lower trophic level than seals and toothed whales (Pauly 1998; Trites 2019). Even though individuals differ in the number of years between sampling, $\delta^{15}N_{AA}$ values for most individuals appear to follow a similar trend within each AA or paired AA. It is not clear whether this is by chance, driven by trends in foraging behaviour, or due to some other factor not considered here. Opposing patterns between AAs and paired AAs (e.g., some individual's measurements decrease for $\delta^{15}N_{Glx-Phe}$ but increase for $\delta^{15}N_{Thr}$ and $\delta^{15}N_{Thr-Phe}$) are also unexplained, but could be due to differing turnover rates among amino acids (Downs et al. 2014).

Taken together, the differences in individual $\delta^{15}N$ AA and $\delta^{13}C$ AA_{ESS} values between sampling occasions indicate considerable flexibility in both distribution and diet, differences not reflected in bulk stable isotope values from some of the same groups (Matthews et al. 2024) but inferred from interannual variation of dentine isotope profiles from different ECA killer whales (Matthews and Ferguson 2014, Matthews et al. 2021). While in the eastern Canadian Arctic, killer whale diet reflects the relative abundance of prey in a given region. For example, around

North Baffin killer whales consume a greater proportion of narwhal than beluga, which are less abundant in that area, and similarly consume higher proportions of bowhead whales in Cumberland Sound where they are more abundant (Ferguson et al. 2012; Higdon et al. 2012; Doniol-Valcroze et al. 2015; Fortune et al. 2020; Biddlecombe and Watt 2024). From these results, killer whales may be equally flexible in the period leading up to their Arctic residency, hunting prey species available in their location at the time. Unfortunately, since individuals are not re-sampled each year, nor are the intervals between sampling occasions consistent for the seven re-sampled individuals in this study, we cannot interpret any group-level or regular inter-annual diet or movement patterns for this population. Nonetheless, this supports previous work suggesting that ECAG1 killer whales are generalist marine mammal predators (Matthews et al. 2024), and aligns with the broad niches and varied diets inferred for other North Atlantic killer whales (Foote et al. 2013; Bourque et al. 2018; Jourdain et al. 2020; Remili et al. 2023).

Some important considerations for this analysis are the extended sampling period, unequal sample sizes between populations, and the lack of stable isotope data for Hudson Bay. Isotopic turnover rates of $\delta^{13}C$ and $\delta^{15}N$ in the skin of bottlenose dolphins (*Tursiops truncatus*), a related odontocete, are estimated to have a mean half-life of 11-48 days (Browning et al. 2013; Giménez et al. 2016), and thus reflect the isotopic composition of their prey in the weeks to months leading up to sampling. The majority of samples were collected in August and early September. For killer whales sampled around Baffin Island, this would be at the beginning or shortly after their arrival in the region, and thus would reflect diet in the period prior to their Arctic residency. However, a few individuals in St. Pierre et Miquelon and Greenland were sampled in June and November, respectively, creating a sampling period of 153 days during which isotopic turnover can occur. Since $\delta^{13}C$ and $\delta^{15}N$ values did not differ with sampling date (in Julian day) I assume here that stable isotope ratios are comparable across the sampling period, but it is important to note that isotopic profiles do not reflect the same time period for all individuals, especially the temporal outliers. On a similar note, bulk $\delta^{13}C$ and $\delta^{15}N$ values sometimes differ between sexes (Foote et al. 2012; Reisinger et al. 2016; Samarra et al. 2017), but AA-specific $\delta^{13}C$ and $\delta^{15}N$ did not for these samples and sex was therefore disregarded in the main analysis. Further, the individual sampled in Naujaat grouped genetically with ECAG2, but unfortunately was not available to be processed for CSIA-AA. As a result, Hudson Bay is not represented in the stable isotope dataset, a broad marine region within which isotopic profiles are

diverse and could differ substantially from Greenland. Finally, I acknowledge that the small sample size of ECAG2 killer whales relative to ECAG1 limits analytical power. In general, Bayesian analysis is better equipped to manage small sample sizes but performs better with informative priors (van de Schoot et al. 2021), whereas here I used default priors. Despite these limitations, the substantial differences between populations shown here, particularly by the WGS PCA, F_{ST}, and $\delta^{15}N$ AA results, provide reasonable evidence that the populations are genetically and ecologically distinct.

Throughout their global distribution, killer whales exhibit diversity in their morphology, diet, behaviour, distribution, and genetics, which have led to the classification of sympatric or neighboring populations (Ford 2009; de Bruyn et al. 2013; Morin et al. 2015, 2024). Discrete categorization is useful for both research and management, but variation both within and across these traits, occurs on a spectrum (Foote 2023). While some populations have reached the degree of differentiation required of ecotypic classification, this designation requires ecological diversification that is not yet known to, or does not, exist in many other killer whale populations. In the Northwest Atlantic, killer whales are challenging to access for study due to their low density, broad distribution, and limited sampling opportunities. Several gaps in knowledge have yet to be filled for these populations, and further information on the social structure, acoustic behaviour, and morphology of these killer whales is required for classification as ecotypes or morphotypes. As a start, this research provides evidence that genetic groups ECAG1 and ECAG2 differ ecologically in diet and distribution.

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Figures

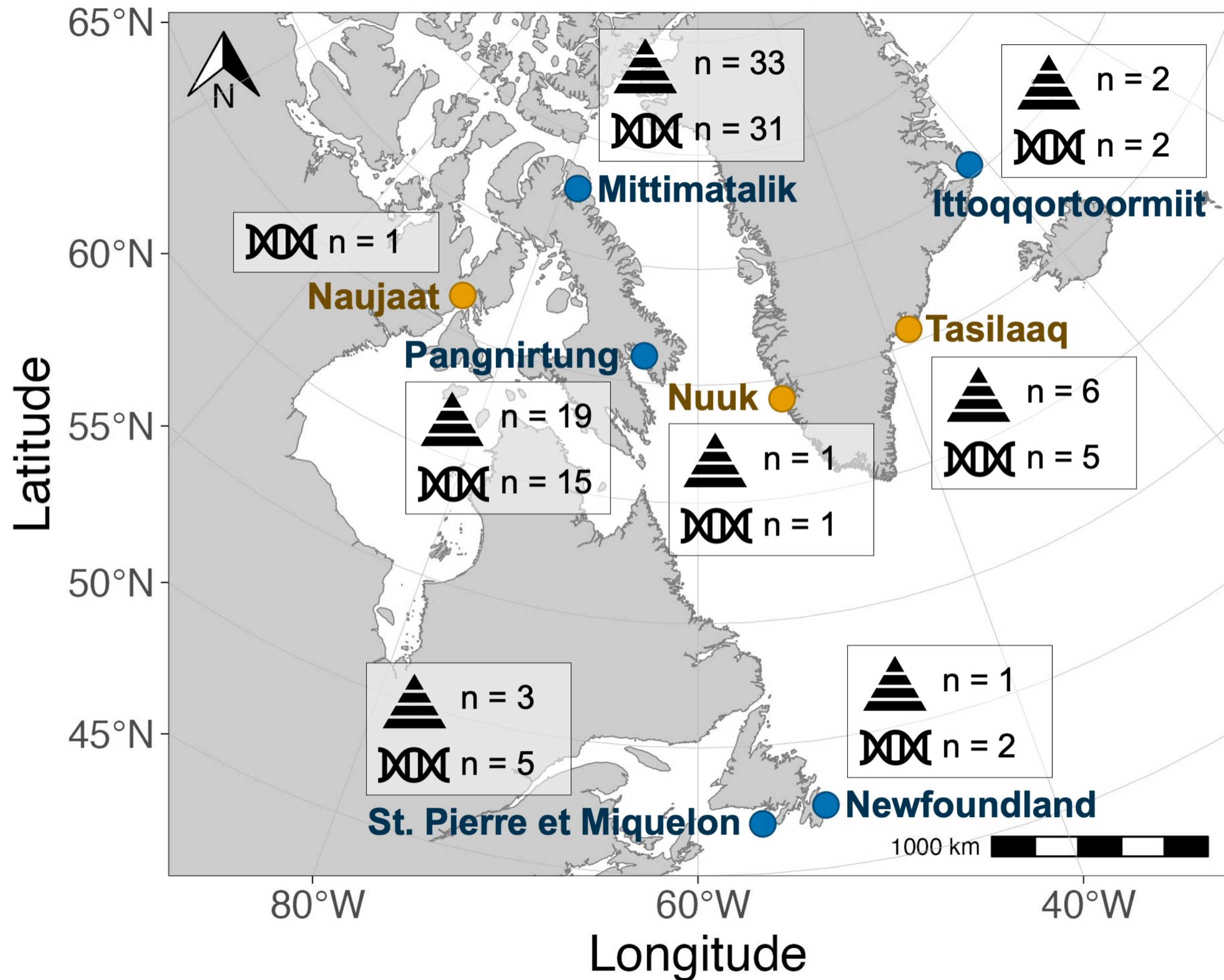


Figure 3.1. Sampling locations and sample size (after removal of duplicates; see Supp. Table 1) in eastern Canada and Greenland where killer whale skin samples were collected for genetic (indicated by DNA icon) and dietary (indicated by trophic triangle icon) analyses between 2002-2022.

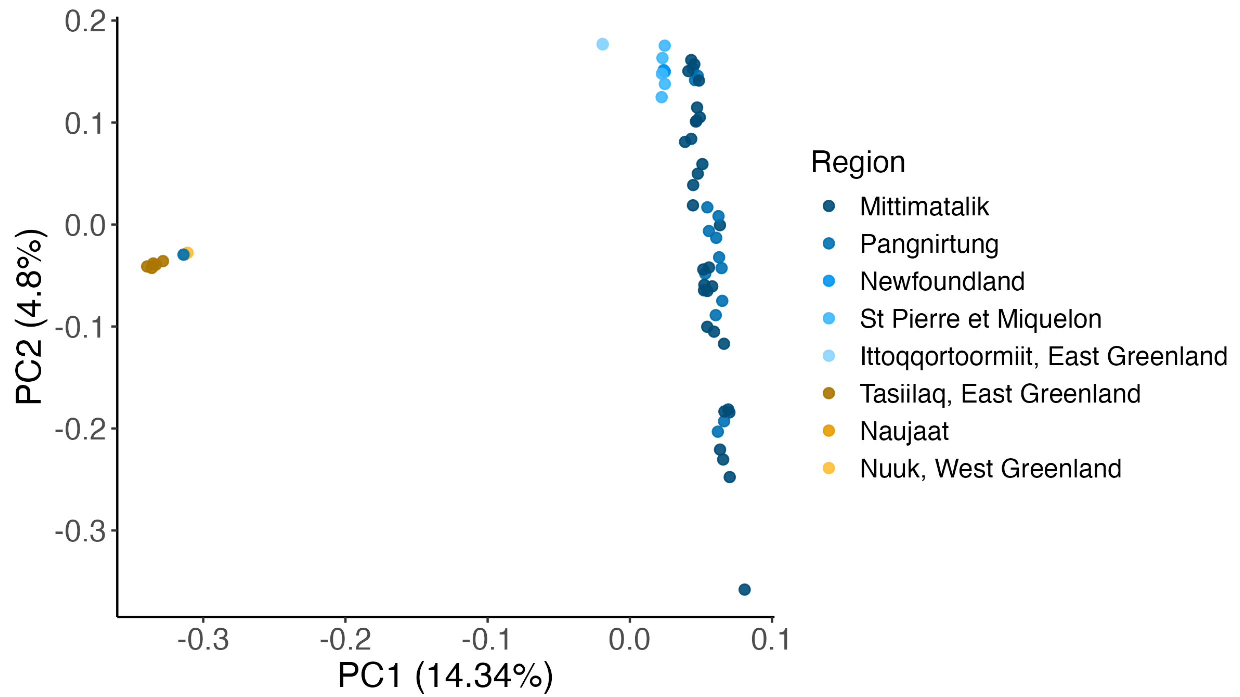


Figure 3.2. Principal component analysis of whole genome sequence data showing the population structure of killer whales (n = 62) in the eastern Canadian Arctic and Greenland.

