



Blubber cortisol in four Canadian beluga whale populations is unrelated to diet

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ABSTRACT: Changing conditions in the Arctic have had severe consequences for many marine mammals. In this study, we examined blubber cortisol using radioimmunoassay in 4 Canadian beluga whale *Delphinapterus leucas* populations. The endangered Cumberland Sound population had higher cortisol levels (mean \pm SE: 0.65 ± 0.11 ng g⁻¹) than populations not at risk: Eastern Beaufort Sea (0.31 ± 0.03 ng g⁻¹; $p < 0.001$), Eastern High Arctic-Baffin Bay (0.32 ± 0.09 ng g⁻¹; $p = 0.004$), and Western Hudson Bay (0.44 ± 0.04 ng g⁻¹; $p = 0.004$). To evaluate if measured cortisol differences were due to differences in diet, we compared stable isotope ratios of carbon and nitrogen ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and dietary fatty acids among populations. Beluga whales from Eastern Beaufort Sea had lower $\delta^{13}\text{C}$ ($p \leq 0.017$) and higher $\delta^{15}\text{N}$ ($p < 0.001$) values than other measured populations, while Western Hudson Bay beluga dietary fatty acid profiles differed from other measured populations ($p < 0.001$). Population and sex were significant predictors of blubber cortisol ($p \leq 0.017$). Females exhibited higher cortisol than males. Despite diet differences among populations, neither stable isotopes nor fatty acids were significant predictors of cortisol, suggesting differences in cortisol levels were unrelated to diet. Other factors, such as increased risk of predation, hunting pressure, vessel traffic, or differences in baseline blubber cortisol concentrations may be contributing to elevated cortisol levels in Cumberland Sound beluga whales. Measuring blubber cortisol in combination with chemical indicators of diet provides a useful method for monitoring population health and can be used to inform management and conservation.

KEY WORDS: Blubber cortisol · Stable isotope analysis · Dietary fatty acids · Arctic · *Delphinapterus leucas*

1. INTRODUCTION

In the past few decades, climate change has prompted drastic changes in the Canadian Arctic and subarctic (Hochheim et al. 2010, Box et al. 2019). Rising temperatures have led to significant declines in seasonal and multi-year sea ice coverage, volume, and thickness (Hochheim et al. 2010, Tivy et al. 2011, Parkinson 2014, Kwok 2018). Heterogeneous rates of decline in sea ice across the Arctic have affected Arctic marine fauna in a multitude of ways, including

declines in body condition, reproductive success, and population size, as well as loss of habitat, distribution shifts, and changes in community structure (Post et al. 2009, Bluhm et al. 2011, Hinzman et al. 2013).

For example, the ranges of boreal invertebrates and fish have shifted northward and, in conjunction with reduced abundance of Arctic species, are altering the structure of marine food webs (Wassmann et al. 2011, Fossheim et al. 2015, Kortsch et al. 2015). Recently, the range of Arctic cod *Boreogadus saida* has contracted due to warming temperatures, and

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they are facing increased competition from boreal species such as capelin *Mallotus villosus* (Kortsch et al. 2015, McNicholl et al. 2016). The range of capelin has shifted northward and they now comprise a greater proportion of Arctic seabird and marine mammal diets (Rose 2005, Gaston et al. 2012, Provencher et al. 2012, Yurkowski et al. 2017). For example, ringed seals *Pusa hispida*, Greenland halibut *Reinhardtius hippoglossoides*, and Arctic char *Salvelinus alpinus* in Cumberland Sound (CS), Nunavut, significantly increased their consumption of capelin from the 1990s to the late 2000s as the availability of capelin increased (Yurkowski et al. 2018). Since many of these changes are driven by changing sea ice dynamics, shifts in the food web are likely to be of particular consequence for species that depend on sea ice to forage on ice-associated prey, such as beluga whales *Delphinapterus leucas* (Burek et al. 2008).

Beluga whales are an ice-adapted cetacean expected to be moderately sensitive to climate change (Laidre et al. 2008, Albouy et al. 2020). Beluga whales display high site fidelity and a low rate of population growth and have a relatively large global population composed of 22 locally adapted populations with a circumpolar distribution (Laidre et al. 2008, Hobbs et al. 2020). Therefore, the effects of climate change may affect some beluga whale populations to a different extent than others. Beluga whales depend on sea ice to forage for prey, as refuge from predators, and to guide migratory movements (Huntington 1999). In addition to the impacts of sea ice decline, beluga whales are subject to pressure from subsistence hunting (COSEWIC 2004), contaminants (Smythe et al. 2018), increased risk of predation (Higdon & Ferguson 2009), and increased anthropogenic activity (Reeves et al. 2014, Halliday et al. 2017). While many Canadian beluga whale populations appear to be abundant, it is challenging to measure and assess changes in their population dynamics, and thus trends in abundance are unclear (Hobbs et al. 2020). Five of 8 Canadian populations are currently listed as special concern, threatened, or endangered by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC), though only 2 populations (CS and St. Lawrence) are listed on the Canadian Species at Risk Act (SARA).

Beluga whale diet differs individually by age, sex, and length, and at the population level by location and latitude (Loseto et al. 2009, Yurkowski et al. 2016, Louis et al. 2021). Males typically occupy a higher trophic position and larger ecological niche than females (Szpak et al. 2020, Louis et al. 2021).

Most beluga whale populations forage mainly on Arctic cod, though their diet is diverse and they are also known to feed on other fish (e.g. redfish *Sebastes marinus*, saffron cod *Eleginus gracilis*, pacific salmon *Onchorhynchus* spp.) and invertebrates (e.g. shrimp *Pandalus borealis*, squid *Gonatus fabricii*, smoothskin octopus *Benthoctopus leioderma*), depending on season and location (Heide-Jørgensen & Teilmann 1994, Loseto et al. 2009, Kelley et al. 2010, Marcoux et al. 2012, Matley et al. 2015, Quakenbush et al. 2015). As capelin increase in abundance in the Arctic, beluga whales in CS, Eastern Beaufort Sea (EBS), and Western Hudson Bay (WHB) have shifted to consume a greater proportion of capelin (Kelley et al. 2010, Marcoux et al. 2012, Watt et al. 2016, Yurkowski et al. 2018, Choy et al. 2020). Whether dietary changes and differences among populations are affecting beluga whale population health is less clear. Arctic cod and capelin occupy a similar isotopic niche, yet they show little overlap in fatty acid profile when co-occurring (McNicholl et al. 2016, Pedro et al. 2020), and some predict that a switch from a cod- to a capelin-dominated diet may lead to declines in body condition of beluga whales (Choy et al. 2020).

