Sexual differences in the foraging ecology of 19th century beluga whales (Delphinapterus leucas) from the Canadian High Arctic

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Abstract

Marine mammals often exhibit significant sexual segregation in their diet and habitat use but these differences have not been studied systematically in historic or ancient populations due to the difficulties associated with determining the sex of skeletal elements based on gross morphology. Using a combined ancient DNA and stable isotope approach, we document a sexual difference in the foraging ecology of late 19th century beluga whales (Delphinapterus leucas) from the Canadian High Arctic. Using two PCR assays that coamplify fragments of the Y-linked SRY and X-linked ZFX genes, we assigned reproducible sex identities to 35 beluga specimens. This provided a basis for investigating sex-specific differences in foraging ecology using stable carbon and nitrogen isotope analyses of bone collagen. These isotopic data demonstrate that although both males and females primarily consumed Arctic cod, males utilized a wider range of prey than females, feeding on high trophic level benthic prey (sculpins) to a greater extent. Because bone collagen integrates prey isotopic compositions over the course of several years these sex-based differences in beluga bone collagen isotopic compositions reflect long-term and sustained sexual differences in foraging.

KEYWORDS

ancient DNA, beluga, foraging ecology, sexual segregation, stable isotopes
Intrapopulation differences in foraging according to sex are particularly widespread, especially among mammals (Ruckstuhl, 2007; Ruckstuhl & Neuhaus, 2005; Wearmouth & Sims, 2008). Owing in part to the difficulties associated with studying marine mammals, however, the majority of studies of sex-based differences in foraging have focused on terrestrial mammals, ungulates in particular (e.g., Bleich, Bowyer & Wehausen, 1997; Kie & Bowyer, 1999; Ruckstuhl, 1998; Ruckstuhl & Neuhaus, 2002). Documenting ecological variation within species or populations according to sex and other variables is an integral part of understanding population dynamics and has important implications for conservation and evolutionary biology (Ruckstuhl & Neuhaus, 2005).

Stable isotopes can provide time-integrated data with respect to the types of foods consumed and the types of habitats in which they were consumed. Consumer carbon isotope compositions ($\delta^{13}C$) are largely conserved between trophic levels, but gradients exist between benthic and pelagic habitats (McConnaughey & McRoy, 1979), as well as inshore and offshore habitats (Cherel & Hobson, 2007; Hobson, 1993; Hobson, Piatt & Pitocchelli, 1994). Additionally, different sources of primary production (e.g., pelagic phytoplankton vs. benthic macroalgae or sea ice algae) often possess distinct carbon isotope compositions (France, Loret, Mathews, & Springer, 1998; Hobson, Ambrose & Renaud, 1995; Miller & Page, 2012), and these are reflected in consumers at higher trophic levels (Daggins, Simenstad & Estes, 1989; Dunton & Schell, 1987; Søreide, Hop, Carroll, Falk-Petersen, & Hegseth, 2006; Wang et al., 2016). Nitrogen isotope compositions ($\delta^{15}N$) reflect trophic position as there is a 3‰–5‰ enrichment of $^{15}N$ between consumers and their prey (Minagawa & Wada, 1984; Post, 2002). There may also be variation in the $\delta^{15}N$ of producers caused by local oceanographic conditions, with areas of upwelling and low oxygen (favoring denitrification), characterized by higher $\delta^{15}N$ than areas where mineralized nitrogen is scarce and $N_2$ fixation dominates (Montoya, 2008). These variations are also reflected in consumers at higher trophic levels (Ruiz-Cooley, Engelhardt & Ortega-Ortiz, 2012; Wallace, Seminoff, Kilham, Spotila, & Dutton, 2006).

Isotopic analyses of animal remains from archaeological and paleontological contexts provide an opportunity to examine the foraging ecology of past animal populations. These studies can generate comparative baselines that are useful in characterizing ecosystem function prior to recent human disturbances associated with industrial scale resource extraction and climate change (Burton et al., 2001; Ostrom et al., 2017; Szpak, Buckley, Darwent, & Richards, 2018). Unlike estimates of animal abundance based on the archaeological record, isotopic data that are generated using archaeological materials can be directly comparable to those produced using modern animal populations. Because archaeological samples for vertebrates consist primarily of bones and teeth, the primary analytical substrate is bone collagen, which reflects the animal’s average diet over a span of multiple years (Hedges, Clement, Thomas, & O’Connell, 2007; Stenhouse & Baxter, 1979; Wild et al., 2000). These data are, therefore, not biased with respect to a limited portion of the year immediately prior to the time at which the tissue was sampled. Nevertheless, there are some significant limitations caused by the fragmentary nature of the archaeological record and important intraspecific traits that may produce ecological variation are often difficult to identify in archaeological samples. For example, for most skeletal elements and taxa, sex cannot be conclusively determined through osteological analyses (Ruscillo, 2003) and this has precluded studies of sexual variation in the foraging ecology of ancient animal populations.

In instances where the sex of faunal remains cannot be determined through osteological analyses, ancient DNA (aDNA) analysis can be used as an alternative sex identification method. Through the use of PCR or high-throughput sequencing methods that screen for the presence/absence of sex-specific alleles or regions, aDNA analyses can assign reliable sex identities to ancient remains of taxa with genetic sex determination systems. Within archaeology and palaeontology, such DNA-based methods have been used to assign sex identities to human remains (Skoglund, Storå, Götherström, & Jakobsson, 2013) and the remains of various nonhuman taxa, including bears (Ursidae; Pagès et al., 2009), cattle (Bos taurus; Svensson, Götherström, & Vrtemark, 2008), horse (Equus ferus caballus; Svensson et al., 2012), Pacific salmonids (Oncorhynchus spp.; Royle et al., 2018), moas (Dinornithiformes; Allentoft, Bunce, Scofield, Hale, & Holdaway, 2010), and turkeys (Meleagris gallopavo; Speller & Yang, 2016). By conducting stable...
isotope analyses on remains whose sex has been identified through aDNA analysis, it is possible to investigate sex-based behavioral and ecological variation within ancient populations. Although a combined genetic/biogeochemical approach has been used to study the migration patterns of ancient humans (Krzewińska et al., 2018), it has not been used to document sexual segregation in foraging or other behaviors among ancient nonhuman animals.