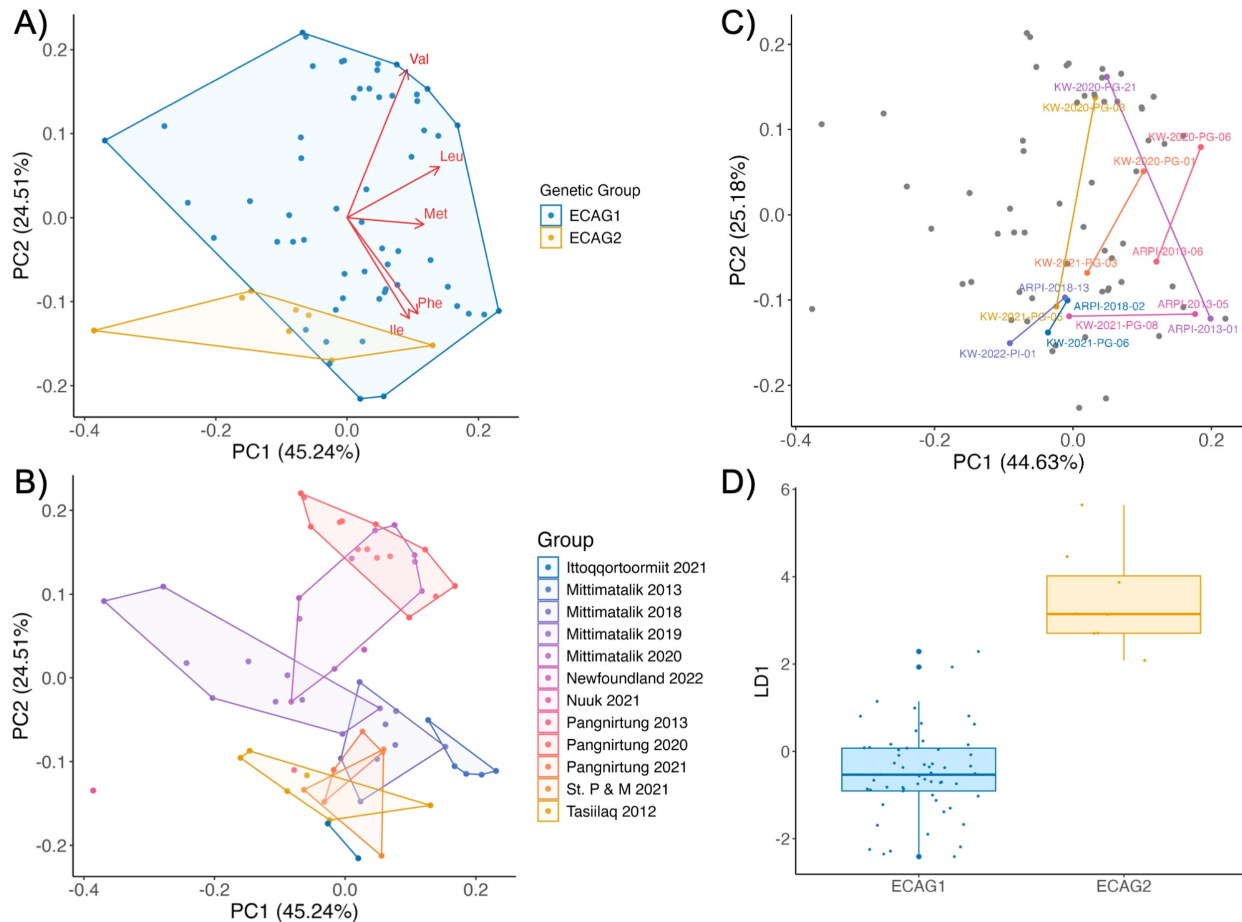


Figure 3.3. Principal component analysis of d^{13}C values for five essential amino acids (AA_{ESS}; Ile, Leu, Met, Phe, Val) in skin biopsies from killer whales in the eastern Canadian Arctic (panels A, B: n = 65, panel C: n = 72), with A) showing genetic groups ECAG1 and ECAG2, and B) showing groups sampled in each location and year. In C), seven individuals that were re-sampled in different years were included in the PCA, with solid lines connecting the values of the same individual on two different sampling occasions and the year of sample indicated in sample name label. Linear discriminant analysis showed separation between ECAG1 and ECAG2 AA_{ESS} values along linear discriminant 1 (D).

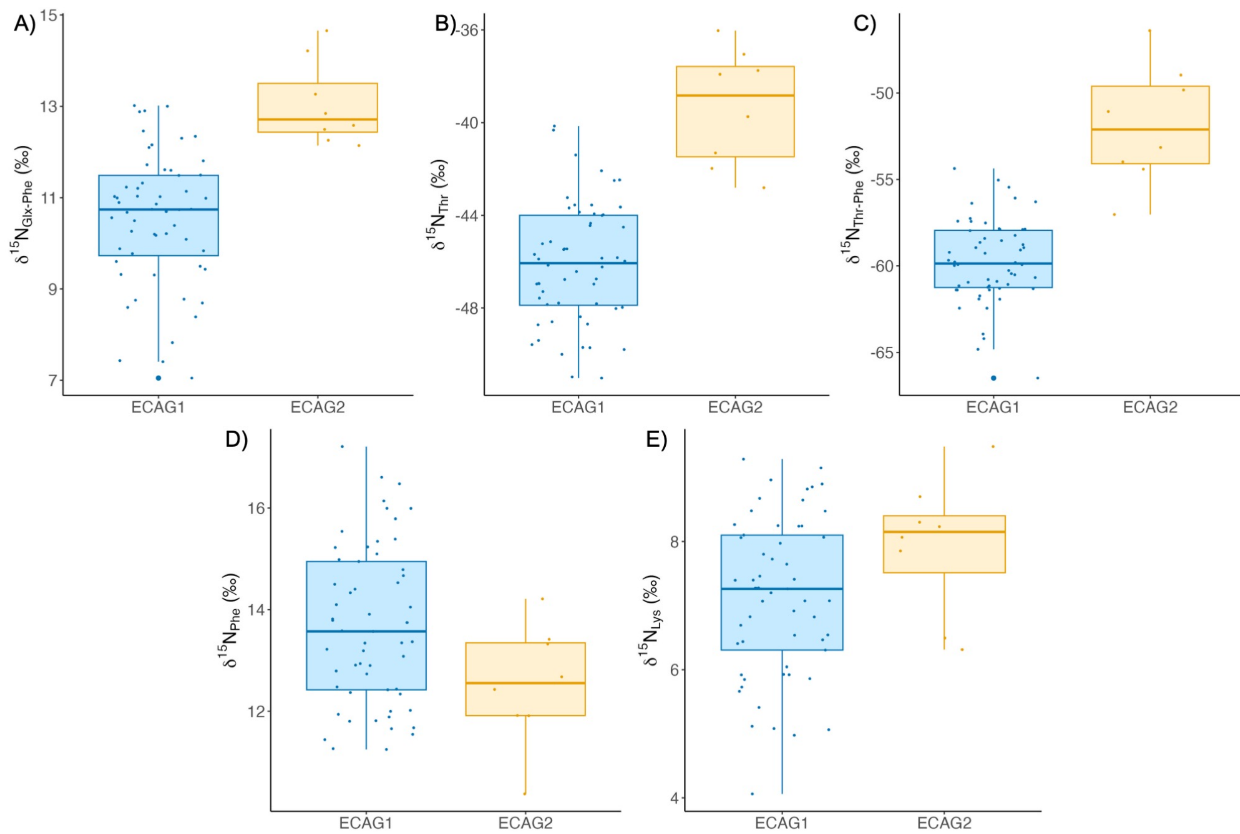


Figure 3.4. A) $d^{15}N_{Glx\text{-}Phe}$, B) $d^{15}N_{Thr}$, C) $d^{15}N_{Thr\text{-}Phe}$, values differed between genetic populations ECAG1 and ECAG2, and D) $d^{15}N_{Phe}$, E) $d^{15}N_{Lys}$ values did not differ between genetic groups ECAG1 and ECAG2 in 65 killer whales skin samples collected between 2002-2022 from eight locations in the Northwest Atlantic.