Cortisol is increasingly being used as an indicator of stress and population health in marine mammals (Atkinson et al. 2015). Glucocorticoid hormones such as cortisol are produced when the hypothalamic-pituitary-adrenal (HPA) axis is activated in response to physiological and nutritional stressors (Sapolsky et al. 2000, Romero 2004). While the short-term release of hormones is beneficial for the organism to respond to the stressor, the continued release of hormones in response to a prolonged stressor (chronic stress) can lead to decreased reproduction, illness, or death (Boonstra et al. 1998, Boonstra 2005, Sheriff et al. 2009). Cortisol concentrations in marine mammals have been measured in several matrices, including hair, claws, baleen, blubber, feces, and breath (Atkinson et al. 2015, Bechshøft et al. 2015, Hunt et al. 2018, Crain et al. 2021). Blubber cortisol concentrations reflect longer time durations than circulating levels of cortisol, as cortisol and other hormones passively diffuse from the bloodstream into adipose tissue, where they accumulate (Mead 1963, Kellar et al. 2006, Champagne et al. 2017). However, the exact rate of diffusion from the bloodstream into blubber is not known. Blubber cortisol was elevated in bottlenose dolphins *Tursiops truncatus* within 60 min in an acute stress situation (Champagne et al. 2018). Conversely, blubber cortisol was not correlated with capture time (within 281 min) in harbour seals *Phoca vitulina* (Kershaw & Hall 2016) or encounter time

(within 300 min) in humpback whales *Megaptera novaengliae* (Mingramm et al. 2020), suggesting that any stress animals may experience from sampling is not reflected in blubber cortisol concentrations. Cortisol also plays a role in regulating energy metabolism, both in fat distribution and storage and by stimulating the mobilization of fat reserves in adipose tissue in situations of nutritional stress to provide energy (Divertie et al. 1991, Douyon & Schteingart 2002, Peckett et al. 2011). Blubber cortisol concentrations have been negatively correlated with nutritional status in marine mammals such as harbour porpoises *Phocoena phocoena* and California sea lions *Zalophus californianus*, and thus may also provide information about an animal's physiological state (Beaulieu-McCoy et al. 2017, Kershaw et al. 2017). Therefore, blubber cortisol concentrations may be useful as a method of monitoring chronically elevated levels of cortisol in beluga whales (Trana et al. 2015, 2016, Loseto et al. 2018). Blubber cortisol concentrations vary with season, size, sex, and age (Kershaw & Hall 2016, Trana et al. 2016, Loseto et al. 2018), which can make comparisons challenging. However, comparing cortisol concentrations among different populations or groups rather than stand-alone measurements of single populations may still serve as a way to assess relative population health (Trana et al. 2016, Loseto et al. 2018, Watt et al. 2021b).

Elevated cortisol and other glucocorticoids have been correlated with diet in circumstances where poor nutrition and low primary prey availability lead to nutritional stress. For example, grizzly bear *Ursus arctos* hair cortisol increased with decreased consumption of nutrient-rich salmon, while fecal glucocorticoids were higher in bears whose diet consisted primarily of vegetation such as leaves, stems, and roots rather than more nutritious berries and meat (Bryan et al. 2013, Stetz et al. 2013). Conversely, cortisol was unrelated to diet in Canada lynx *Lynx canadensis* whose preferred prey was limited, suggesting that the stress response to dietary changes may be species-specific (Burstahler et al. 2019). In marine mammals, integrating hormone and diet analysis may help assess population nutrition and health (Fleming et al. 2018). For example, fecal glucocorticoid concentrations in southern resident killer whales *Orcinus orca* were related to the abundance of their primary prey, Chinook salmon *Oncorhynchus tshawytscha* (Ayles et al. 2012). The growth of this endangered population appears to be limited by the nutritional impact of low salmon availability during key life-history periods, such as pregnancy, sug-

gesting a means of mitigating recovery (Wasser et al. 2017). Therefore, combined information from hormone and dietary analysis can better inform marine mammal conservation and management (Fleming et al. 2018).

Stable isotopes and fatty acids are biomarkers frequently used to infer marine mammal diet (Thiemann et al. 2009, Newsome et al. 2010). Carbon stable isotope ratios ($\delta^{13}\text{C}$) are conserved between predator and prey and thus reflect feeding habitat (such as nearshore or offshore) through the carbon signatures of primary producers, while nitrogen stable isotope ratios ($\delta^{15}\text{N}$) increase predictably from prey to predator and are used to infer trophic level (Peterson & Fry 1987, Hobson et al. 1995, Newsome et al. 2010). Based on epidermal turnover rate, stable isotope ratios of beluga whale skin are expected to reflect diet over the previous 2–3 mo (St. Aubin et al. 1990). Dietary fatty acids are used to inform marine mammal diet by comparing fatty acids present in predator adipose tissues to potential prey species, since fatty acids not biosynthesized in predator tissues are transferred from prey to predator with little modification (Iverson et al. 1997, 2004). Fatty acid turnover in blubber occurs over the course of several weeks to months and thus represents diet over this period (Kirsch et al. 2000, Iverson et al. 2004, Budge et al. 2006). Although blubber cortisol, blubber fatty acids, and skin stable isotopes incorporate and turn over in tissues at different rates, we used a multi-year data set with samples collected over a 3 mo time frame across 26 yr, therefore reflecting long-term trends in seasonal diet and cortisol concentrations rather than short-term correlations.