Beluga whales (*Delphinapterus leucas*) are the most abundant Arctic odontocete, with a discontinuous circumpolar distribution (Stewart & Stewart, 1989). In the Canadian Arctic, seven distinct populations are recognized according to the use of different core areas during the summer months, as well as genetic differences (Brown Gladden, Ferguson, Friesen, & Clayton, 1999; COSEWIC, 2004). The Eastern High Arctic/Baffin Bay population summers in the Canadian High Arctic, particularly around Somerset Island (Smith & Martin, 1994), and overwinters in Baffin Bay and West Greenland (Heide-Jørgensen et al., 2003; Figure 1). Belugas consume a variety of different benthic and pelagic prey (fish and invertebrates) throughout their range (Kleinenberg, Yablokov, Bel’kovich et al., 1969). In the eastern Canadian Arctic, Arctic cod (*Boreogadus saida*) is the primary prey of belugas in the spring–summer and while the fall–winter diet is unknown, Greenland halibut (*Reinhardtius hippoglossoides*) is suspected to be an important prey item (Marcoux, McMeans, Fisk, & Ferguson, 2012; Matley, Fisk & Dick, 2015).

Sexual segregation in habitat use and intrapopulation variation in diet, particularly with respect to trophic level, have been observed in belugas (Loseto, Richard, Stern, Orr, & Ferguson, 2006). Belugas exhibit sexual dimorphism with males reaching an asymptotic length that is 40–100 cm longer than females, depending on the stock (Sergeant & Brodie, 1969); males are approximately 40% heavier than females (Michaud, 2005). Beluga trophic level (as reflected in muscle and liver δ15N) in the Beaufort Sea has been positively correlated with length and although there are no differences have been found in δ15N according to sex, this may have been due to small sample size for females (Loseto, Stern & Ferguson, 2008). Similarly, Dehn and Follmann (2007) found no differences in beluga δ15N according to sex, but also pointed out that this may have been due to small sample size. Marcoux et al. (2012) found that older belugas in Cumberland Sound had higher skin δ13C and δ15N values than younger individuals for both sexes. In northwestern Alaska, beluga diets (assessed on the basis of stomach contents) are broadly similar across

**FIGURE 1** Map showing the location of Elwin Bay and the summering (shaded area around Somerset Island) and wintering areas (shaded areas around the North Water Polynya [NOW] and Disko Bay) of the Eastern High Arctic beluga stock (Heide-Jørgensen et al., 2017).
ages and sexes with subtle differences; older individuals consumed significantly larger saffron cod (*Eleginus gracilis*) than younger individuals and males consumed more large fish (sculpins) than females (Lowry, Frost, & Seaman, 1986).

Due to recent changes in the length of the open water season and the extent of summer sea ice in the Arctic (Mueller, Gillett, Monahan, & Zwiers, 2018; Stroeve et al., 2012), it is possible that sexual segregation among marine mammal populations that are dependent on these habitats may be impacted (Laidre et al., 2008). Assessing this impact, however, requires adequate baselines for comparison. The warming in the North American Arctic today represents a reversal of a long-term cooling trend that has characterized much of the region through the Holocene (Briner et al., 2016; Miller et al., 2010). Peak cooling seems to have occurred towards the end of the Little Ice Age (ca. 1350–1850 AD) in the 19th century (Miller, Wolfe, Briner, Sauer, & Nesje, 2005; Moore, Hughen, Miller, & Overpeck, 2001; Thomas, Szymanski & Briner, 2010). To understand if ecosystems have been impacted by recent warming, a deep time perspective needs to be employed that recognizes the range of variation that existed over the course of more than a few years or decades. Archaeological and paleontological specimens represent the only source of information about the ecology of higher trophic level vertebrates over hundreds or thousands of years. In this study, we sought to evaluate sex-based dietary variation among late 19th century belugas through the stable carbon and nitrogen isotope analysis of bone collagen from animals harvested in the Canadian High Arctic for which sex had been determined through aDNA analysis.

## 2 | MATERIALS AND METHODS

### 2.1 | Materials

Complete beluga crania and mandibles were collected from a historic whaling site on Elwin Bay, Somerset Island, in the central Canadian Arctic Archipelago (Figure 1; Friesen, Savelle & Smith, 1998). Elwin Bay is an estuarine area heavily used by the Canadian High Arctic beluga stock in the summer months before they overwinter off the west coast of Greenland near Disko Bay or in the North Water Polynya in Baffin Bay (Heide-Jørgensen et al., 2017). The belugas analyzed in this study were harvested at Elwin Bay between 1874 and 1898 (Reeves & Mitchell, 1987). A large number of complete crania and mandibles were collected by James Savelle in 1992 and these have been stored at McGill University since their collection.