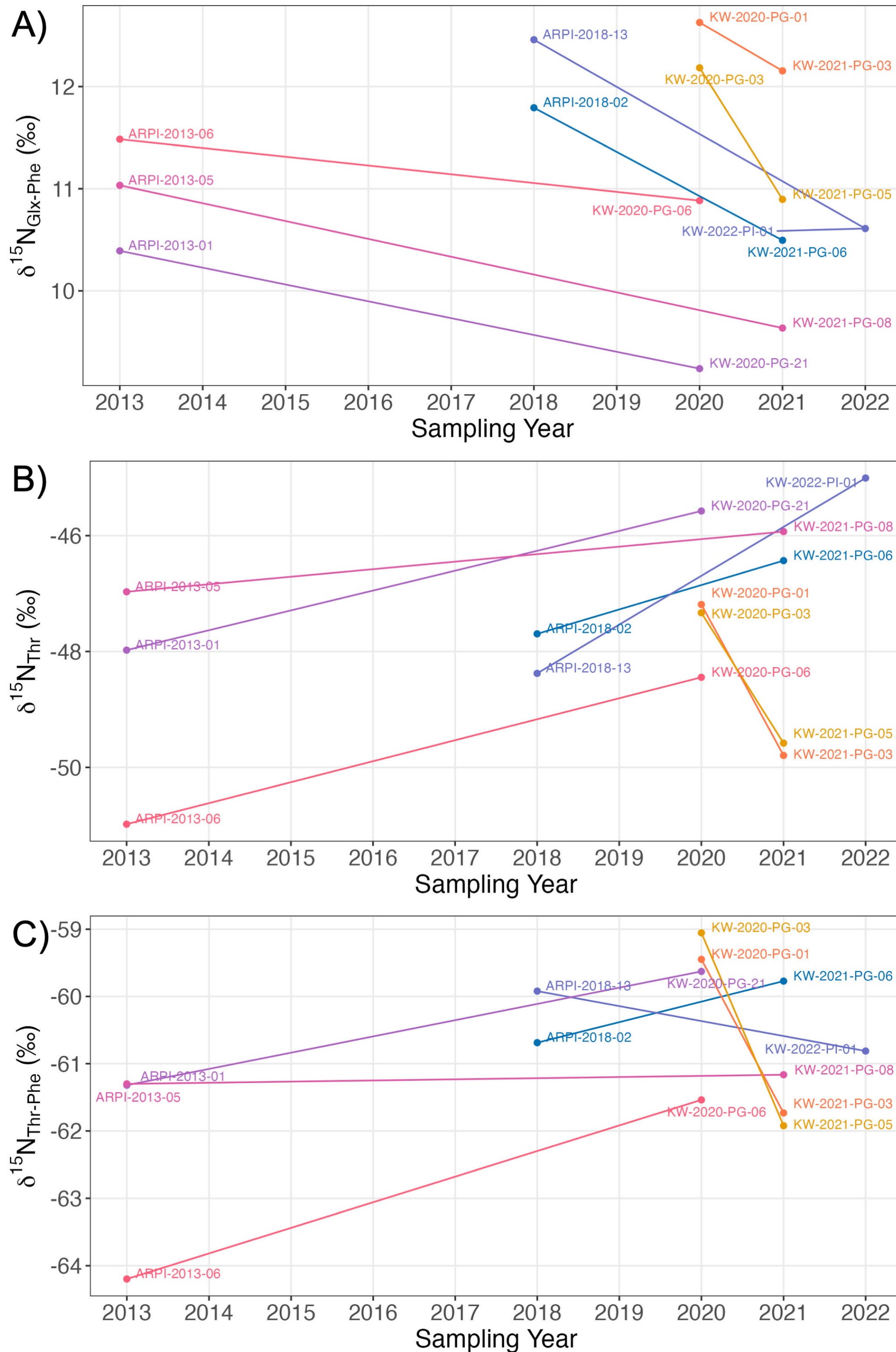


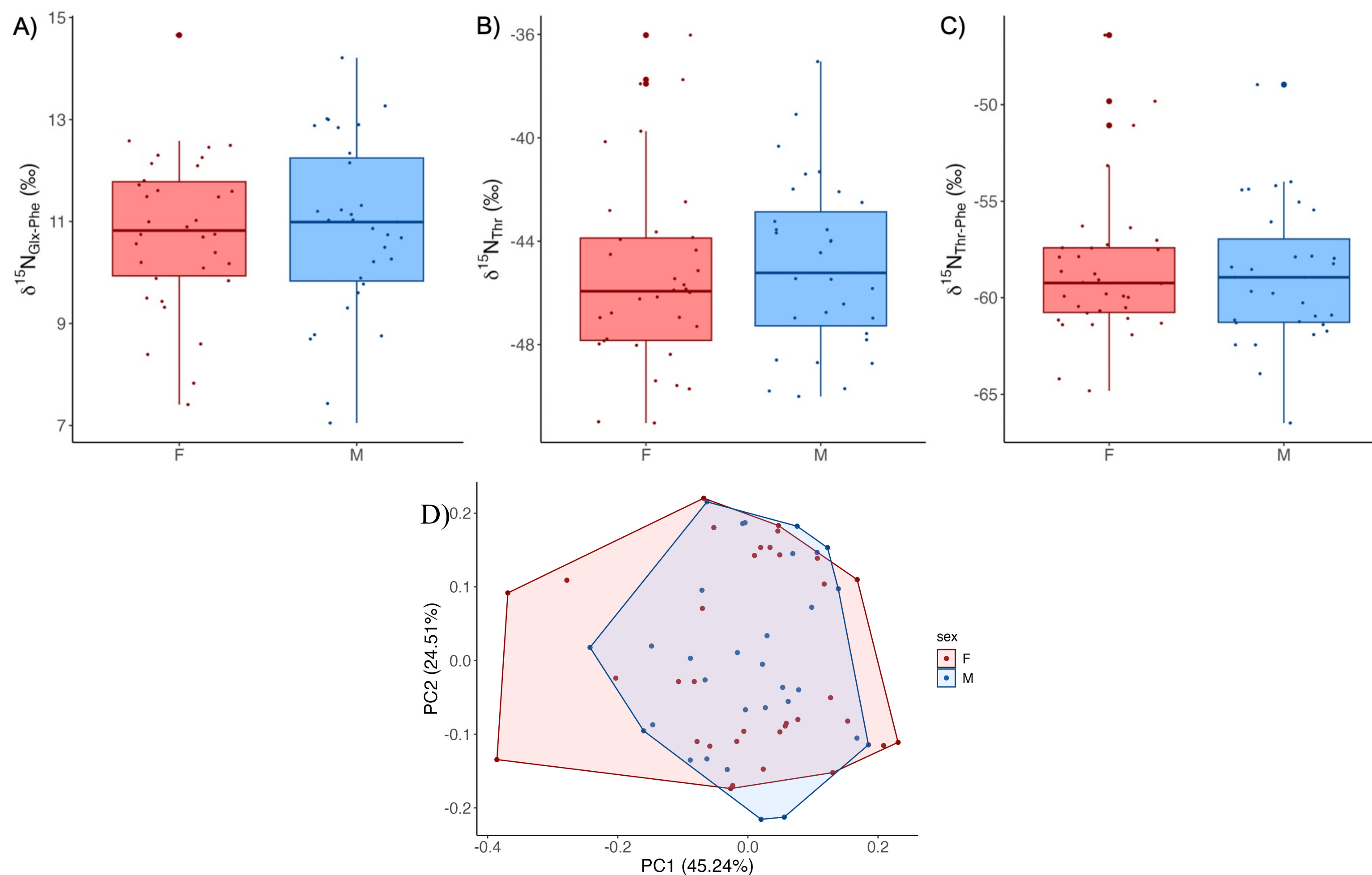
Figure 3.5. Skin A) d$^{15}N_{Glx\text{-}Phe}$, B) d$^{15}N_{Thr}$, and C) d$^{15}N_{Thr\text{-}Phe}$ values for seven re-sampled killer whales, with solid lines connecting samples from the same individual in different sampling years. Re-sampled individuals were determined genetically from whole genome sequences.

Tables

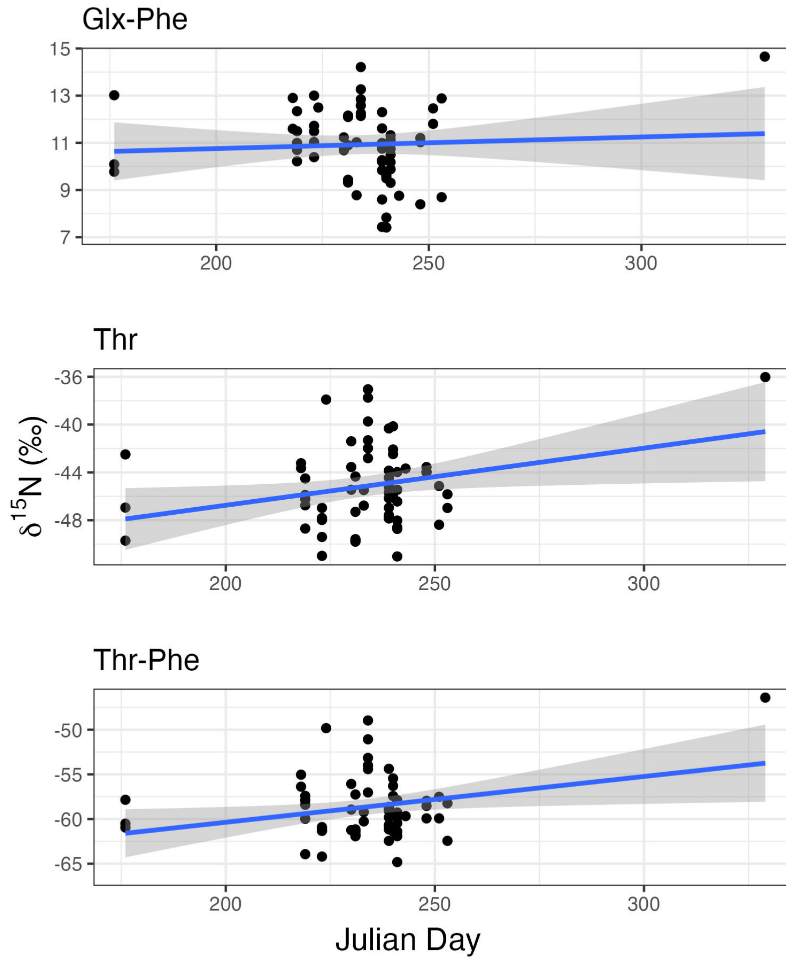
Table 3.1. Results from Bayesian multivariate (d^{13}C of essential amino acids Ile, Leu, Met, Phe, and Val) and univariate (d^{15}N of trophic and source amino acids Glx-Phe, Thr, Thr-Phe, Phe, Lys) models examining differences in compound-specific stable isotope ratios between genetic groups ECAG1 and ECAG2, measured in 65 killer whale skin samples collected between 2002-2022 from 8 locations across the Northwest Atlantic. For each AA or paired AA examined, ECAG2 was interpreted to significantly differ from ECAG1 when the 95% Bayesian confidence interval (L = lower limit, U = upper limit) for ECAG2 did not overlap with zero. AAs that differed are in italics and indicated with an asterisk.

Model	Coefficients	Estimate	Est. Error	L-95% C.I.	U-95% C.I.	
δ^{13}C AA_{ESS} ~ genetic group	Ile Intercept	-15.38	0.09	-15.56	-15.19	
	Leu Intercept	-25.09	0.09	-25.27	-24.91	
	Met Intercept	-21.99	0.12	-22.22	-21.75	
	Phe Intercept	-26.83	0.09	-27.01	-26.65	
	Val Intercept	-18.91	0.26	-19.41	-18.40	
	Ile Genetic Group ECAG2	-0.54	0.27	-1.06	-0.02	*
	Leu Genetic Group ECAG2	-1.54	0.26	-2.05	-1.03	*
	Met Genetic Group ECAG2	-0.05	0.34	-0.72	0.62	
	Phe Genetic Group ECAG2	0.59	0.26	0.09	1.10	*
	Val Genetic Group ECAG2	-2.33	0.73	-3.77	-0.89	*
$\delta^{15}N_{Glx\text{-}Phe}$ ~ genetic group	Intercept	10.53	0.19	10.16	10.89	
	Genetic Group ECAG2	*2.53*	*0.54*	*1.48*	*3.58*	*
$\delta^{15}N_{Thr}$ ~ genetic group	Intercept	-45.97	0.36	-46.69	-45.25	
	Genetic Group ECAG2	*6.64*	*1.04*	*4.58*	*8.68*	*
$\delta^{15}N_{Thr\text{-}Phe}$ ~ genetic group	Intercept	-59.65	0.35	-60.35	-58.96	
	Genetic Group ECAG2	*7.80*	*1.01*	*5.81*	*9.78*	*
$\delta^{15}N_{Phe}$ ~ genetic group	Intercept	13.68	0.20	13.28	14.08	
	Genetic Group ECAG2	-1.15	0.58	-2.30	0.00	
$\delta^{15}N_{Lys}$ ~ genetic group	Intercept	7.13	0.16	6.81	7.45	
	Genetic Group ECAG2	0.80	0.47	-0.11	1.73	