The cortisol data used in this study includes a subset of data previously published in a methodological study by Trana et al. (2015). In addition, we included data from 3 other beluga populations on cortisol, fatty acids, and stable isotopes to explore variation in cortisol concentrations by year, sex, and study population and evaluate associations between blubber cortisol concentrations and diet. The first objective of this study was to compare blubber cortisol levels among 4 Canadian beluga whale populations of differing location/latitude and conservation status (Table 1): CS, EBS, Eastern High Arctic-Baffin Bay (HA), and WHB (see Fig. 1). The second objective was to test whether dietary differences, inferred from dietary biomarkers, were associated with differences in blubber cortisol in these 4 populations. We used $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ and dietary fatty acid analysis to examine differences in diet. We hypothesized that (1) the 4 populations would demonstrate different levels of

Table 1. Characteristics of 4 Canadian beluga whale populations, including the most recent estimated population size, current conservation status in Canada, and location of summering and wintering areas

Population	Estimated population size	Conservation status ^e	Summering areas	Wintering areas
Cumberland Sound	1151 ^a	Endangered	Clearwater Fjord (Northwest Cumberland Sound) ^f	Cumberland Peninsula (Southeast Cumberland Sound) ^f
Eastern High Arctic-Baffin Bay	21 213 ^b	Special concern	Somerset Island, Lancaster Sound, Peel Sound ^h	North Water polynya, Baffin Bay, West Greenland ^h
Eastern Beaufort Sea	39 258 ^c	Not at risk	Eastern Beaufort Sea ^g	Bering Sea ⁱ
Western Hudson Bay	54 473 ^d	Not at risk	Nelson, Seal, and Churchill River Estuaries ⁱ	Hudson Strait ⁱ

^aWatt et al. (2021a); ^bInnes et al. (2002); ^cHarwood & Smith (2002); ^dMatthews et al. (2017); ^eCOSEWIC (2021); ^fRichard & Stewart (2009); ^gHauser et al. (2014); ^hRichard et al. (2001b); ⁱRichard et al. (1990); ^jCitta et al. (2017)

blubber cortisol concentrations aligning with increased level of conservation status and that (2) differences in diet among populations would contribute to differences in blubber cortisol concentrations.

2. MATERIALS AND METHODS

2.1. Study populations

The CS beluga whale population is located along southeastern Baffin Island, Nunavut (NU). Beluga

whales reside in CS year-round, summering mainly in the estuaries of Clearwater Fjord, and undergo a short migration to the mouth of the sound in Cumberland Peninsula for the winter (Fig. 1) (Richard & Stewart 2009). This population's diet consists primarily of Arctic cod and Greenland halibut, with a recent shift to capelin (Marcoux et al. 2012, Watt et al. 2016, Yurkowski et al. 2018). CS beluga whales were designated as endangered by COSEWIC in 2020 following a severe population decline from over 8000 individuals to less than 1000 throughout the 1900s due to hunting for subsistence and sale to the Hudson Bay

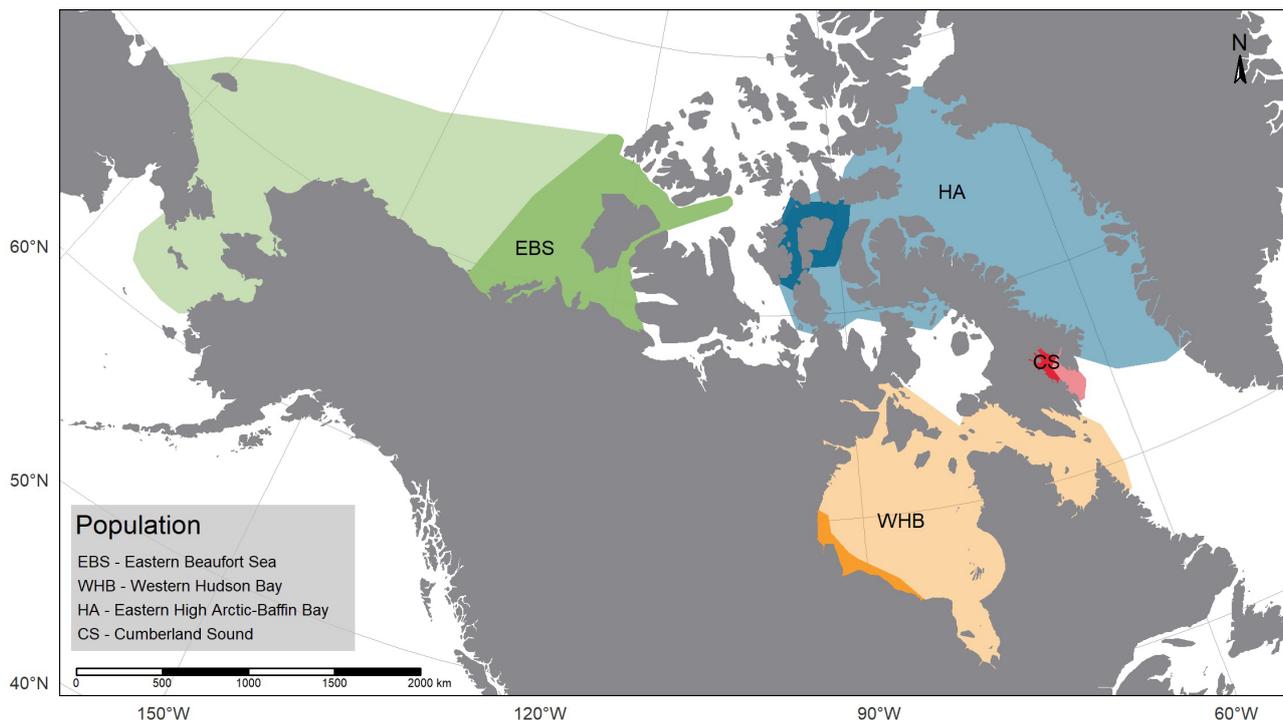


Fig. 1. Geographic extent of 4 beluga whale populations in the Canadian Arctic. Shaded areas represent the summering (dark colored) and wintering (light colored) areas for Cumberland Sound, Eastern Beaufort Sea, Eastern High Arctic-Baffin Bay, and Western Hudson Bay

Company (DFO 2005, Stewart 2018, COSEWIC 2021). The population was listed as threatened on SARA in 2017, and recent surveys suggest the population is currently in decline (Watt et al. 2021a). The community of Pangnirtung continues to harvest CS beluga whales for subsistence, with the annual harvest quota set at 41 since 2002 (Stewart 2018).