A total of 39 bones were sampled by breaking off large chunks of bone with vice grips or a hammer. These chunks were subsequently sampled for isotopic and aDNA analysis. Since two anatomical elements were sampled, it is possible that some of the mandibles could belong to the same individual as some of the crania, but this is unlikely to influence the results of this study. On the basis of the number of beluga crania (the most abundant skeletal element) at Elwin Bay, the minimum number of individuals (MNI) was 380 and the sample for this study consisted of 13 crania and 26 left mandibles. To estimate the likelihood of resampling, we simulated 10,000 resampling events in which 13 and 26 random numbers between 1 and 380 were compared, checking for replicates between the sets (approximating sampling the same individual twice). There were zero resamplings in 39.4% of the simulations, one resampling in 39.2% of the simulations, two resamplings in 16.2% of the simulations, and three resamplings in 4.7% of the simulations. Therefore, assuming that there are 380 crania and 380 corresponding left mandibles at this site, there is a 95% probability that in our set of 39 samples, there are two or fewer individuals that have been sampled twice. The MNI, however, provides only a measure of the *minimum* number of individuals required to produce a given assemblage and does not speak to the most likely number of individuals (MLNI) represented, which is significantly larger than the MNI, especially for large assemblages (Adams & Konigsberg, 2004; Fieller & Turner, 1982). For example, Allen and Guy (1984) analyzed faunal remains from a site in Papua New Guinea and determined the MLNI for wallabies to be 1,100 when the MNI was 242 and the MLNI for parrotfish to be 369 when the MNI was 123; similar results were obtained using alternative methods of MLNI estimation (Krantz, 1968; Koike, 1979). Performing
the same simulations again with 10,000 draws but assuming each mandible and cranium could be drawn from a set of 500 or 1,000 individuals decreases the probability of resampling the same individual considerably (Table 1). We cannot derive an MLNI estimation for Elwin Bay because this requires paired elements to be matched and this would not be feasible for an assemblage of this size (Lyman, 2006), but we know from historic records that at least 10,985 belugas were killed at Elwin Bay between 1874 and 1898 (Reeves & Mitchell, 1987) and the site contains thousands of beluga bones on the surface (Figure 2). The MLNI for the site is, therefore, far in excess of 380, making the resampling of multiple individuals highly improbable. The actual probabilities of resampling are even lower than those presented in Table 1 because these calculations assume that there is one cranium for every left mandible at the site and this is never the case in practice (Adams & Konigsberg, 2004). Some mandibles lack corresponding crania and some crania lack corresponding mandibles, making it impossible for these individual animals to be resampled. Consequently, it is improbable that we have resampled individual animals twice in this assemblage.

2.2 | Ancient DNA analysis

2.2.1 | Decontamination and DNA extraction

All pre-PCR procedures were conducted in a dedicated ancient DNA laboratory in the Department of Archaeology, Simon Fraser University (Burnaby, Canada), and followed strict contamination control protocols (Yang & Watt,

![Figure 2](Image)

**Figure 2** Surface of Elwin Bay littered with thousands of beluga bones as it appeared in 1992 when the samples were collected.

**Table 1** Probability of resampling the same individual in our data set for three different numbers of individual animals present at the site (assuming there are no unpaired skeletal elements). Note that the MNI was 380 but the actual number of individuals represented is likely much greater.

<table>
<thead>
<tr>
<th>Number of individuals resampled</th>
<th>380 individuals</th>
<th>500 individuals</th>
<th>1,000 individuals</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.394</td>
<td>0.502</td>
<td>0.712</td>
</tr>
<tr>
<td>1</td>
<td>0.392</td>
<td>0.353</td>
<td>0.248</td>
</tr>
<tr>
<td>2</td>
<td>0.162</td>
<td>0.120</td>
<td>0.036</td>
</tr>
<tr>
<td>3</td>
<td>0.047</td>
<td>0.023</td>
<td>0.005</td>
</tr>
<tr>
<td>4</td>
<td>0.005</td>
<td>0.002</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>0.001</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>
Chunks of bone (0.561–5.273 g) were removed from each of the 19th century beluga specimens and decontaminated prior to DNA extraction using a modified version of the protocol described by Yang, Liu, Chen, and Speller (2008). The exterior surface of the samples was removed through abrasion with sandpaper dampened with a 100% commercial bleach solution (5%–10% w/v NaOCl). The samples were then rinsed with distilled water and submerged in a 100% commercial bleach solution for 7–10 min, followed by distilled water for 30–60 s, 1 M HCl for 30–60 s, 1 M NaOH for 30–60 s, distilled water for 30–60 s, and distilled water again for 10 min. The samples were then UV irradiated in a crosslinker for 30 min on two sides and left to air dry. Following decontamination, the samples were powdered with a decontaminated hammer or Dremel 7300 MiniMite rotary tool (Dremel, Racine, Wisconsin).

DNA was extracted from the powdered samples using a modified silica-spin column method (Yang et al., 2008). Bone powder (188–740 mg) was incubated overnight at 50°C in 5 mL of lysis buffer (0.5 M EDTA pH 8.0, 0.5% SDS, and 0.5 mg/mL proteinase K) in a rotating hybridization oven. Following incubation, the samples were centrifuged, and 4 mL of the resulting supernatant was concentrated to <100 μl using an Amicon Ultra-4 10 or 30 kDa molecular weight cut-off (MWCO) centrifugal filter (Millipore, Billerica, MA). The concentrated extracts were then purified using a QIAquick PCR Purification Kit (Qiagen, Valencia, CA). One to two blank extraction controls were processed in tandem with each batch of samples and subjected to amplification.

### 2.2.2 | PCR sex identification assays

The sex of individual cetaceans and other therian mammals is determined through an XY genetic sex determination system (Wallis, Waters, & Graves, 2008). Within XY genetic sex determination systems, males are the heterogametic sex (XY) and females are the homogametic sex (XX). We therefore designed multiple primer pairs targeting various X- and Y-linked markers. To evaluate the primers' efficacy and sensitivity we applied them to a subset of the 19th century beluga samples.