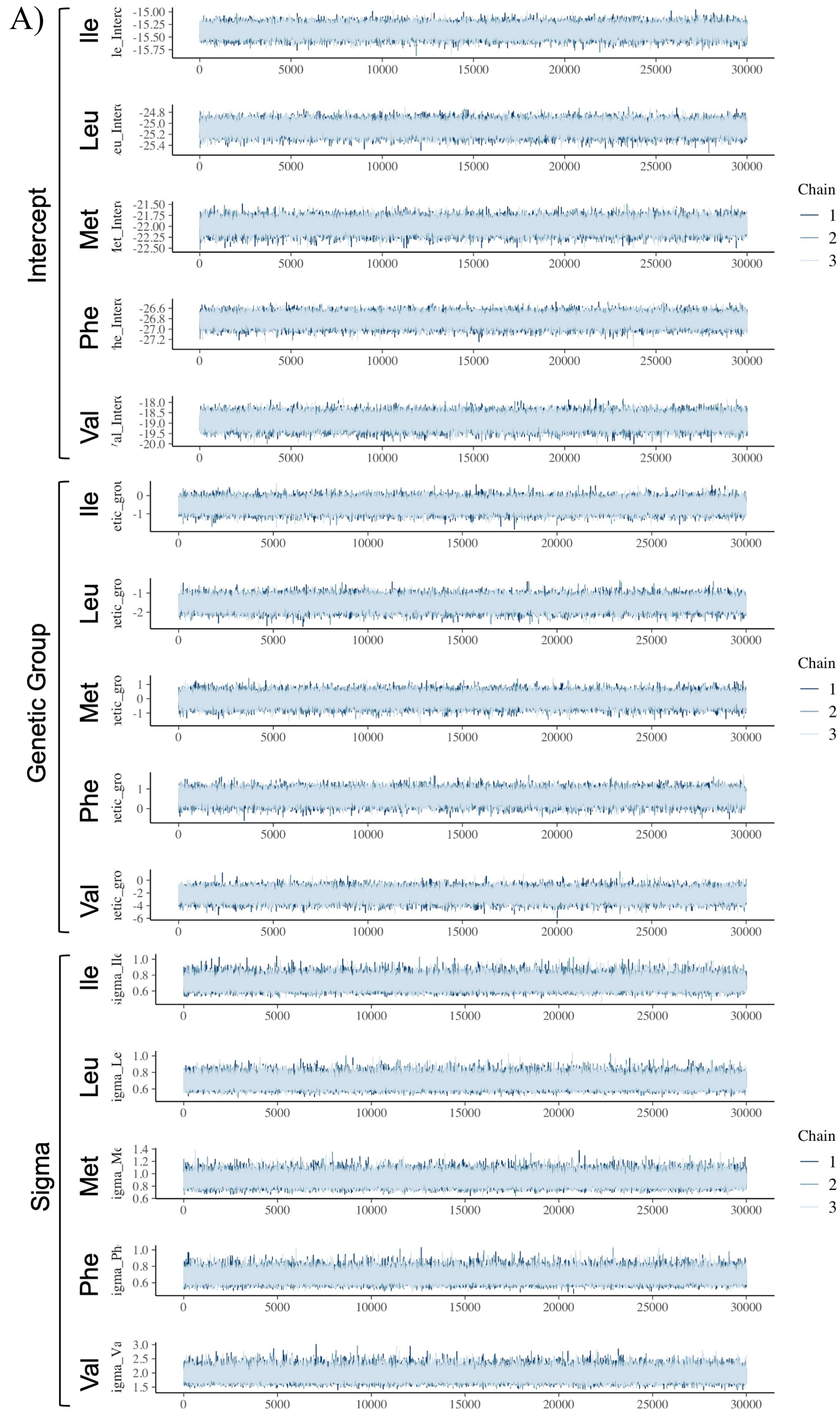
Supplemental Material

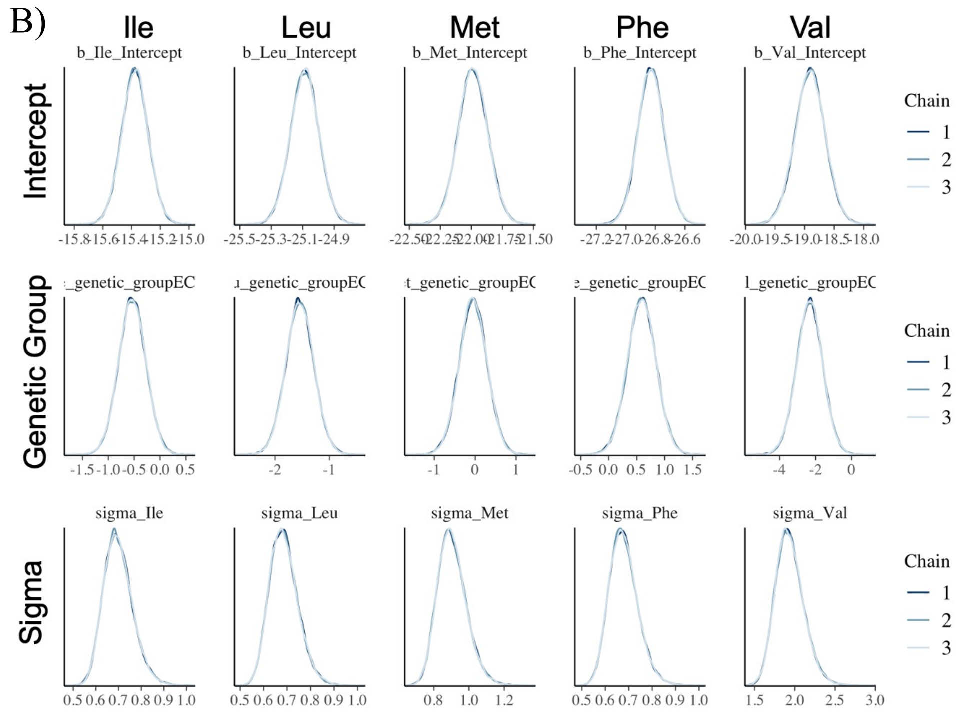


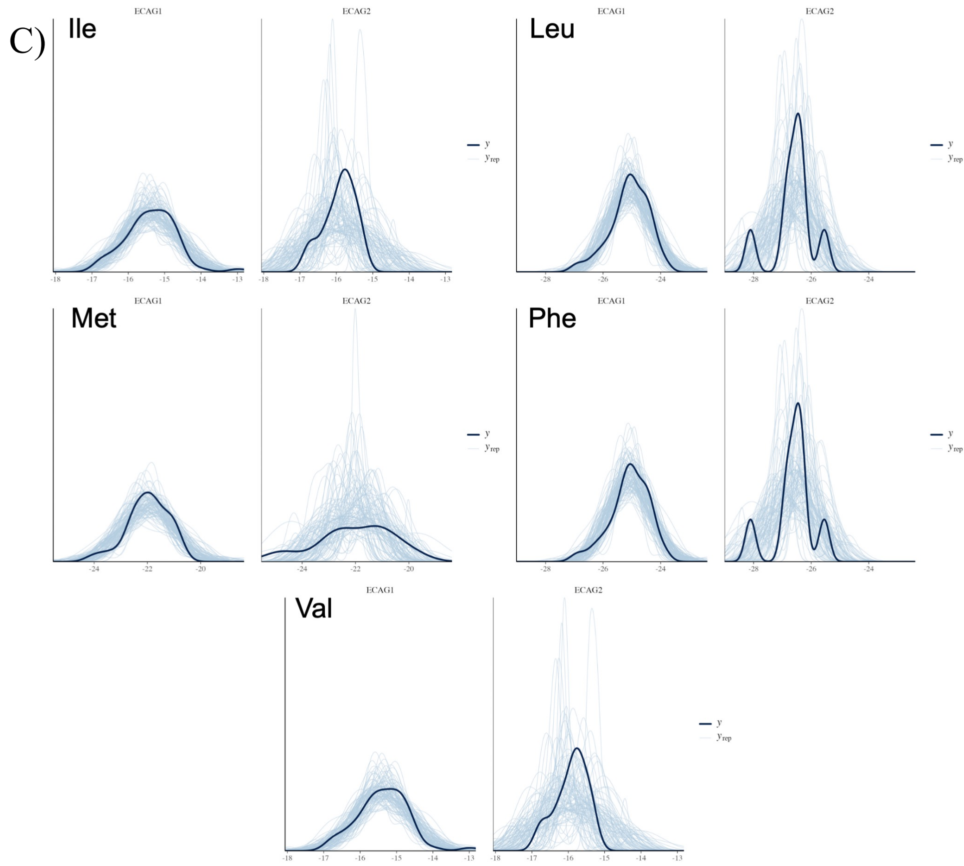
Supp. Figure 3.1. Boxplots showing no difference in A) $\delta^{15}N_{Glx\text{-}Phe}$, B) $\delta^{15}N_{Thr}$, and C) $\delta^{15}N_{Thr\text{-}Phe}$ values, and D) PCA showing substantial overlap in $\delta^{13}C$ AA_{ESS} values indicating no difference, between male (n = 31) and female (n = 34) killer whales sampled in 8 locations in the Northwest Atlantic between 2002-2022.

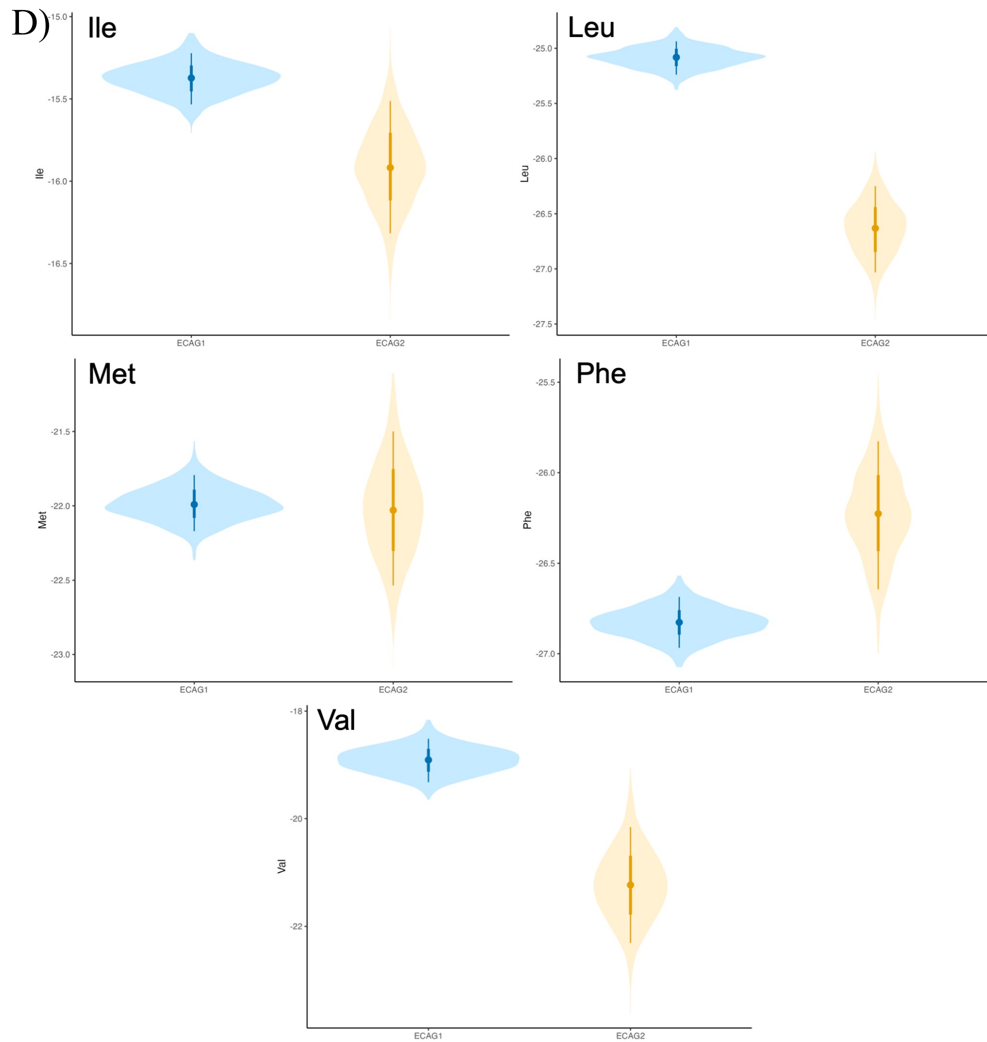


Supp. Figure 3.2. Julian Day was not a significant co-variate of genetic group for $\delta^{15}N_{Glx\text{-}Phe}$, $\delta^{15}N_{Thr}$, or $\delta^{15}N_{Thr\text{-}Phe}$ for 60 killer whales sampled between 2002-2022 in the Northwest Atlantic. Exact sampling date was not known for 5 individuals, but they were known to be sampled in July and August (Julian dates 182-243).