EBS beluga whales are located off the northern coast of Inuvialuit Settlement Area, Yukon Territory, Canada, and Alaska, USA. The EBS beluga whale summering grounds include the Mackenzie River estuary, Amundsen Gulf, M'Clure Strait, and Viscount Melville Sound (Richard et al. 2001a, Hauser et al. 2014). For winter, these whales migrate south to the Bering Sea (Citta et al. 2017). Their diet consists primarily of Arctic cod and capelin, supplemented by Canadian eelpout *Lycodes polaris*, Greenland halibut, shrimp, and octopus (Loseto et al. 2009, Quakenbush et al. 2015, Choy et al. 2020). This population is considered abundant and is designated as not at risk by COSEWIC, but the population trend is unknown, and hunted whales are exhibiting a trend of declining body growth rates (COSEWIC 2004, Harwood et al. 2014). This population is harvested for subsistence by several communities in Canada and the USA at a sustainable rate (COSEWIC 2004, Harwood et al. 2014).

The HA beluga whale population resides in the Eastern Canadian Archipelago and northern Baffin Bay. These beluga whales spend summer in Lancaster Sound, Peel Sound, and estuaries around Somerset Island and migrate to the North Water Polynya of Baffin Bay and down the western coast of Greenland for winter (Smith & Martin 1994, Richard et al. 2001a, Hobbs et al. 2020). Their diet is not well studied, but stomach content analysis suggests that they mainly feed on Arctic cod and amphipods (Matley et al. 2015). HA beluga whales are designated as special concern by COSEWIC (2004, 2021) due to past overharvesting by commercial whaling in Canada and overexploitation by Greenland hunters, but the harvest is now considered sustainable and the population appears to be depleted but stable (Hobbs et al. 2020).

The WHB population includes beluga whales that use the western portion of Hudson Bay along the coast of Manitoba and NU. Hudson Bay beluga whales spend winter in the Hudson Strait, and in summer divide into the genetically distinct Western, Eastern, and James Bay populations (Richard et al. 1990, de March & Postma 2003, Turgeon et al. 2012, COSEWIC 2016). Once the sea ice retreats, the WHB population spends summer in the Nelson, Seal, and Churchill River estuaries (Richard et al. 1990, Tur-

geon et al. 2012, Smith et al. 2017). Unlike the northern populations, capelin is the main prey of WHB beluga whales, supplemented by other fish (Sergeant 1973, Kelley et al. 2010, Breton-Honeyman et al. 2016). WHB is estimated to be the largest population of beluga whales globally and appears to be stable (Matthews et al. 2017, Hobbs et al. 2020). Despite past concerns for WHB beluga whales due to high removals from commercial whaling and the threat of increasing shipping and hydroelectric dams (COSEWIC 2004), the WHB population is designated as not at risk by COSEWIC (2021).

2.2. Sample collection and storage

Samples of non-calf beluga whales were collected during summer (July–September) by subsistence hunters. Whales were hunted opportunistically from 1984–2010 by hunters in areas surrounding the communities of Hendrickson Island, Northwest Territories (NT), East Whitefish, NT, Kendall Island, NT, and Paulatuk, NT (approximately 69° 21'–69° 29' N; representing EBS beluga whales), Pangnirtung, NU (approximately 66° 08' N; CS beluga whales), Grise Fiord, NU, and Resolute, NU (approximately 76° 25'–76° 41' N; HA beluga whales), and Arviat, NU (approximately 61° 06' N; WHB beluga whales). Although we do not have information on specific pursuit times, blubber cortisol levels generally reflect longer-term chronic stress (Loseto et al. 2018). We do not expect that hunt length affected blubber cortisol concentrations, since blubber cortisol concentrations were neither correlated with capture time in harbour seals (Kershaw & Hall 2016) nor with encounter time in humpback whales (Mingramm et al. 2020). Body length (cm) was measured when possible. Sex was either recorded in the field upon collection or later determined by genetic analysis, with the latter taking precedence (Petersen et al. 2012). Tissue samples including blubber and skin (muktuk) were collected, frozen, and archived in –40°C freezers at the Freshwater Institute, Winnipeg, MB. We requested that hunters collect the sample from close to the dorsal ridge near the midline, though exact sampling locations may vary. We used approximately 0.5 g of skin from the sample for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotope analysis. For cortisol analysis, we removed the outer edges of blubber samples to avoid sampling oxidized blubber and used a 1 g subsample of blubber that spanned from skin to muscle to maintain consistency in cortisol with blubber depth (Trana et al. 2015). For fatty acid analysis, we used a 0.5 g subsample from

the innermost layer of blubber available. Blubber and skin samples were freeze-dried for a minimum of 36 h to remove water.

Since blubber samples can degrade over time and affect hormone concentrations, we used only high-quality blubber samples that showed no visible discoloration or other signs of degradation (Trana et al. 2015). A total of 364 samples were classified as high quality and used for analysis (Supplement 1 at www.int-res.com/articles/suppl/m698p171_supp1.xlsx), though not all samples were processed for each measure (Table S1 in Supplement 2 at www.int-res.com/articles/suppl/m698p171_supp2.pdf). Stable isotope analysis was run on 341 skin samples, while fatty acids and cortisol concentrations were measured in 236 and 326 blubber samples, respectively (Table S1). Sex was determined in 352 individuals, and body length was measured in 307 individuals, with a mean (\pm SE) length of 373.7 ± 3.6 cm.

2.3. Cortisol analysis

A version of methods used to extract blubber progesterone (Kellar et al. 2006) was used to extract cortisol, modified to accommodate a larger sample mass. Modifications included an increased blubber subsample size, associated solvent amounts, and evaporation times. These adjustments were made to ensure detection of cortisol within blubber tissue. Samples were homogenized with solvents and a metal rod, rather than a homogenization machine, and reusable glass tubes were used for some steps rather than disposable tubes.