Based on the results of these pilot experiments, we selected three primer pairs to include in two PCR sex identification assays that, following Rosel (2003), coamplify fragments of the X-specific zinc finger protein, X-linked (ZFX) and Y-specific sex determining region Y (SRY) genes. The primers targeting the SRY fragments amplified by the assays were designed using a published beluga SRY sequence obtained from GenBank (Accession number: AB108518) (Nishida, Goto, Pastene, Kanda, & Koike, 2007). The ZFX primers included in the assays were designed using a partial ZFX sequence (GenBank Accession Number: MN095756) generated from one of the 19th century belugas samples (DL20) using a primer pair (ZFX0582F and ZFX0923R) designed by Bérubé and Palsbøll (1996). To promote the preferential amplification of SRY, the SRY primers in each assay were designed to target fragments shorter than the co-amplified ZFX fragment (Speller & Yang, 2016). In the first assay, primers Bel-SRY-F603 and Bel-SRY-R677 coamplify a 75 bp fragment of SRY alongside a 116 bp fragment of ZFX, which was targeted with primers ZFX106F

### TABLE 2 | Primers included in the PCR sex identification assays used in this study.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Primer</th>
<th>Sequence (5’–3’)</th>
<th>Amplicon size</th>
</tr>
</thead>
<tbody>
<tr>
<td>SRY</td>
<td>Bel-SRY-F603&lt;sup&gt;a&lt;/sup&gt;</td>
<td>CCCTACTGTGGACGGACAAC</td>
<td>75 bp</td>
</tr>
<tr>
<td></td>
<td>Bel-SRY-R677</td>
<td>CCGGACGCTCTTCTTACAT</td>
<td></td>
</tr>
<tr>
<td>SRY</td>
<td>Bel-SRY-F537</td>
<td>TGACCGGTAGGATTACAGCC</td>
<td>92 bp</td>
</tr>
<tr>
<td></td>
<td>Bel-SRY-R628</td>
<td>GCTACCGTTGTCCGGTACAC</td>
<td></td>
</tr>
<tr>
<td>ZFX</td>
<td>ZFX106F</td>
<td>ACCAACATGCTCTTATCCACCA</td>
<td>116 bp</td>
</tr>
<tr>
<td></td>
<td>ZFX221R</td>
<td>GTCTTTGGTGGAATGAAATTAG</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup>F denotes a forward primer while R denotes a reverse primer.
and ZFX221R (Table 2). The second assay, which also includes primers ZFX106F and ZFX221R, co-amplifies the aforementioned 116 bp fragment of ZFX in tandem with a 92 bp fragment of SRY that overlaps with the 75 bp SRY fragment amplified in the first assay. The 92 bp SRY fragment amplified in the second assay was amplified with primers Bel-SRY-F537 and Bel-SRY-R628 (Table 2). In both assays, the coamplification of ZFX functions as both an internal positive control and means for identifying samples as female. The successful amplification of this internal positive control and the lack of an SRY amplicon suggests the failure to amplify SRY is due to the Y-chromosome being biologically absent on account of a sample being female rather than a lack of amplifiable template DNA.

2.2.3 | PCR amplification and sex identification

To assign sex identities to the beluga samples, we applied both PCR sex identification assays to each sample. PCR amplifications were performed in a Mastercycler Personal or Gradient thermal cycler (Eppendorf, Mississauga, Canada) in a 30 μl reaction volume containing 1.5× PCR Gold Buffer (Applied Biosystems, Carlsbad, CA), 2 mM MgCl₂, 0.2 mM of each dNTP, 0.3 μM of each primer, 1 mg/mL BSA, 1.5–5 μl DNA sample, and 0.38–0.63 U/μl AmpliTaq Gold (Applied Biosystems, Carlsbad, CA). The thermal conditions for the PCRs consisted of an initial denaturation step at 95°C for 12 min followed by 60 cycles at 95°C for 30 s (denaturation), 56°C for 30 s (annealing), and 70°C for 40 s (extension), and a final extension step at 72°C for 7 min. To monitor for contamination, a negative PCR control was included in each PCR run. All PCR amplifications and post-PCR procedures were conducted in a laboratory physically separated from the aDNA laboratory.

Following amplification, 5 μl of PCR product was pre-stained with SYBR Green I (Life Technologies, Eugene, OR), electrophoresed on a 2% agarose gel, and visualized with a Dark Reader transilluminator (Clare Chemical Research, Dolores, CO). Samples were assigned a sex identity using a modified version of the criteria outlined by Royle et al. (2018) by visually analyzing the electrophoresis gels of the PCR products generated by both assays. Briefly, a sample was identified as a male if SRY was amplified with both assays, whereas a female sex identity was assigned if ZFX was amplified with both assays, but SRY failed to amplify. A sample was not assigned a sex identity if the assays yielded inconsistent results or failed to yield amplified DNA. To verify the sex identities assigned to the samples, repeat amplification and sexing of a subset of the samples (n = 14) with both assays was conducted by an independent analyst working within the same laboratory.

2.3 | Genetic analysis of modern beluga samples

We assessed our sex identification method’s reliability by applying it to whole blood samples collected from nine belugas of known phenotypic sex held at Marineland of Canada (Niagara Falls, Canada) and the Vancouver Aquarium (Vancouver, Canada). All of the whole blood samples were drawn by trained veterinary personnel during routine veterinary examinations or necropsies and mixed with EDTA to prevent coagulation. For each individual, DNA was extracted from 100 μl of whole blood with EDTA using a DNeasy Blood and Tissue Extraction Kit (Qiagen, Valencia, CA), following the manufacturer’s directions.

To evaluate its sensitivity, we subsequently applied our sex identification method to dilution series of one each of the modern female (MB7) and male (MB9) samples. A Nanodrop 2000c spectrophotometer (Thermo Fisher Scientific, Waltham, MA) operating in pedestal mode was used to measure in triplicate the concentration of DNA in both samples. Based on this analysis, the concentration of DNA in these two samples is 3.6 ± 0.1 (MB7) and 3.1 ± 0.1 (MB9) ng/μl, respectively (mean ± SD). Dilution series consisting of six concentrations (1:1, 1:10, 1:100, 1:1000, 1:10,000, and 1:100,000) were then prepared for each sample through a 10-fold serial dilution with ultrapure water. PCRs on each of the six concentrations in both dilution series were carried out using 3 μl of DNA solution.
Consequently, the approximate amount of total genomic DNA included in the reaction volumes used for the amplification of the dilution series ranged from 10.8 ng to 0.108 pg (MB7) and 9.3 ng to 0.093 pg (MB9).