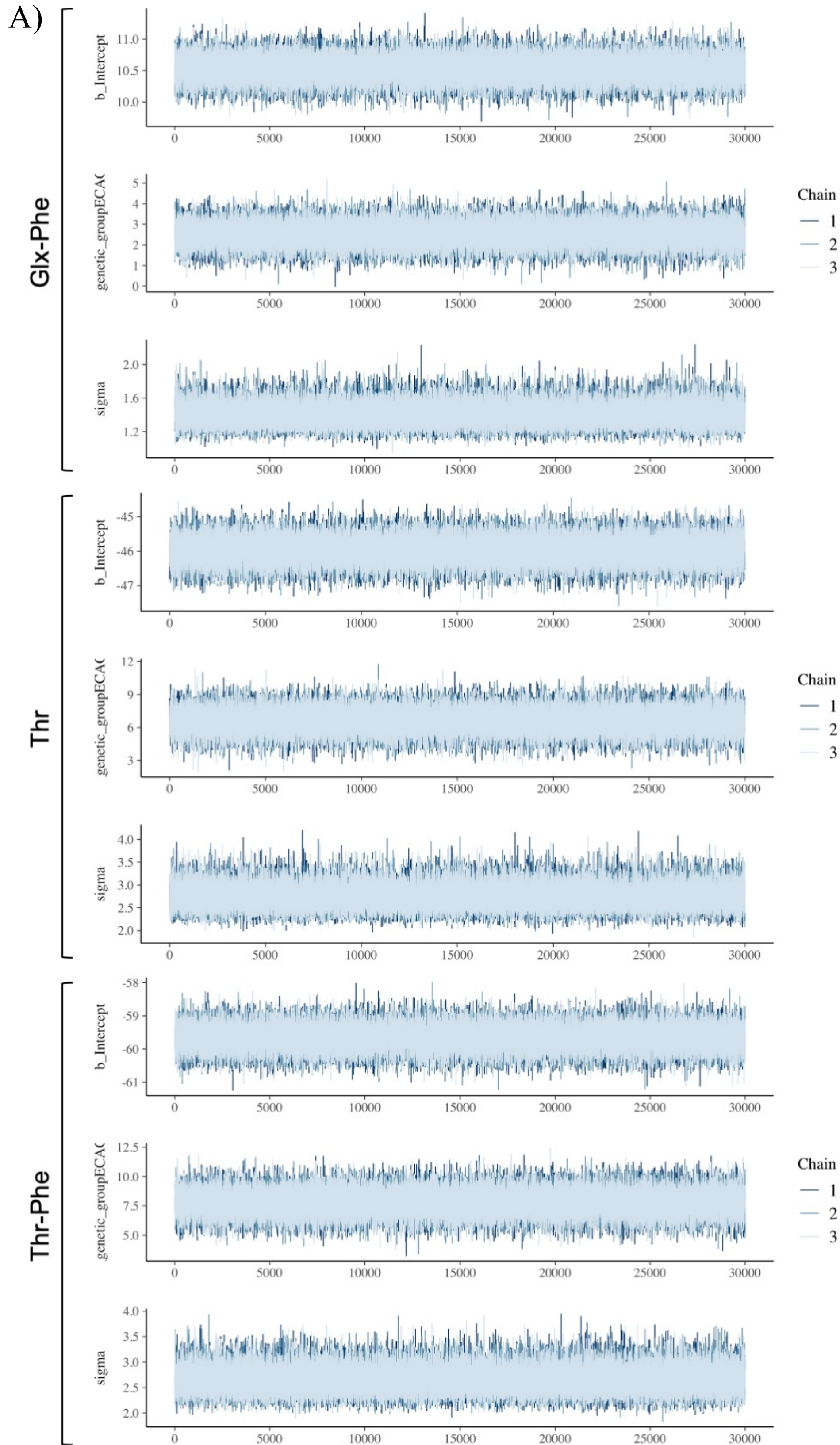

A)
Intercept
Genetic Group
Sigma
Ile
Leu
Met
Phe
Val
Chain
1
2
3
0
5000
10000
15000
20000
25000
30000

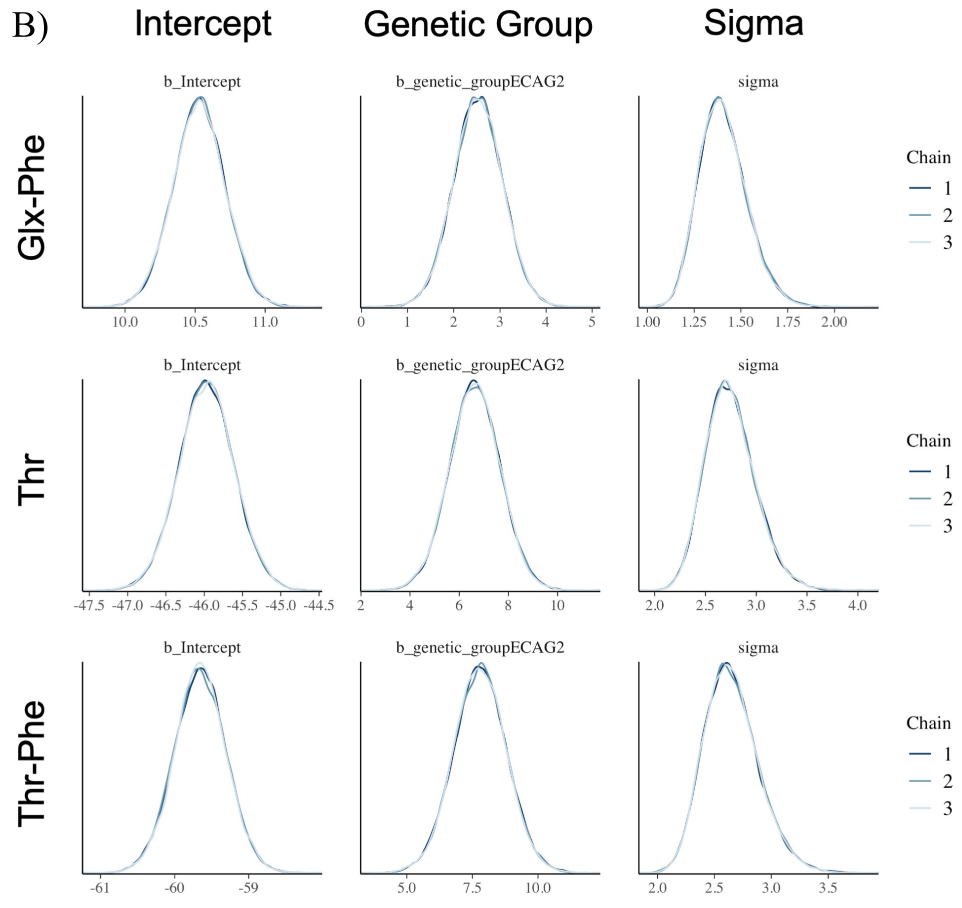

B)
Ile
Leu
Met
Phe
Val
b_Ile_Intercept
b_Leu_Intercept
b_Met_Intercept
b_Phe_Intercept
b_Val_Intercept
Intercept
Chain
1
2
3
-15.8-15.6-15.4-15.2-15.0
-25.5-25.3-25.1-24.9
-20.0-19.5-19.0-18.5-18.0
_genetic_groupEC
genetic_groupEC
Genetic Group
-1.5-1.0-0.5 0.0 0.5
-2 -1
-1 0 1
-0.5 0.0 0.5 1.0 1.5
-4 -2 0
sigma_Ile
sigma_Leu
sigma_Met
sigma_Phe
sigma_Val
Sigma
0.5 0.6 0.7 0.8 0.9 1.0
0.8 1.0 1.2
1.5 2.0 2.5 3.0


C)
Ile
Leu
Met
Phe
Val
ECAG1
ECAG2
y
y_{rep}

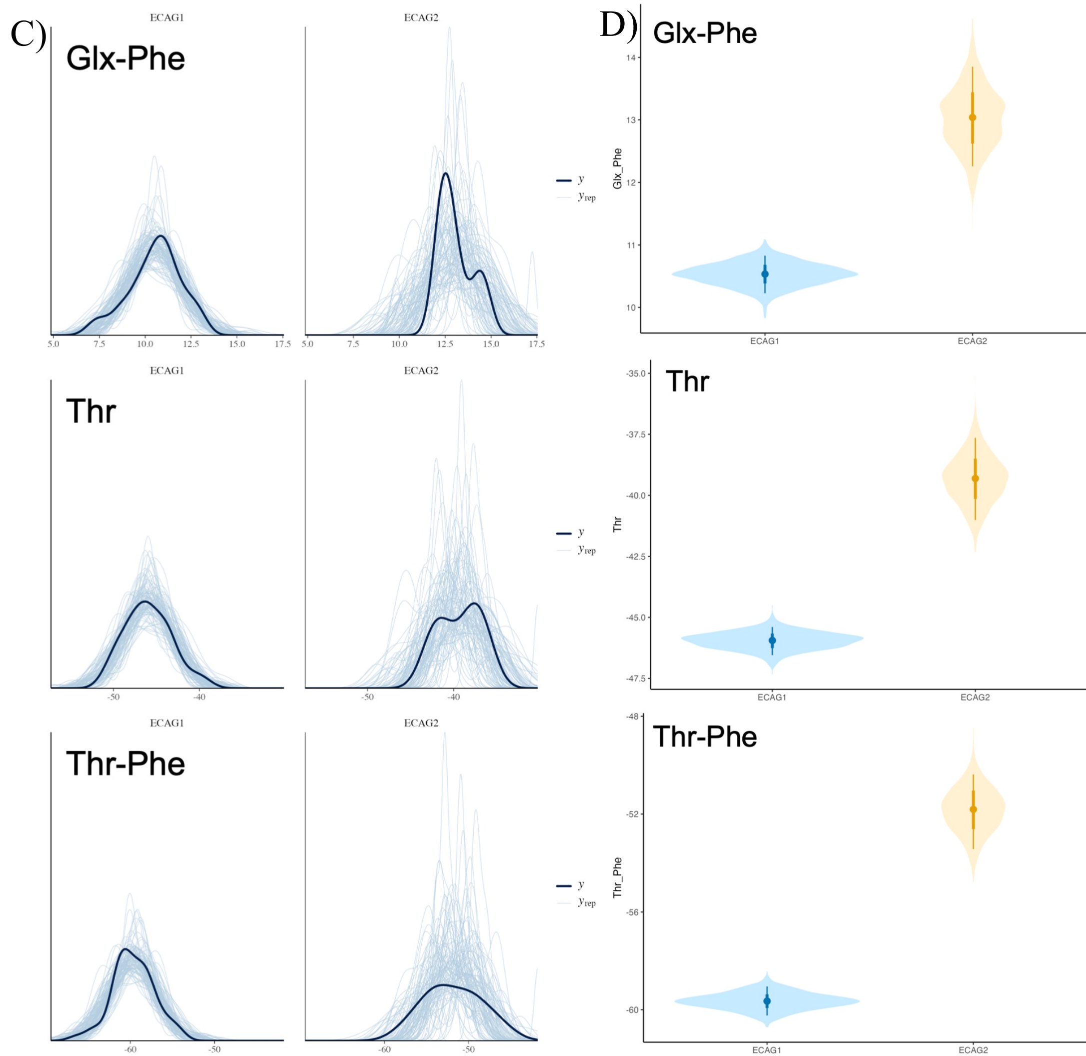


Supp. Figure 3.3. Diagnostic plots and posterior predictive plots for multivariate Bayesian model examining differences in five $\delta^{13}C$ AA_{ESS} (Ile, Leu, Met, Phe, Val) between two genetic groups (ECAG1, ECAG2). The A) trace plots of model draws from three chains for the intercept, ECAG2, and sigma (standard deviation of the posterior distribution) for the five $\delta^{13}C$ AA_{ESS}, and B) the posterior distribution of the five $\delta^{13}C$ AA_{ESS} generated from the three chains, both indicate convergence of the three model chains. The posterior predictive plots, C) posterior distribution of the mean AA value from the model (thick dark blue line) overlayed on the distribution of the mean AA value of 100 datasets simulated from the model (thin light blue lines) for ECAG1 and ECAG2, and D) the spread of model draws for ECAG1 and ECAG2, indicate lower certainty and model predictive ability for ECAG2 posterior estimates than ECAG1.