Cortisol concentrations were measured using radioimmunoassay (RIA), described in detail by Trana et al. (2015) and summarized here. Samples were first measured into 1 g subsamples. To remove cortisol from the lipid-rich matrix, blubber samples were freeze-dried, vortexed, centrifuged, and evaporated with ethanol, acetone, ethyl ether, acetonitrile, and hexane, consecutively. The sample was evaporated until dry in a nitrogen evaporator. Each extracted sample was then dissolved with 250 μ l of RIA buffer composed of 10 ml phosphate buffer (71.6 g $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$, 15.3 g $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ in 1 l milli-Q [ultrapure] water), 0.9 g NaCl, 0.5 g bovine serum albumin, and 90 ml milli-Q water. Sample tubes were prepared by mixing 100 μ l of sample mixture with 100 μ l tritiated cortisol (5000 disintegrations per minute, dpm; Net 396250 mCi, specific activity 72.45 Ci mmol^{-1} ; PerkinElmer), and 100 μ l of 1:3200 dilution of cortisol antibody was added (Fitzgerald

Industries; product code 20-CR50). Cross-reactivity of the antibody used was 100% for cortisol, 5.7% for 11-deoxycortisol, 3.3% for corticosterone, 36% for prednisolone, and <0.7% for cortisone, according to the manufacturer. All assay components were the same as those validated for measurement of fecal glucocorticoid metabolites in Richardson's ground squirrels *Urocitellus richardsonii* (Hare et al. 2014). The mixture was incubated, and 100 μ l of a charcoal-dextran buffer solution (2.5 g charcoal, 0.25 g dextran in 50 ml RIA buffer solution) was added to the mixture before vortexing, incubating, and centrifuging the solution. The supernatant was decanted into scintillation vials and mixed with scintillation fluid (Ultima Gold, PerkinElmer). A scintillation counter (Tri-Carb[®] 3110TR, PerkinElmer) was used to count radioactivity for 5 min. Each extracted sample was divided to create duplicates for processing through RIA, and each assay contained a standard concentration curve measured in triplicate of 10 concentrations ranging from 0.05–25 ng ml^{-1} (Trana et al. 2015). Extraction efficiency was determined by adding 250 000 dpm of radioactively labeled cortisol to 10 pooled samples (composed of a mixture of 40 subsamples) prior to extraction. Inter-assay variation was measured by including a pooled sample in each of 22 assays, and intra-assay variation was measured by including 20 pooled samples within one assay. The mean of the pooled sample groups was divided by the standard deviation (expressed as a percent) to calculate sampling variability among samples. Inter-assay variation was 14% ($n = 22$), intra-assay variation was 6% ($n = 20$), and mean extraction efficiency was $77 \pm 4\%$. Serial dilution of samples presented good parallelism with the standard curve (see Trana et al. 2015, their Fig. 2), and sample quenching was less than 1% (Trana et al. 2015). Cortisol values adjusted for extraction efficiency were used for all analyses. Blubber cortisol (ng g^{-1}) was calculated as: interpolated cortisol (ng ml^{-1}) \times ml of RIA buffer / wet mass of blubber sample.

2.4. Stable isotope analysis

Skin samples were rinsed with distilled water and chopped finely before freeze-drying. They were homogenized into a coarse powder using a mortar and pestle and analyzed at the University of Windsor Chemical Tracers Lab. Lipids were extracted using a 2:1 mixture of chloroform to methanol (McMeans et al. 2009), and approximately 0.5 mg of the prepared

sample was wrapped in a tin capsule. Ratios of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were measured on a continuous flow isotope ratio mass spectrometer (Delta V Advantage, Thermo Electron).

In recent decades, atmospheric $\delta^{13}\text{C}$ has decreased due to increased CO_2 from the burning of ^{13}C -depleted fossil fuels (referred to as the Suess effect; Sonnerup et al. 1999). We corrected $\delta^{13}\text{C}$ values for the oceanic Suess effect by applying a correction value of 0.02‰ yr^{-1} with 1982 as the baseline, as suggested for the North Atlantic (Sonnerup et al. 1999, Körtzinger et al. 2003).

2.5. Dietary fatty acid analysis

We used the Folch procedure (Folch et al. 1957) for lipid extraction with modifications recommended by Budge et al. (2006). We extracted lipids from the blubber subsample with 2:1 chloroform–methanol containing 0.01% butylated hydroxytoluene. We removed the blubber and added 3.5 ml of sodium chloride to the solution, then used a solution of dichloromethane with 0.01% butylated hydroxytoluene and Hilditch (a mixture of sulfuric acid and dry methanol) heated for 1 h at 100°C to produce fatty acid methyl esters (FAMES). We added hexane, distilled water, and sodium sulfate and placed the sample under an evaporative nitrogen stream. We used an Agilent Technologies 7890A GC system to analyze the FAME samples. We used Supelco (37 component FAME mix) and Nucheck (54 component mix GLC-463) standards and identified FAME by comparison to known standard mixtures and retention time.

Although many fatty acids are biosynthesized in predator tissues, only fatty acids that are known to transfer from prey to predator are useful for analyzing diet (Iverson et al. 2004). A total of 29 dietary fatty acids were identified and used to evaluate diet in this study (Table S2). For individuals that were interpreted as having undetectable amounts of a given fatty acid due to the detection limit of gas chromatography, we added an arbitrary small number (0.00001; Kenkel 2006). We divided percent fatty acid values by the percentage of the reference fatty acid C18:0 and log-transformed the standardized values (Budge et al. 2006).

2.6. Statistical analysis

Statistical analysis was performed in R version 3.6.3 (R Core Team 2020).

2.6.1. Cortisol

Cortisol data were log-transformed to meet the assumptions of normality and homogeneity of variance. We used an ANOVA followed by a Tukey's HSD test to investigate whether blubber cortisol concentrations differed among the 4 populations. Since sex was significant in the preliminary analysis, we used Student's *t*-tests to determine whether cortisol differed between sexes. We used a linear mixed-effect model with year as a fixed effect and population as a random effect to examine whether cortisol changed significantly over time. For this model and all subsequent linear mixed-effect models, we used the function 'lmer' in the package 'lme4' (Bates et al. 2015) with the default family 'gaussian' and the link function 'identity'. We used the package 'lmerTest' (Kuznetsova et al. 2017) to obtain p-values for the linear mixed-effect models.