All DNA extractions and PCR setups involving the modern beluga samples and the dilution series were conducted in a laboratory in the Centre for Forensic Research, Simon Fraser University (Burnaby, Canada) dedicated to the analysis of modern samples with nondegraded DNA. PCR amplification and sex identification of the modern samples and dilution series were carried out in the same manner as described for the 19th century samples, with a few modifications. First, the cycle number for PCRs involving the undiluted modern samples was reduced to 40 cycles. Second, the concentration of polymerase in the reaction volumes used for the amplification of the modern samples and dilution series was reduced to 0.025 U/μl. Negative PCR controls were included in each of the PCR runs performed with the modern samples and dilution series. The negative controls amplified alongside the dilution series included 3 μl of the ultrapure water used to dilute the samples.

2.4 | Stable isotope analysis

Bone collagen was extracted and purified using a modified version of the method presented by Beaumont, Beverly, Southon, and Taylor (2010). Bone surfaces were cleaned by abrasion with a dental drill equipped with a diamond-tipped cutting wheel. Chunks of bone (250–400 mg) were soaked in 2:1 chloroform-methanol (v:v) under sonication for 1 hr to remove lipids (Folch, Lees & Stanley, 1957). Samples were then air dried for 24 hr and demineralized in 0.5 M HCl at 4°C. After demineralization, samples were rinsed to neutrality with Type I water and then the insoluble collagen residue was solubilized in 10⁻³ M HCl at 75°C for 48 hr. The resulting solution was filtered through a 5–8 μm filter (Elkay Laboratory Products, Hampshire, UK) to remove insoluble residues and then filtered using a 30 kDa MWCO ultrafilter (Pall Corporation, Port Washington, NY) to remove low molecular weight compounds (Brown, Nelson, Vogel, & Southon, 1988). The >30 kDa fraction was freeze-dried, and the collagen yield (mass collagen/initial bone mass) was calculated. Collagen integrity was monitored using collagen yields and elemental compositions (wt% C, wt% N, C:N ratios). On the basis of experimental studies, ancient collagen samples that have isotopic compositions that have not been modified by postburial degradation or contamination possess collagen yields >1%, wt% C > 13%, wt% N > 4.5%, and an atomic C:N ratio between 2.9 and 3.6 (Ambrose, 1990; DeNiro, 1985; van Klinken, 1999). Given their recent geological age and the superb organic preservation in the High Arctic, collagen integrity was excellent and no samples failed any of these criteria.

Carbon and nitrogen isotopic and elemental compositions were determined using a Vario MICRO cube elemental analyzer coupled to an Isoprime isotope ratio mass spectrometer (IRMS; Elementar, Hanover, Germany). Sample carbon and nitrogen isotope compositions were calibrated relative to the VPDB and AIR scales using USGS40 ($\delta^{13}C = -26.39\%\ ± 0.04\%$, $\delta^{15}N = -4.52\%\ ± 0.06\%$) and USGS41 ($\delta^{13}C = +37.63\%\ ± 0.05\%$, $\delta^{15}N = +47.57\%\ ± 0.11\%$) (Qi, Coplen, Geilmann, Brand, & Böhlke, 2003). A suite of internal standards with similar elemental compositions to bone collagen were analyzed alongside the samples for quality assurance (Supplementary Material). Standard uncertainty was determined to be ±0.19‰ for $\delta^{13}C$ and ±0.26‰ for $\delta^{15}N$ (Szpak, Metcalfe, & Macdonald, 2017).

2.5 | Data treatment

Male and female beluga $\delta^{13}C$ and $\delta^{15}N$ were compared using a multivariate analysis of variance (MANOVA). Correlations between $\delta^{13}C$ and $\delta^{15}N$ values were assessed using Spearman’s $\rho$. Total niche width of the male and female belugas was estimated using the convex hull area or total area (TA)—the minimum area polygon encompassing all points in bivariate ($\delta^{13}C$, $\delta^{15}N$) space (Layman, Quattrochi, Peyer, & Allgeier, 2007). TA was calculated using the SIBER package in R (Jackson, Inger, Parnell, & Bearhop, 2011). Although the convex hull is thought to be less well-suited to approximating the isotopic niche width than the standard bivariate ellipse because it is much more sensitive
to extreme values (Jackson et al., 2011), when focusing on intraspecific variation we felt that it was important to capture these extremes.

To compare these late 19th century belugas to modern populations, we adjusted the $\delta^{13}C$ values of modern individuals to account for the change in atmospheric and oceanic dissolved inorganic carbon (DIC) that has occurred since the late 19th century due to industrialization (the “Suess Effect”; Keeling, Mook & Tans, 1979; Quay, Tilbrook & Wong, 1992). Modern beluga $\delta^{13}C$ values were adjusted by $\delta^{13}C_{\text{Suess}}$ according to Equation 1 (Hilton et al., 2006):

$$\Delta^{13}C_{\text{Suess}} = (a_{\text{waterbody}}) \times \left( e^{(y - 1850)b} \right)$$

where $a$ is the annual rate at which $\delta^{13}C$ has declined for a particular water body, $y$ is the year that the sample was collected, and $b$ defines the global oceanic decrease in $\delta^{13}C$ determined to be 0.027‰ (Gruber et al., 1999). We have used a value of 0.023‰ for $a$ based on the values for the North Atlantic presented by Sonnerup et al. (1999). Typically, these annual decreases in $\delta^{13}C$ that have been observed are within 0.005 ‰ of this value (Eide, Olsen, Ninneemann, & Eldevik, 2017; Matthews & Ferguson, 2015).