A)
Glx-Phe
b_Intercept
genetic_groupECAC
sigma
Chain
1
2
3
Thr
Thr-Phe



B)
Intercept
Genetic Group
Sigma
Glx-Phe
Thr
Thr-Phe
b_Intercept
b_genetic_groupECAG2
sigma
Chain
1
2
3
10.0 10.5 11.0
0 1 2 3 4 5
1.00 1.25 1.50 1.75 2.00
-47.5 -47.0 -46.5 -46.0 -45.5 -45.0 -44.5
2 4 6 8 10
2.0 2.5 3.0 3.5 4.0
-61 -60 -59
5.0 7.5 10.0
2.0 2.5 3.0 3.5



Supp. Figure 3.4. Diagnostic plots and posterior predictive plots for univariate Bayesian model examining differences in $\delta^{15}N_{Glx\text{-}Phe}$, $\delta^{15}N_{Thr}$, and $\delta^{15}N_{Thr\text{-}Phe}$ between genetic groups ECAG1 and ECAG2. The A) trace plots of model draws from three chains for the intercept, ECAG2, and sigma (standard deviation of the posterior distribution) for $\delta^{15}N_{Glx\text{-}Phe}$, $\delta^{15}N_{Thr}$, and $\delta^{15}N_{Thr\text{-}Phe}$, and B) the posterior distribution of $\delta^{15}N_{Glx\text{-}Phe}$, $\delta^{15}N_{Thr}$, and $\delta^{15}N_{Thr\text{-}Phe}$ generated from the three chains, both indicate convergence of model chains. The posterior predictive plots, C) posterior distribution of the mean AA value from the model (thick dark blue line) overlayed on the distribution of the mean AA value of 100 datasets simulated from the model (thin light blue lines) for ECAG1 and ECAG2, and D) the spread of model draws for ECAG1 (blue) and ECAG2 (orange), indicate lower certainty and model predictive ability for ECAG2 posterior estimates than ECAG1.

Supp. Table 3.1. List of killer whale skin samples collected for whole genome sequencing (WGS; n = 89) and compound-specific stable isotope analysis of amino acids (CSIA-A; n = 81) between 2002-2022 from eight locations across the Northwest Atlantic: Mittimatalik (Pond Inlet), Naujaat, and Pangnirtung in Nunavut, Canada; Newfoundland and St. Pierre et Miquelon (St. P&M), France on the southern Atlantic coast of Canada; Nuuk in West Greenland; and Ittoqqortoormiit and Tasiilaq in East Greenland. Individuals re-sampled in the same year or in different years are indicated by their sample ID, and were removed from the WGS analysis (WGS duplicate removed; n = 24) and CSIA-AA (CSIA duplicate removed; n = 16). Close kin were also removed for the WGS analysis (WGS close kin removed; n = 3). Individuals that were determined from the WGS analysis to be in ECAG2 are highlighted in grey, and individuals determined to be in ECAG1 are not highlighted.

Sample ID	Year	Location	Sex	Duplicate ID(s)	WGS	WGS Duplicate Removed	WGS Close Kin Removed	CSIA	CSIA Duplicate Removed
B045	2002	Newfoundland	M		x				
ARRB_xx_1291	2009	Naujaat	F		x				
48335	2012	Tasiilaq, EG	F		x		x		
48336	2012	Tasiilaq, EG	F		x			x	
48337	2012	Tasiilaq, EG	M		x			x	
48338	2012	Tasiilaq, EG	F		x		x	x	
48339	2012	Tasiilaq, EG	M		x		x	x	
48340	2012	Tasiilaq, EG	M		x				
48341	2012	Tasiilaq, EG	M		x			x	
48342	2012	Tasiilaq, EG	F		x			x	
ARPG-2013-01	2013	Pangnirtung	F		x			x	
ARPI-2013-01	2013	Mittimatalik	F	KW_2020_PG_21	x			x	
ARPI-2013-02	2013	Mittimatalik	M		x				
ARPI-2013-03	2013	Mittimatalik	F	ARPI-2013-04, KW-2021-PG-11	x			x	
ARPI-2013-04	2013	Mittimatalik	F	ARPI-2013-03, KW-2021-PG-11	x	x		x	x
ARPI-2013-05	2013	Mittimatalik	M	KW-2021-PG-08, KW-2021-PG-XX	x			x	
ARPI-2013-06	2013	Mittimatalik	F	KW-2020-PG-06, KW-2020-PG-18	x			x	

ARPI-2013-07	2013	Mittimatalik	M	KW-2021-PG-09	x		x	
ARPI-2018-01	2018	Mittimatalik	F		x		x	
ARPI-2018-02	2018	Mittimatalik	M	ARPI-2018-17, KW-2021-PG-06	x	x	x	x
ARPI-2018-03	2018	Mittimatalik	F				x	
ARPI-2018-05	2018	Mittimatalik	M				x	
ARPI-2018-06	2018	Mittimatalik	F				x	
ARPI-2018-07	2018	Mittimatalik	F	KW-2020-PI-12	x	x		
ARPI-2018-10	2018	Mittimatalik	F	ARPI-2018-14	x		x	
ARPI-2018-11	2018	Mittimatalik	F	KW-2020-PI-08	x	x		
ARPI-2018-13	2018	Mittimatalik	F	KW-2022-PI-01	x		x	
ARPI-2018-14	2018	Mittimatalik	F	ARPI-2018-10	x	x		
ARPI-2018-15	2018	Mittimatalik	M	ARPI-2018-16	x		x	
ARPI-2018-16	2018	Mittimatalik	M	ARPI-2018-15	x	x		
ARPI-2018-17	2018	Mittimatalik	M	ARPI-2018-02, KW-2021-PG-06	x	x		
ARPI-2018-18	2018	Mittimatalik	M		x		x	
ARPI-2019-01	2019	Mittimatalik	M		x		x	
ARPI-2019-02	2019	Mittimatalik	M		x		x	
ARPI-2019-03	2019	Mittimatalik	M	ARPI-2019-05	x		x	
ARPI-2019-04	2019	Mittimatalik	F		x		x	
ARPI-2019-05	2019	Mittimatalik	M	ARPI-2019-03	x	x		
ARPI-2019-06	2019	Mittimatalik	F		x		x	
ARPI-2019-07	2019	Mittimatalik	M		x		x	
ARPI-2019-08	2019	Mittimatalik	M		x		x	
ARPI-2019-09	2019	Mittimatalik	F		x		x	
ARPI-2019-10	2019	Mittimatalik	M		x		x	
ARPI-2019-11	2019	Mittimatalik	F		x		x	
KW-2019-01	2019	St. P&M, FR	M		x			
KW-2019-02	2019	St. P&M, FR	M		x			
KW-2020-PG-01	2020	Pangnirtung	M	KW-2020-PG-12, KW-2021-PG-03	x	x	x	x
KW-2020-PG-02	2020	Pangnirtung	F		x		x	

KW-2020-PG-03	2020	Pangnirtung	F	KW-2020-PG-07, KW-2021-PG-05	x	x		x	x
KW-2020-PG-04	2020	Pangnirtung	M	KW-2020-PG-20	x			x	
KW-2020-PG-05	2020	Pangnirtung	M		x			x	
KW-2020-PG-06	2020	Pangnirtung	F	ARPI-2013-06, KW-2020-PG-18	x	x		x	x
KW-2020-PG-07	2020	Pangnirtung	F	KW-2020-PG-03, KW-2021-PG-05	x	x		x	x
KW-2020-PG-08	2020	Pangnirtung	F					x	
KW-2020-PG-09	2020	Pangnirtung	F	KW-2020-PG-19	x			x	
KW-2020-PG-10	2020	Pangnirtung	M					x	
KW-2020-PG-11	2020	Pangnirtung	F		x			x	
KW-2020-PG-12	2020	Pangnirtung	M	KW-2020-PG-01, KW-2021-PG-03	x	x		x	x
KW-2020-PG-13	2020	Pangnirtung	M					x	
KW-2020-PG-14	2020	Pangnirtung	M					x	
KW-2020-PG-15	2020	Pangnirtung	F		x			x	
KW-2020-PG-16	2020	Pangnirtung	F		x			x	
KW-2020-PG-17	2020	Pangnirtung	M	KW-2020-PG-23	x			x	
KW-2020-PG-18	2020	Pangnirtung	F	ARPI-2013-06, KW-2020-PG-06	x	x		x	x
KW-2020-PG-19	2020	Pangnirtung	F	KW-2020-PG-09	x	x		x	x
KW-2020-PG-20	2020	Pangnirtung	M	KW-2020-PG-04	x	x		x	x
KW-2020-PG-21	2020	Pangnirtung	F	ARPI-2013-01	x	x		x	x
KW-2020-PG-22	2020	Pangnirtung	F		x			x	
KW-2020-PG-23	2020	Pangnirtung	M	KW-2020-PG-17	x	x		x	x
KW-2020-PG-24	2020	Pangnirtung	M		x			x	
KW-2020-PI-02	2020	Mittimatalik	F		x			x	
KW-2020-PI-03	2020	Mittimatalik	M		x			x	
KW-2020-PI-05	2020	Mittimatalik	F	KW-2020-PI-06	x			x	
KW-2020-PI-06	2020	Mittimatalik	F	KW-2020-PI-05	x	x		x	x
KW-2020-PI-07	2020	Mittimatalik	F		x			x	
KW-2020-PI-08	2020	Mittimatalik	F	ARPI-2018-11	x			x	
KW-2020-PI-10	2020	Mittimatalik	M		x			x	
KW-2020-PI-12	2020	Mittimatalik	F	ARPI-2018-07	x			x	

KW-2020-PI-13	2020	Mittimatalik	M		x			x	
KW-2020-PI-14	2020	Mittimatalik	M		x			x	
KW-2020-PI-15	2020	Mittimatalik	F		x			x	
GRNL-KW-2021-01	2021	Ittoqqortoormiit, EG	F		x			x	
GRNL-KW-2021-02	2021	Nuuk, WG	F		x			x	
GRNL-KW-2021-03	2021	Ittoqqortoormiit, EG	M		x			x	
KW-2021-PG-03	2021	Pangnirtung	M	KW-2020-PG-01, KW-2020-PG-12	x			x	
KW-2021-PG-04	2021	Pangnirtung	F		x			x	
KW-2021-PG-05	2021	Pangnirtung	F	KW-2020-PG-03, KW-2020-PG-07	x			x	
KW-2021-PG-06	2021	Pangnirtung	M	ARPI-2018-02, ARPI-2018-17	x			x	
KW-2021-PG-08	2021	Pangnirtung	M	ARPI-2013-05, KW-2021-PG-XX	x	x		x	x
KW-2021-PG-09	2021	Pangnirtung	M	ARPI-2013-07, ARPI-2013-03	x	x			
KW-2021-PG-11	2021	Pangnirtung	F	ARPI-2013-03, ARPI-2013-04	x	x			
KW-2021-PG-XX	2021	Pangnirtung	M	ARPI-2013-05, KW-2021-PG-08	x	x		x	x
Or21-1	2021	St. P&M, FR	F		x			x	
Or21-2	2021	St. P&M, FR	M		x			x	
Or21-3	2021	St. P&M, FR	M		x			x	
KW-2022-PI-01	2022	Mittimatalik	F	ARPI-2018-13	x	x		x	x
KW-Nfld-22-25	2022	Newfoundland	M		x				

Supp. Table 3.2. Raw compound-specific $\delta^{15}N$ and $\delta^{13}C$ stable isotope ratios from 81 killer whale skin samples collected from the Northwest Atlantic between 2002-2022. $\delta^{15}N_{Glx-Phe}$, $\delta^{15}N_{Thr}$, and $\delta^{15}N_{Thr-Phe}$ differ between consumer tissues and their prey and thus are used to infer trophic level, while $\delta^{15}N_{Phe}$ and $^{15}N_{Lys}$ are "source" AAs used to infer distribution. Ile, Leu, Met, Phe, and Val are considered "essential" AAs since they cannot be produced by consumers, and thus their $\delta^{13}C$ values reflect the source carbon and are used to infer distribution.