2.6.2. Dietary biomarkers

Residuals of stable isotope data did not meet the assumptions of normality or homogeneity of variance ($\delta^{13}\text{C}$: Shapiro-Wilk, $p < 0.001$, Levene's test, $p = 0.021$; $\delta^{15}\text{N}$: Shapiro-Wilk, $p < 0.001$, Levene's test, $p = 0.005$). Therefore, we used Kruskal-Wallis 1-way ANOVA followed by a Dunn post hoc test to investigate whether $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ differed among the 4 populations (CS, EBS, HA, and WHB). We used the packages SIBER (Jackson et al. 2011) and 'rjags' (Plummer 2021) to calculate standard ellipse areas (the bivariate equivalent to standard deviations) for the 4 populations and for males and females from all populations together. Since some populations have a small sample size, we used the standard ellipse areas corrected for small sample size (SEA_c). We evaluated the similarity in diet between populations by calculating the overlapping area of the SEA_c of populations and sexes. We also used Bayesian standard ellipse areas (SEA_B) to calculate and compare isotopic niche widths between populations and sexes.

We used a principal component analysis (PCA) with a covariance matrix to reduce the dimensionality of the 29 fatty acids using the function 'prcomp' in the base stats package (R Core Team 2020) and visualized the PCA results using the package 'factoextra' (Kassambara & Mundt 2016). We used the first principal component (PC1) for further analysis. Since we had only 2 blubber samples of high quality from HA that were analyzed for fatty acid composition, this population was excluded from the fatty acid analysis.

Residuals of PC1 did not meet the assumptions of normality (Shapiro-Wilk: $p < 0.001$). Therefore, we used a Kruskal-Wallis 1-way ANOVA followed by a Dunn post hoc test to determine if dietary fatty acid composition differed among the 3 populations (CS, EBS, and WHB). In our preliminary analysis, sex was a significant factor predicting cortisol levels. Therefore, we used Wilcoxon rank sum tests to determine whether $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and PC1 differed between sexes.

2.6.3. Linear mixed-effects models

Two linear mixed-effects models were used to investigate whether differences in diet contributed to differences in blubber cortisol concentration. In the first model, we used population (CS, EBS, HA, and WHB), sex, length, $\delta^{13}\text{C}$, and $\delta^{15}\text{N}$ as factors. In the second model, we used population (CS, EBS, and WHB), sex, length, PC1, and PC2 as factors. Since length was not significant, we removed it from both models, and since PC2 was not significant, we removed it from the second model. We used year as a random effect in both models, since year was not correlated with cortisol. We also ran a third model with 3 populations (CS, EBS, WHB), sex, length, $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and PC1 to investigate whether results would differ when both dietary biomarkers were included in the same model. The results of the third model did not change or add to the conclusions drawn from the first 2 models. The factors that were statistically significant in the first 2 models, with stable isotope ratios and PC1 included separately, were also statistically significant in the third model, with dietary biomarkers in the same model. Similarly, the factors that were non-significant in the first 2 models were non-significant in the third. Therefore, to include HA in the analysis, we used the first 2 models with the dietary biomarkers included as factors separately, and ruled out the third model. We calculated the conditional R^2 for the generalized linear mixed-effects model using the package 'MuMIn' (Bartoń 2020)

3. RESULTS

3.1. Cortisol

Blubber cortisol differed among the 4 populations ($F_{3,322} = 7.9$, $p < 0.001$; Fig. 2A). CS beluga whales (untransformed mean \pm SE: $0.65 \pm 0.11 \text{ ng g}^{-1}$) had higher cortisol concentrations than EBS ($0.31 \pm 0.03 \text{ ng g}^{-1}$, $p < 0.001$), HA ($0.32 \pm 0.09 \text{ ng g}^{-1}$, $p = 0.004$),

and WHB ($0.44 \pm 0.04 \text{ ng g}^{-1}$, $p = 0.004$), while EBS, HA, and WHB were similar ($p > 0.82$; Fig. 2A). Females had higher cortisol than males overall (females: $0.56 \pm 0.05 \text{ ng g}^{-1}$, males: $0.36 \pm 0.03 \text{ ng g}^{-1}$; $t_{312} = -4.60$, $p < 0.001$) and in WHB ($t_{138} = -4.6$, $p < 0.001$), but sexes did not differ significantly in the other 3 populations ($p > 0.26$; Fig. 2A). Cortisol concentrations were not correlated with year ($F_{1,300.4} = 0.6$, $p = 0.44$; Fig. 3).

3.2. Stable isotope analysis

Values of $\delta^{13}\text{C}$ differed among populations ($\chi^2_3 = 75.7$, $p < 0.001$; Fig. 4). EBS ($-18.04 \pm 0.04\text{‰}$) had lower $\delta^{13}\text{C}$ values than CS ($-17.78 \pm 0.06\text{‰}$, $p = 0.017$), HA ($-17.55 \pm 0.09\text{‰}$, $p < 0.001$), and WHB ($-17.53 \pm 0.05\text{‰}$, $p < 0.001$; Fig. 2B). WHB had greater $\delta^{13}\text{C}$ values than CS ($p = 0.034$), but no other pairwise comparisons were significant ($p > 0.38$; Fig. 2B). $\delta^{15}\text{N}$ also differed among populations ($\chi^2_3 = 101.1$, $p < 0.001$; Fig. 4), with EBS ($17.30 \pm 0.04\text{‰}$) greater than CS ($16.69 \pm 0.13\text{‰}$, $p < 0.001$), HA ($16.46 \pm 0.12\text{‰}$, $p < 0.001$), and WHB ($16.62 \pm 0.05\text{‰}$, $p < 0.001$), while CS, HA, and WHB were similar ($p > 0.47$; Fig. 2C). When all populations were considered together, females had higher $\delta^{13}\text{C}$ than males (females: $-17.67 \pm 0.06\text{‰}$, males: $-17.81 \pm 0.04\text{‰}$; $W = 10162$, $p = 0.026$; Fig. 2B). Conversely, males had higher $\delta^{15}\text{N}$ than females overall (females: $16.55 \pm 0.06\text{‰}$, males: $17.05 \pm 0.04\text{‰}$; $W = 17428$, $p < 0.001$; Fig. 2C). The SEA_c of each population and for males and females varied between 3.24 and 6.75‰^2 , while the overlapping area ranged from 1.77 – 4.96‰^2 (Table S3, Fig. 4A). The SEA_B of the 4 populations and of males and females from all populations together also showed substantial overlap (Figs. 4B & S1B).