To assess the relative contribution of different prey items to the diet, we sourced prey $\delta^{13}C$ and $\delta^{15}N$ values from published literature summarized in Table 3. The $\delta^{13}C$ values of these prey were adjusted for the Suess Effect as described above.

### 3 | RESULTS

#### 3.1 | Genetic analyses

##### 3.1.1 | 19th century samples

DNA was successfully amplified with at least one of the assays from 38 of the 39 analyzed samples (Figure 3). Consistent results across both assays were obtained for 35 of the 38 samples that yielded DNA (Table S3). Of these 35 samples that could be assigned a sex identity using the outlined criteria, 15 were identified as female and 20 were identified as male (Tables 4 and S3). The sex identities assigned to all 10 of the identified samples that underwent repeat amplification and sex identification were successfully reproduced by an independent analyst (Table S3).

#### TABLE 3 Isotopic compositions for potential beluga prey items.

<table>
<thead>
<tr>
<th>Common name</th>
<th>Scientific name</th>
<th>Location</th>
<th>Year</th>
<th>$\delta^{13}C$ (%)$_{VPDB}$</th>
<th>$\delta^{15}N$ (%)$_{AIR}$</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arctic cod</td>
<td><em>Boreogadus saida</em></td>
<td>Resolute Bay</td>
<td>2010</td>
<td>$-17.1 \pm 0.7$</td>
<td>$+14.6 \pm 0.7$</td>
<td>Landry <em>et al.</em> (2018a)</td>
</tr>
<tr>
<td>Shorthorn sculpin</td>
<td><em>Myoxocephalus scorpius</em></td>
<td>Resolute Bay</td>
<td>2013</td>
<td>$-16.0 \pm 0.6$</td>
<td>$+16.7 \pm 0.6$</td>
<td>Landry <em>et al.</em> (2018a)</td>
</tr>
<tr>
<td>Greenland halibut</td>
<td><em>Reinhardtius hippoglossoides</em></td>
<td>Davis Strait</td>
<td>1996</td>
<td>$-18.5 \pm 0.6$</td>
<td>$+16.6 \pm 0.4$</td>
<td>Watt and Ferguson (2015)</td>
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<tr>
<td>Boreoatlantic armhook squid</td>
<td><em>Gonatus fabricii</em></td>
<td>Davis Strait</td>
<td>1999</td>
<td>$-18.5 \pm 0.7$</td>
<td>$+16.0 \pm 0.4$</td>
<td>Watt and Ferguson (2015)</td>
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<tr>
<td><em>Themisto libellula</em></td>
<td></td>
<td>Allen Bay$^b$</td>
<td>2010</td>
<td>$-19.3 \pm 1.0$</td>
<td>$+9.6 \pm 1.0$</td>
<td>Matley <em>et al.</em> (2015)</td>
</tr>
<tr>
<td><em>Onisimus sp.</em></td>
<td></td>
<td>Allen Bay</td>
<td>2010</td>
<td>$-14.8 \pm 0.9$</td>
<td>$+8.6 \pm 1.0$</td>
<td>Matley <em>et al.</em> (2015)</td>
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<td>2010</td>
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<td>$+9.8 \pm 1.8$</td>
<td>Matley <em>et al.</em> (2015)</td>
</tr>
</tbody>
</table>

$^a$This value has been adjusted to account for the Suess Effect as described in the text.

$^b$Allen Bay is immediately west of Resolute Bay on Cornwallis Island.
Among the identified males, the amplification of the \textit{SRY} fragment tended to be stronger than that of the coamplified \textit{ZFX} fragment, indicating \textit{SRY} was preferentially amplified (Figure 3). No sex identity was assigned to the remaining three samples (DL9, DL12, and DL14) that yielded DNA as the assays yielded discordant results for these samples (Table S3). Repeat amplification by an independent analyst also failed to assign a sex identity to these samples, as well as the one sample that failed to yield amplified DNA (Table S3). No DNA was amplified from any of the blank extraction or negative PCR controls.

### 3.1.2 Modern samples

DNA was amplified with both assays from each of the modern beluga samples, with the assays yielding consistent results for each sample (Figure 4). As such, genetic sex identities could be confidently assigned to each of the modern beluga samples (Table 5). Each of the nine modern beluga samples’ known phenotypic sex and assigned genetic sex identity were congruent (Table 5).

The two assays generated amplicons and concordant results for the 1:1, 1:10, 1:100, 1:1000 dilutions in both dilution series, enabling each of these dilutions in both series to be assigned a sex identity (Figures S1 and S2). The genetic sex identities assigned to each of these dilutions of MB7 and MB9 matched their known phenotypic sex. No sex
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<th>Isotope lab ID</th>
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<th>δ¹³C (‰)</th>
<th>δ¹⁵N (‰)</th>
<th>wt% C</th>
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</tbody>
</table>
identities could be assigned to the 1:10,000 or 1:100,000 dilutions of either MB7 or MB9 due to one or both PCR assays failing to generate amplicons when applied to these dilutions (Figures S1 and S2). The concentration of DNA in the greatest dilution (1:1000) of both samples that could be assigned a sex identity is approximately 3.6 ± 0.1 (MB7) and 3.1 ± 0.1 (MB9) pg/μl. As such, the total amount of total genomic DNA included in the reaction volume used for the amplification of the 1:1000 dilutions of MB7 and MB9 was approximately 10.8 and 9.3 pg, respectively. No DNA was amplified from any of the negative PCR controls amplified in tandem with the modern beluga samples or the dilution series.