	$\delta^{15}N_{AA}$					$\delta^{13}C_{AA}$				
Sample ID	**Glx-Phe**	**Thr**	**Thr-Phe**	**Phe**	**Lys**	**Ile**	**Leu**	**Met**	**Phe**	**Val**
48336	12.58	-39.74	-53.15	13.41	8.23	-16.08	-26.41	-21.14	-26.22	-21.38
48337	14.21	-41.98	-54.41	12.43	8.70	-15.49	-26.58	-22.88	-26.92	-20.84
48338	12.26	-37.75	-51.07	13.32	8.30	-15.76	-26.41	-21.29	-25.74	-21.21
48339	12.84	-37.05	-48.96	11.91	7.86	-15.74	-26.31	-22.62	-26.03	-21.06
48341	13.27	-41.31	-53.99	12.68	8.07	-15.86	-26.77	-22.51	-26.82	-21.40
48342	12.14	-42.81	-57.02	14.21	9.48	-15.36	-25.55	-20.02	-25.46	-20.01
ARPG-2013-01	12.50	-37.91	-49.82	11.92	6.49	-16.31	-26.89	-21.08	-26.06	-20.97
ARPI-2013-01	10.39	-47.98	-61.32	13.34	6.69	-14.62	-24.23	-21.16	-25.40	-18.69
ARPI-2013-03	11.72	-49.40	-61.07	11.67	6.44	-14.50	-23.84	-21.64	-25.19	-18.31
ARPI-2013-04	11.91	-49.71	-62.05	12.34	6.27	-15.08	-24.08	-21.94	-25.49	-18.42
ARPI-2013-05	11.03	-46.97	-61.30	14.33	6.82	-14.90	-24.44	-20.94	-25.45	-19.12
ARPI-2013-06	11.48	-50.98	-64.20	13.22	7.07	-15.26	-24.39	-21.75	-25.71	-18.82
ARPI-2013-07	13.00	-47.82	-60.90	13.08	8.10	-14.92	-24.63	-21.14	-25.48	-18.85
ARPI-2018-01	10.75	-47.85	-60.79	12.94	7.08	-14.82	-25.12	-22.24	-26.28	-20.96
ARPI-2018-02	11.79	-47.70	-60.69	12.99	7.87	-14.78	-25.00	-22.90	-26.56	-20.34
ARPI-2018-03	7.43	-45.23	-62.44	17.21	8.26	-15.52	-24.76	-20.69	-26.54	-20.23
ARPI-2018-05	10.20	-45.69	-60.68	14.99	7.65	-14.98	-25.01	-21.02	-26.81	-20.96
ARPI-2018-06	11.61	-47.79	-59.81	12.02	7.40	-14.74	-24.35	-20.87	-26.24	-19.55
ARPI-2018-10	11.80	-45.14	-57.51	12.37	7.26	-15.03	-24.62	-21.59	-26.38	-20.19
ARPI-2018-13	12.46	-48.38	-59.92	11.54	7.27	-14.92	-24.68	-23.22	-26.48	-20.69
ARPI-2018-15	8.69	-45.83	-62.44	16.61	8.85	-15.36	-25.04	-20.98	-26.50	-20.04
ARPI-2018-18	12.88	-46.97	-58.23	11.26	7.07	-14.66	-24.62	-22.68	-27.14	-19.10
ARPI-2019-01	10.74	-43.55	-58.94	15.39	7.41	-15.24	-25.77	-21.71	-27.54	-20.51

ARPI-2019-02	10.68	-45.45	-61.24	15.79	8.48	-14.65	-24.85	-21.89	-26.94	-19.23
ARPI-2019-03	11.23	-41.40	-56.07	14.67	8.82	-15.52	-25.90	-22.08	-27.42	-20.02
ARPI-2019-04	9.32	-47.30	-61.40	14.10	5.41	-16.73	-26.28	-23.63	-28.08	-20.82
ARPI-2019-06	11.02	-46.78	-59.21	12.44	5.92	-16.64	-26.98	-23.86	-28.71	-21.73
ARPI-2019-07	8.78	-45.48	-60.27	14.79	6.83	-16.27	-26.75	-22.56	-27.99	-21.57
ARPI-2019-08	11.14	-48.60	-61.39	12.79	5.08	-15.66	-25.87	-22.66	-27.80	-20.68
ARPI-2019-09	9.88	-51.03	-64.82	13.79	5.66	-15.19	-26.43	-22.83	-28.32	-21.41
ARPI-2019-10	11.32	-48.73	-61.91	13.19	6.46	-15.44	-25.29	-21.24	-26.91	-21.09
ARPI-2019-11	10.75	-48.03	-60.45	12.42	5.12	-15.53	-25.70	-22.56	-27.32	-20.87
KW-2020-PG-01	12.63	-47.19	-59.45	12.26	7.55	-15.08	-24.48	-22.00	-26.47	-16.88
KW-2020-PG-02	11.60	-43.64	-56.38	12.74	8.67	-15.06	-23.96	-21.26	-26.66	-16.44
KW-2020-PG-03	12.18	-47.33	-59.05	11.72	7.71	-15.64	-24.41	-22.53	-27.00	-16.91
KW-2020-PG-04	12.90	-43.23	-55.04	11.80	8.24	-15.71	-24.08	-22.09	-26.92	-17.31
KW-2020-PG-05	12.34	-46.75	-58.41	11.65	7.27	-15.39	-24.53	-21.28	-26.34	-16.34
KW-2020-PG-06	10.88	-48.45	-61.54	13.09	6.89	-15.19	-24.19	-20.84	-26.27	-16.09
KW-2020-PG-07	12.52	-47.05	-58.53	11.49	7.91	-16.20	-25.09	-22.17	-27.07	-17.07
KW-2020-PG-08	11.00	-44.51	-57.41	12.90	7.46	-15.88	-24.50	-22.02	-26.82	-17.15
KW-2020-PG-09	10.70	-46.23	-59.98	13.74	7.80	-15.79	-24.71	-21.49	-27.31	-16.66
KW-2020-PG-10	10.21	-48.70	-63.93	15.24	9.29	-14.95	-24.44	-22.94	-26.24	-16.16
KW-2020-PG-11	11.49	-45.89	-57.89	12.00	7.40	-16.31	-25.35	-22.26	-27.27	-17.24
KW-2020-PG-12	12.48	-46.79	-59.02	12.23	7.47	-16.27	-24.91	-23.02	-27.44	-17.01
KW-2020-PG-13	10.86	-44.44	-58.94	14.50	8.24	-16.18	-25.23	-21.82	-27.14	-16.62
KW-2020-PG-14	10.99	-47.57	-61.16	13.58	7.73	-16.27	-25.20	-22.17	-26.91	-16.45
KW-2020-PG-15	12.30	-46.96	-58.77	11.81	7.28	-15.84	-24.94	-22.45	-26.79	-16.44
KW-2020-PG-16	9.84	-46.16	-58.64	12.48	8.06	-16.79	-25.36	-22.49	-27.07	-16.81
KW-2020-PG-17	10.27	-40.32	-54.37	14.05	8.65	-16.68	-25.65	-22.09	-27.16	-16.61
KW-2020-PG-18	8.99	-47.07	-61.75	14.68	7.28	-16.36	-25.17	-22.15	-27.20	-16.09
KW-2020-PG-19	9.41	-45.36	-59.77	14.41	7.22	-16.35	-25.13	-22.16	-27.31	-15.88
KW-2020-PG-20	11.16	-45.80	-58.52	12.72	7.45	-16.36	-25.11	-22.48	-27.09	-15.93
KW-2020-PG-21	9.24	-45.58	-59.63	14.05	7.19	-15.91	-24.95	-21.88	-26.75	-15.84
KW-2020-PG-22	8.60	-43.86	-59.08	15.22	7.97	-15.89	-25.18	-21.85	-26.78	-16.26
KW-2020-PG-23	10.41	-41.26	-55.56	14.30	6.61	-15.95	-25.28	-22.48	-27.35	-16.35
KW-2020-PG-24	8.76	-43.68	-59.67	16.00	8.47	-15.67	-24.52	-21.17	-26.62	-16.00

KW-2020-PI-02	9.43	-44.34	-57.25	12.91	5.93	-15.75	-24.89	-21.87	-27.01	-16.09
KW-2020-PI-03	9.60	-42.08	-55.45	13.37	5.86	-15.14	-24.33	-22.02	-26.90	-15.89
KW-2020-PI-05	7.41	-40.15	-56.29	16.14	6.41	-15.54	-25.49	-21.53	-27.37	-16.68
KW-2020-PI-06	7.85	-40.46	-56.86	16.40	9.39	-15.33	-25.24	-21.44	-27.14	-16.66
KW-2020-PI-07	9.50	-42.47	-57.42	14.95	6.92	-14.86	-25.07	-20.96	-27.03	-15.96
KW-2020-PI-08	7.83	-45.85	-61.39	15.54	6.04	-15.24	-25.01	-20.76	-27.12	-16.02
KW-2020-PI-10	9.30	-43.97	-57.88	13.91	5.73	-15.44	-24.40	-21.68	-27.33	-16.30
KW-2020-PI-12	10.17	-45.45	-59.27	13.82	8.07	-15.65	-25.14	-22.50	-27.24	-21.41
KW-2020-PI-13	11.20	-44.00	-58.53	14.53	8.90	-15.83	-24.30	-24.15	-27.02	-19.04
KW-2020-PI-14	11.03	-43.55	-57.96	14.41	8.96	-15.71	-25.02	-22.04	-26.87	-19.87
KW-2020-PI-15	8.39	-43.93	-59.93	15.99	9.15	-16.11	-25.22	-22.09	-27.32	-19.83
GRNL-KW-2021-01	10.56	-45.98	-57.87	11.89	5.85	-15.34	-26.05	-21.61	-26.16	-21.61
GRNL-KW-2021-02	14.66	-36.03	-46.40	10.37	6.31	-16.73	-28.11	-24.74	-26.65	-22.93
GRNL-KW-2021-03	7.05	-50.00	-66.48	16.48	6.30	-12.98	-25.39	-23.36	-26.93	-20.18
KW-2021-PG-03	12.15	-49.79	-61.73	11.94	4.98	-14.82	-24.88	-22.55	-26.59	-19.65
KW-2021-PG-04	12.10	-49.71	-61.15	11.44	4.06	-14.86	-24.90	-21.73	-26.51	-20.11
KW-2021-PG-05	10.90	-49.58	-61.92	12.34	6.54	-14.56	-25.00	-22.46	-27.15	-21.26
KW-2021-PG-06	10.50	-46.43	-59.77	13.34	5.92	-15.10	-25.58	-22.09	-26.55	-21.65
KW-2021-PG-08	9.64	-45.93	-61.16	15.23	6.16	-15.21	-25.61	-21.49	-26.48	-21.16
KW-2021-PG-XX	9.58	-46.73	-61.54	14.81	5.97	-14.87	-25.58	-21.84	-26.52	-20.60
OR21-1	10.09	-46.94	-60.52	13.57	5.06	-14.68	-25.08	-21.57	-26.67	-19.80
OR21-2	9.77	-42.50	-57.84	15.35	8.25	-15.21	-26.17	-22.25	-26.50	-20.94
OR21-3	13.02	-49.70	-60.95	11.25	6.54	-14.01	-25.47	-22.53	-25.97	-20.17
KW-2022-PI-01	10.61	-45.01	-60.81	15.80	7.68	-14.72	-26.45	-23.09	-26.54	-20.40
KW-Nfld-22-25	9.89	-39.09	-54.19	15.10	7.20	-16.19	-25.28	-21.12	-26.35	-18.94

Supp. Table 3.3. Results from Bayesian multivariate ($\delta^{13}C$ of essential amino acids Ile, Leu, Met, Phe, and Val) and univariate ($\delta^{15}N$ of trophic and source amino acids Glx-Phe, Thr, Thr-Phe) models examining differences in compound-specific stable isotope ratios between genetic groups ECAG1 and ECAG2 and sex, measured in 65 killer whale skin samples collected between 2002-2022 from 8 locations across the Northwest Atlantic. For each AA or paired AA examined, ECAG2 was interpreted to significantly differ from ECAG1, and males to significantly differ from females, when the 95% Bayesian confidence interval (L = lower limit, U = upper limit) for ECAG2 or males did not overlap with zero. AAs that differed are in italics and indicated with an asterisk.