3.3. Dietary fatty acids

PC1 explained 38.2% (eigenvalue = 22.10) of the variation in the 29 dietary fatty acids, while the second principal component (PC2) explained 17.8% of the variation (Fig. S2). The fatty acids with the greatest contribution to PC1 were 22:4n3 (45.3%), 22:2n6 (38.1%), 22:5n6 (5.5%), 16:4n3 (3.6%), and 22:1n7 (1.4%), and the fatty acids with the greatest contribution to PC2 were 22:2n6 (54.6%), 22:4n3 (24.8%), 22:5n6 (14.4%), 21:5n3 (2.7%), and 22:4n6 (0.7%; Table S2, Fig. S2). PC1 differed among the 3 populations ($\chi^2_2 = 30.4$, $p < 0.001$). WHB differed signifi-

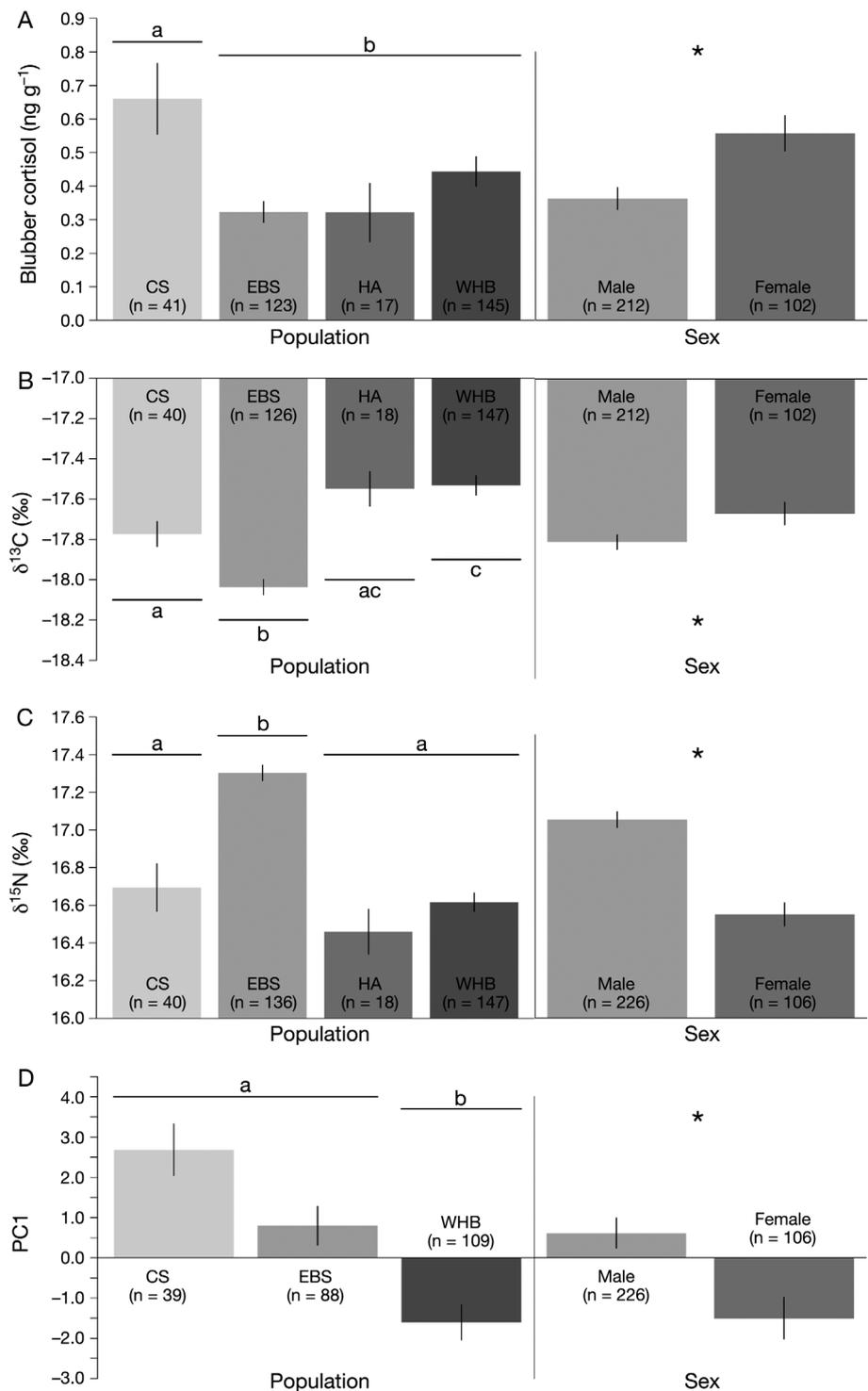


Fig. 2. Mean (\pm SE) (A) blubber cortisol concentration, (B) skin $\delta^{13}\text{C}$, (C) skin $\delta^{15}\text{N}$, and (D) the first principle component (PC1) of a principle component analysis on 29 dietary fatty acids of beluga whales grouped by population and sex from Cumberland Sound (CS), Eastern Beaufort Sea (EBS), Eastern High Arctic-Baffin Bay (HA), and Western Hudson Bay (WHB). Letters: significant differences among populations; asterisks: significant differences between sexes. CS had higher cortisol than the other 3 populations ($p \leq 0.004$). EBS had lower $\delta^{13}\text{C}$ ($p \leq 0.17$) and higher $\delta^{15}\text{N}$ ($p < 0.001$) than the other 3 populations, and WHB had higher $\delta^{13}\text{C}$ than CS ($p = 0.034$). WHB differed from the CS and EBS in dietary fatty acids ($p < 0.01$)

cantly from CS ($p < 0.001$) and EBS ($p < 0.001$), while CS and EBS did not differ ($p = 0.07$; Fig. 2D). When all populations were considered together, fatty acids in males differed significantly from females ($W = 7342$, $p = 0.001$; Fig. 2D).