3.2 | Stable isotopes

Bone collagen $\delta^{13}$C was $-13.4\% \pm 0.4\%$ (range $-14.2\%$ to $-12.6\%$) for females and $-13.2\% \pm 0.4\%$ (range $-14.3\%$ to $-12.6\%$) for males; $\delta^{15}$N was $+17.3\% \pm 1.2\%$ (range $+15.1\%$ to $+19.1\%$) for females and $+17.5\%$
The δ¹³C and δ¹⁵N values did not differ between male and female belugas (MANOVA, $F_{[2,32]} = 1.24$, $p = .30$; Wilk’s $Λ = 0.93$, partial $η^2 = 0.07$), however, the δ¹³C and δ¹⁵N values were strongly correlated for females (Spearman’s $ρ = −0.80$, $p < .001$, $n = 15$), but not for males (Spearman’s $ρ = 0.04$, $p = .87$, $n = 20$) (Figure 5). The convex hull area was 3.23‰² for male belugas and 2.70‰² for female belugas.

On the basis of a comparison of the beluga δ¹³C and δ¹⁵N values adjusted for trophic level discrimination (δ¹³C = +3.7‰ and δ¹⁵N = +3.6‰) (Szpak, Orchard, McKechnie, & Gröcke, 2012) to potential prey items adjusted for the Suess Effect, Arctic cod were the primary prey of both male and female belugas (Figure 6).
4 | DISCUSSION

4.1 | Authenticity of ancient DNA data

In this study, we assigned reproducible sex identities to 35 archaeological beluga remains using two PCR assays. The high proportion (90%) of samples that were successfully assigned reproducible sex identities likely reflects our method's sensitivity and the excellent DNA preservation expected for remains less than 150 years old from the High Arctic (Hofreiter et al., 2015). A depositional context favorable to DNA preservation as well as other evidence suggests our aDNA data is authentic and not the result of systematic contamination. First, all pre-PCR procedures were conducted in a dedicated aDNA laboratory physically separated from the post-PCR laboratory (Poinar, 2003). Second, the modern beluga samples as a contamination source was excluded by receiving and processing them in a separate laboratory after the analysis of the 19th century samples had been completed. Third, the samples were decontaminated through a combination of physical abrasion, UV irradiation, as well as bleach, HCl, and NaOH washes (Yang & Watt, 2005). Fourth, the failure to amplify DNA from any of the blank extraction and negative PCR controls indicates there was a lack of systematic contamination (Poinar, 2003). Fifth, the sex identities assigned to the samples were confirmed using two independent PCR assays and in the case of 10 samples were successfully reproduced by an independent analyst through repeat amplification (Poinar, 2003). Sixth, the excellent collagen preservation exhibited by the samples provides independent evidence for the preservation of biomolecules within the remains (Poinar, 2003).

4.2 | Reliability of sex identifications

Studies of modern cetaceans of known sex have demonstrated reliable sex identities can be assigned to individuals using PCR assays that, similar to ours, coamplify SRY and ZFX (Jayasankar, Anoop, & Rajagopalan, 2008; Rosel, 2003). The assignment of each of the analyzed modern belugas to the correct sex with our sex identification method, suggests our method is similarly reliable. Nonetheless, the PCR-based sex identification of ancient cetaceans through coamplification assays is complicated by the DNA degradation that characterizes such specimens (Sinding et al., 2016). Notably, by increasing the potential for Y-chromosome drop-out caused by stochastic variation (Kim et al., 2013; Taberlet et al., 1996) and competition from coamplified X-chromosome fragments (Sinding et al., 2016), DNA degradation increases the likelihood of male samples being erroneously identified as females. However, we were able to correctly identify the sex of two samples using only 10.8 pg and 9.3 pg of total genomic DNA, indicating our method is reliable even when the amount of starting DNA template is relatively low.

Our method’s reliability at low DNA concentrations reflects both its sensitivity and the incorporation of measures aimed at mitigating the potential for Y-chromosome dropout caused by the aforementioned factors. First, the use of two assays for sex identification increases the probability of detecting SRY dropout resulting from stochastic processes (Taberlet et al., 1996). Second, the observed preferential amplification of SRY reduces the potential for Y-chromosome dropout by limiting amplification competition from the coamplified ZFX fragment (Royle et al., 2018; Speller & Yang, 2016). In addition, although not a problem limited to ancient specimens (King & Stevens, 2019), our method reduces the potential for erroneous sex identifications caused by SRY dropout due to primer-template mismatches by using different SRY primers in each assay (Royle et al., 2018). The coamplification of a fragment ZFX in each assay also contributes to the method reliability in instances where the amount of template DNA is low. Namely, it provides a quick means for assessing whether the failure to amplify SRY is indeed due to a sample being female rather than degradation or inhibition (Royle et al., 2018; Speller & Yang, 2016).
4.3 | Foraging ecology of 19th century belugas

A number of studies have attempted to utilize Arctic marine mammal bone collagen isotopic compositions to examine environmental or dietary variation throughout the Late Holocene (Clark, Horstmann, de Vernal, Jensen, & Misarti, 2019; Outridge, Hobson & Savelle, 2009; Szpak et al., 2018; Szpak, Savelle, Conolly, & Richards, 2019) or over the last century (Nelson, Quakenbush, Mahoney, Taras, & Wooller, 2018; Skovrind et al., 2019). The $\delta^{13}C$ and $\delta^{15}N$ values of the late 19th century belugas from Elwin Bay were not found to differ significantly from belugas collected in the 1990s from West Greenland (Disko Bay and NOW areas; Skovrind et al., 2019) after accounting for the Suess Effect (MANOVA, $F_{[2,50]} = 1.10, p = .34$; Wilk’s $\Lambda = 0.96$, partial $\eta^2 = 0.04$) (Figure 7). These areas in West Greenland should correspond to the wintering grounds of the belugas that migrate to the area around Somerset Island in the summer (Figure 1; Heide-Jørgensen et al., 2017). This stability in $\delta^{13}C$ and $\delta^{15}N$ values suggests that the foraging ecology of these belugas has remained relatively constant between the late 19th and late 20th centuries or if there have been shifts in the prey consumed, these shifts have occurred between species with comparable isotopic compositions. There is a larger amount of variation in the late 19th compared to late 20th century belugas (convex hull area of 4.8‰$^2$ compared to 1.4‰$^2$) but this may simply be a product of sample size and the fact that the 19th century animals were harvested over a period of 25 years whereas the late 20th century animals were only collected between 1990 and 1994. Alternatively, it is possible that this beluga population was more narrowly focused on one particular prey (e.g., Arctic cod) in the 1990s than they were in the late 19th century but this hypothesis requires further testing with larger data sets. Nelson et al. (2018) analyzed the $\delta^{13}C$ and $\delta^{15}N$ values of bone collagen from Cook Inlet (Alaska) belugas collected between 1967 and 2007 and found $\delta^{15}N$ values declined by 1‰–2‰ and $\delta^{13}C$ values declined by 3‰ over this period. The fact that significant changes may be observed in one population but not another is not surprising given the diversity of prey consumed by the different beluga populations in North America (COSEWIC, 2004).