Model	Coefficients	Estimate	Est. Error	L-95% C.I.	U-95% C.I.	
$\delta^{13}C\ AA_{ESS}$ ~ genetic group + sex	Ile Intercept	-15.43	0.13	-15.68	-15.18	
	Leu Intercept	-25.09	0.13	-25.34	-24.85	
	Met Intercept	-21.92	0.16	-22.24	-21.59	
	Phe Intercept	-26.83	0.12	-27.08	-26.59	
	Val Intercept	-18.94	0.35	-19.63	-18.25	
	Ile Genetic Group ECAG2	-0.53	0.27	-1.05	0.00	
	Ile Sex Male	0.11	0.18	-0.23	0.46	
	Leu Genetic Group ECAG2	*-1.54*	*0.26*	*-2.06*	*-1.03*	*
	Leu Sex Male	0.01	0.17	-0.33	0.35	
	Met Genetic Group ECAG2	-0.06	0.34	-0.74	0.62	
	Met Sex Male	-0.14	0.23	-0.59	0.31	
	Phe Genetic Group ECAG2	*0.59*	*0.26*	*0.08*	*1.10*	*
	Phe Sex Male	0.01	0.17	-0.33	0.34	
	Val Genetic Group ECAG2	*-2.32*	*0.74*	*-3.78*	*-0.86*	*
	Val Sex Male	0.06	0.49	-0.90	1.03	
$\delta^{15}N_{Glx-Phe}$ ~ genetic group + sex	Intercept	10.43	0.26	9.92	10.94	
	Genetic Group ECAG2	*2.55*	*0.54*	*1.49*	*3.62*	*
	Sex Male	0.19	0.35	-0.50	0.89	
$\delta^{15}N_{Thr}$ ~ genetic group + sex	Intercept	-46.36	0.50	-47.33	-45.39	
	Genetic Group ECAG2	*6.72*	*1.04*	*4.68*	*8.78*	*
	Sex Male	0.79	0.68	-0.56	2.14	
$\delta^{15}N_{Thr-Phe}$ ~ genetic group + sex	Intercept	-59.68	0.48	-60.62	-58.73	
	Genetic Group ECAG2	*7.80*	*1.01*	*5.81*	*9.80*	*
	Sex Male	0.05	0.67	-1.27	1.36	

Supp. Table 3.4. Results from Bayesian multivariate ($\delta^{13}C$ of essential amino acids Ile, Leu, Met, Phe, and Val) and univariate ($\delta^{15}N$ of trophic and source amino acids Glx-Phe, Thr, Thr-Phe) models examining differences in compound-specific stable isotope ratios between genetic groups ECAG1 and ECAG2 and with Julian day, measured in 60 killer whale skin samples collected between 2002-2022 from 8 locations across the Northwest Atlantic. Exact sampling date was not known for 5 individuals, but they were known to be sampled in July and August (Julian dates 182-243). Variables are interpreted to be significant when the 95% Bayesian confidence interval (L = lower limit, U = upper limit) did not overlap with zero. AAs that differed are in italics and indicated with an asterisk.

Model	Coefficients	Estimate	Est. Error	L-95% C.I.	U-95% C.I.	
$\delta^{13}C$ AA_{ESS} ~ genetic group + Julian day	Ile Intercept	-13.1	0.93	-14.92	-11.27	
	Leu Intercept	-23.83	1.05	-25.91	-21.76	
	Met Intercept	-18.98	1.35	-21.67	-16.34	
	Phe Intercept	-24.71	1.03	-26.73	-22.70	
	Val Intercept	-17.11	3.02	-23.03	-11.15	
	Ile Genetic Group ECAG2	-0.38	0.23	-0.84	0.08	
	Ile Julian day	-0.01	0.00	-0.02	0.00	
	Leu Genetic Group ECAG2	*-1.50*	*0.27*	*-2.03*	*-0.98*	*
	Leu Julian day	-0.01	0.00	-0.01	0.00	
	Met Genetic Group ECAG2	*0.11*	*0.34*	*-0.55*	*0.78*	
	Met Julian day	-0.01	0.01	-0.02	0.00	
	Phe Genetic Group ECAG2	*0.73*	*0.26*	*0.22*	*1.23*	*
	Phe Julian day	-0.01	0.00	-0.02	0.00	
	Val Genetic Group ECAG2	*-2.30*	*0.76*	*-3.80*	*-0.80*	*
	Val Julian day	-0.01	0.01	-0.03	0.02	
$\delta^{15}N_{Glx\text{-}Phe}$ ~ genetic group + Julian day	Intercept	11.59	2.11	7.44	15.75	
	Genetic Group ECAG2	*2.51*	*0.54*	*1.45*	*3.56*	*
	Julian day	0.00	0.01	-0.02	0.01	
$\delta^{15}N_{Thr}$ ~ genetic group + Julian day	Intercept	-51.63	3.99	-59.48	-43.85	
	Genetic Group ECAG2	*6.38*	*1.01*	*4.40*	*8.38*	*
	Julian day	0.02	0.02	-0.01	0.06	
$\delta^{15}N_{Thr\text{-}Phe}$ ~ genetic group + Julian day	Intercept	-65.15	3.73	-72.49	-57.81	
	Genetic Group ECAG2	*7.50*	*0.95*	*5.63*	*9.35*	*
	Julian day	0.02	0.02	-0.01	0.06	

4. General Conclusion

In the eastern Canadian Artic and surrounding Northwest Atlantic, killer whales are a difficult species to study because of their low density, broad distribution, long-range movements, and the limited season in which sampling can occur. Despite these challenges, collaborative research with Inuit communities over the last 15 years has produced a sufficiently large photograph database and tissue inventory to investigate longstanding questions about the demographics, population structure, and ecology of eastern Canadian Arctic killer whales using complementary physical and molecular methods.

The objective of my second chapter was to evaluate population abundance, growth rate, and demographic structure of killer whales seasonally resident in the eastern Canadian Arctic. I used Bayesian capture-mark-recapture models to estimate the population abundance and growth rate, comparing model results between photo-identification and genetic-identification data types. The photo-identification data indicated a population abundance of 215 individuals and a growth rate of approximately 1.7% per year. These estimates are reasonable compared to the previous abundance estimate (Lefort et al. 2020) and growth rates estimated for other killer whale populations (e.g. Brault and Caswell 1993; Olesiuk et al. 2005; Matkin et al. 2014; Towers 2019; Jordaan et al. 2020; DFO 2022), and align with the apparent increase from sightings reports. The genetic-identification data produced estimates that were biologically implausible compared to the photo-identification data, likely due to lower data availability and a large break between the first and subsequent sampling years. I determined sex from whole genome sequences (Smith et al. 2018) and estimated individual age using epigenetic aging (Parsons et al. 2023), which produced a demographic structure skewed towards juveniles and with few reproductive adults. Finally, I supplemented the demographic structure produced from molecular markers, which did not include calves, by counting the proportion of calves and young juveniles in groups relative to older adults in photographs of group encounters. While the proportion of calves per group is comparable to the proportion of calves in a stable or growing population, it is difficult to draw any conclusions about population growth from the demographic structure due to the patchwork nature of this analysis and the nominal differences in age-class ratios between stable and growing populations (Olesiuk et al. 2005).

In my third chapter, I explored ecological differences between two genetically discrete killer whale populations in the western North Atlantic, inferring diet and distribution using compound-specific stable isotope analysis (CSIA) of amino acids (AA). Using a principal component analysis of whole genome sequences to evaluate population structure, I confirmed that genetic groupings, with the inclusion of new samples, aligned with those identified in Garroway et al. (2024). Bayesian multivariate analysis of $\delta^{13}C$ in five essential amino acids (AA_{ESS}), used as a proxy for geographic distribution, pointed to moderately overlapping, yet distinct $\delta^{13}C$ AA_{ESS} profiles, suggesting differing spatial distribution between the two populations. Analysis of $\delta^{15}N$ in "trophic" and "source" amino acids, used to infer trophic position of consumers, indicated that ECAG1 is feeding at a higher trophic level than ECAG2. This trophic difference is consistent with knowledge from stomach contents, fatty acid analysis, and previous stable isotope analyses that ECAG1 killer whales consume primarily, if not exclusively, marine mammals, while ECAG2 whales consume both marine mammals and fish. Overall, these results revealed ecological differentiation between the genetic populations, which aligns with the ecological and genetic divergence observed in other North Atlantic and global killer whale populations.

All analyses in this thesis depended on archived photographs and biopsy samples collected during dedicated field sampling programs, from collaboration across provincial and national boundaries, and thanks to the generous contributions of independent parties. This work highlights the value of long-term sampling programs, and emphasizes the importance of cooperation and collaboration with Inuit communities and local research partners. Due to the difficulties in sampling killer whales mentioned previously, inconsistent temporal and spatial coverage of photographs and biopsies across the sampling region reduced the power of the analyses presented here. Continued annual collection of killer whale photographs, biopsies for epigenetic aging, and drone images will help to improve the accuracy of demographic ratios and rates. Similarly, collection of killer whale biopsies, particularly in Hudson Bay, Atlantic Canada, and the East and West coasts of Greenland, will help to resolve genetic structure and population-level differences in diet and distribution. A focus on the acoustic behaviour, social dynamics, and morphology of these killer whales will further contribute to our understanding of ecological divergence (or similarities) between populations, and whether labels such as ecotype or morphotype would be appropriate.

Characterizing the abundance, dynamics, and ecology of top predators is understand their role in, and potential impact on, ecosystems, particularly when that predator occupies different ecological niches in different settings. As species' ranges and interactions shift with climate change, conserving ecosystem diversity and intraspecies genetic variation is equally as important as preserving individual species (Walther 2010; Pauls et al. 2013; Gallagher et al. 2013; Schmidt et al. 2023). As apex predators, killer whales have the potential to exert influence at the ecosystem level (Estes et al. 1998; Springer et al. 2003), and a growing population in the eastern Canadian Arctic could have a significant numerical and behavioral impact on vulnerable Arctic-endemic cetaceans (Ferguson et al. 2012; Breed et al. 2017; Westdal et al. 2017; Matthews et al. 2020; Lefort et al. 2020). They appear to be generalist predators, flexible in both their diet and distribution (Matthews et al. 2021, 2024; this thesis), and may be behaviourally adaptable to a changing Arctic ecosystem. However, the population remains small and faces additional threats from contaminants, harvest, and low within-population genetic diversity (Desforges et al. 2024; Garroway et al. 2024; this thesis), and thus may require conservation protection. Although the population appears to be growing and may currently benefit from increased access to Arctic prey, both killer whales and their prey face multiple anthropogenic impacts (Burek et al. 2008; Laidre et al. 2008) so advantages gained could be short-lived. In a broader context, they are genetically differentiated and occupy a distinct ecological niche from other killer whales in the North Atlantic (Garroway et al. 2024; Matthews et al. 2024; this thesis), exhibiting intra-specific diversity that is important to maintain (Pauls et al. 2013). Accordingly, discussions regarding potential predator management should also consider the conservation needs of this population.

Overall, this work contributes to our understanding of killer whale ecology in the Northwest Atlantic, quantifying the apparent population growth in the eastern Canadian Arctic and describing the genetic and ecological differentiation of populations that aligns with the diversity of killer whale observed globally.

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