The combination of the data generated through our DNA-based sex identification technique and biogeochemical analyses demonstrates subtle long-term differences in the types of prey utilized by male and female belugas in the late 19th century. While the average isotopic compositions of males and females were nearly identical to one another, the presence of a strong correlation between $\delta^{13}C$ and $\delta^{15}N$ for females but not for males suggests a difference in foraging behavior. Because these data were obtained from bone collagen, with its slow rate of turnover

**FIGURE 7** Comparison of the $\delta^{13}C$ and $\delta^{15}N$ values of 19th century belugas from Elwin Bay to late 20th century belugas collected in the Disko Bay and North Water Polynya regions of West Greenland (data from Skovrind et al., 2019).
(Hedges et al., 2007), differences between male and female belugas must reflect sustained variation in foraging ecology, integrating prey consumption through all seasons. Additionally, the fact that these belugas were harvested over an approximately 25-year period means that any differences cannot be ascribed to short-term temporal variation in diet or prey isotopic compositions.

The isotopic compositions of both male and female belugas suggest that their diets were dominated by Arctic cod, consistent with other lines of evidence about this population in the late 20th and early 21st centuries (Matley et al., 2015; Welch, Crawford & Haakon, 1993). Males, however, relied on a more diverse range of prey as evidenced by the fact that their convex hull area was 20% larger than that of the females. The correlation between δ13C and δ15N for females suggests that they supplemented their Arctic cod-focused diet primarily with Greenland halibut and/or armhook squid and the amphipod *Onisimus* sp. Some males, on the other hand, appeared to rely to a greater extent on shorthorn sculpin, the most abundant benthic fish in the High Arctic (Landry et al., 2018b), or prey with similar isotopic compositions to these fish. The more restricted diet of females may be driven by avoidance of predation risk. Female belugas travel with their offspring for periods of two to three years, with nursing lasting for 24 months (Brodie, 1971). In the Beaufort Sea, female belugas with calves spent more time in open water habitats near the mainland relative to larger males, which spent more time in areas of closed sea ice cover in the CAA (Loseto et al., 2006). The subtle differences in male and female beluga isotopic compositions may therefore reflect differences in the availability of prey in the areas used most frequently by either sex.

Outridge, Hobson, and Sevelle (2005) documented a negative, but much weaker correlation between δ13C and δ15N in 19th century beluga teeth from Elwin Bay, but this relationship was absent from beluga teeth sampled in the 1990s from the same region. Similarly, the data reported by Matley et al. (2015) for a modern population showed no correlation between beluga liver or muscle δ13C and δ15N sampled in Resolute Bay, Cornwallis Island. The weak correlation in the 19th century belugas reported by Outridge et al. (2005) was likely a product of the amalgamation of male and female specimens—disaggregating our data according to sex and analyzing all individuals together also produced a weak correlation (\( r = -0.33, p = .05 \)). The lack of any correlation between δ13C and δ15N in the belugas from the 1990s presented by Outridge et al. (2005) and Matley et al. (2015), however, suggests that there may have been a very subtle change in the types of prey exploited by males and females over the last 100 years. Specifically, females may be consuming more higher trophic level benthic prey (e.g., shorthorn sculpin) or more lower trophic level pelagic prey (e.g., *Themisto libellula*) today than they did in the late 19th century. Either of these possibilities would serve to obscure the linear correlation that we observed for 19th century female belugas. Notably, in Cumberland Sound belugas have been observed to consume proportionately more pelagic forage fish (capelin, *Mallotus villosus*) and less Greenland halibut, a shift which has been directly linked to the northward expansion of capelin populations driven by recent warming in the Arctic (Yurkowski et al., 2017). A third possibility is that both males and females have reduced the diversity of prey that they consume, with both sexes relying to a much greater extent on one focal resource, such as Arctic cod. This hypothesis is consistent with the lower amount of isotopic variation observed in the late 20th century belugas relative to those from Elwin Bay.

As with most marine mammals, beluga diets are relatively poorly understood, especially during the winter months when the animals move into open water (Marcoux et al., 2012). The combined isotopic and genetic data presented here for 19th century belugas document a sustained sexual difference in foraging ecology and provide a comparative baseline with which to assess long-term changes in beluga diet and habitat use. The combination of genetic and biogeochemical techniques has great potential for developing an understanding of intrapopulation ecological variation in historic and ancient vertebrates.

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REFERENCES


Reeves, R. R., & Mitchell, E. (1987). *Distribution and migration, exploitation, and former abundance of white whales (Delphinapterus leucas) in Baffin Bay and adjacent waters*. Canadian Special Publication of Fisheries and Aquatic Sciences 99. Ottawa, Canada: Department of Fisheries and Oceans.


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