3.4. Linear mixed-effects models

In the first model, population and sex were significant predictors of blubber cortisol, while $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ were not (Table 2). Similarly, in the second

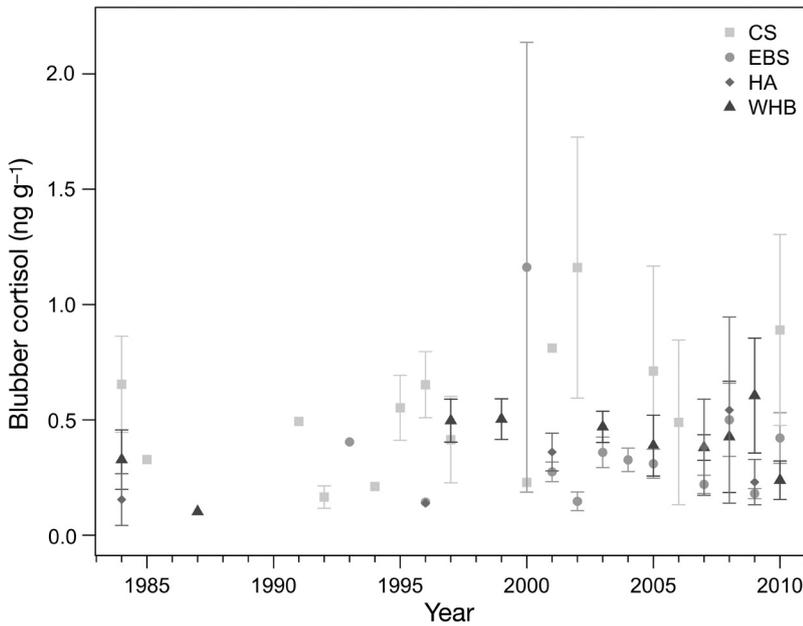


Fig. 3. Average (\pm SE) blubber cortisol concentration of beluga whales from Cumberland Sound (CS), Eastern Beaufort Sea (EBS), Eastern High Arctic-Baffin Bay (HA), and Western Hudson Bay (WHB) from 1984–2010. Blubber cortisol concentrations were not significantly correlated with year ($p = 0.44$)

model, population and sex were significant predictors of blubber cortisol, while PC1 was not (Table 2).

4. DISCUSSION

We found statistically significant differences in blubber cortisol concentrations among the 4 beluga whale

populations. CS whales had slightly elevated blubber cortisol concentrations while the other populations did not differ, suggesting CS beluga whales may be experiencing marginally higher prolonged elevation in circulating levels of cortisol relative to the other 3 populations studied. The cortisol concentrations measured here were comparable to reported concentrations of blubber cortisol in beluga whales and other whales (Trana et al. 2016, Loseto et al. 2018, Mingramm et al. 2020, Watt et al. 2021b), though lower than concentrations reported in some other cetaceans (e.g. Kellar et al. 2015, Kershaw et al. 2017). All 4 populations had average cortisol concentrations similar to healthy whales harvested from EBS in 2006–2007 ($0.25 \pm 0.04 \text{ ng g}^{-1}$) but marginally lower than whales experiencing prolonged stress from an ice-entrapment event ($1.76 \pm 0.32 \text{ ng g}^{-1}$; Trana et al. 2016). Loseto et al. (2018)

also found similar blubber cortisol concentrations in harvested EBS beluga whales between 2007 and 2010 ($\sim 0.8 \text{ ng g}^{-1}$). Similar blubber cortisol concentrations were reported in narwhals *Monodon monoceros*, a close beluga whale relative, in Baffin Bay, NU, prior to an increase in industrial vessel traffic ($0.81 \pm 0.45 \text{ ng g}^{-1}$), while slightly higher concentrations were reported after an increase in traffic

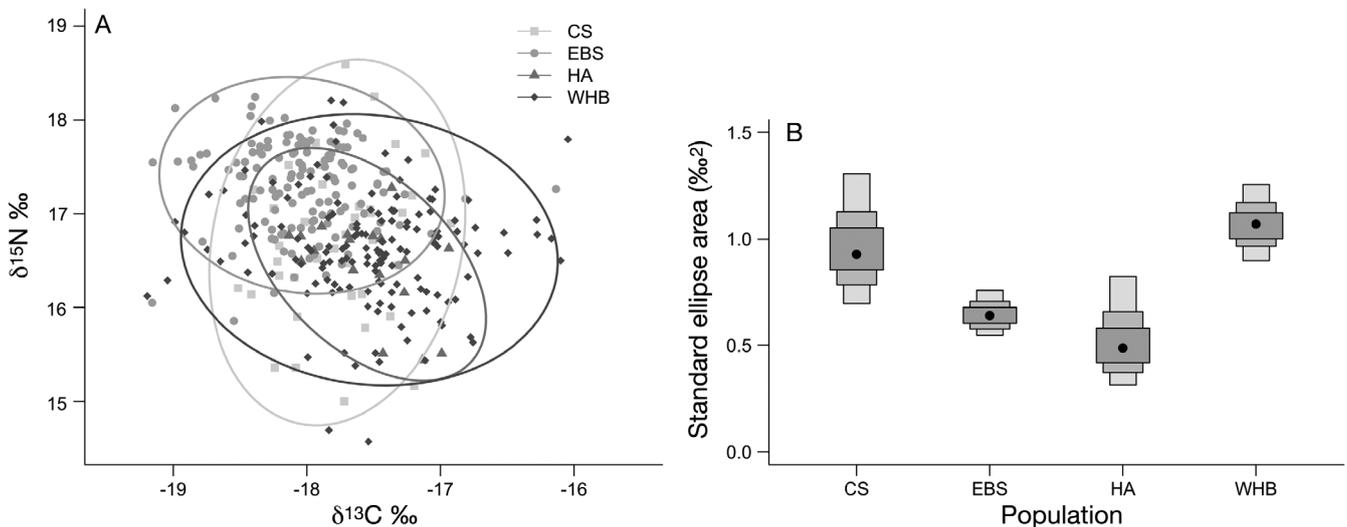


Fig. 4. (A) $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ biplot of skin samples from individual beluga whales from Cumberland Sound (CS), Eastern Beaufort Sea (EBS), Eastern High Arctic-Baffin Bay (HA), and Western Hudson Bay (WHB; populations in grayscale). Ellipses indicate standard ellipse area corrected for small sample size, representing dietary niche. (B) Density plot indicating the Bayesian isotopic niche area for each population, with boxes indicating 50, 75, and 95% credibility intervals. See Table S1 for sample sizes

Table 2. Sample size, factor degrees of freedom, *F*-statistics, *p*-values, and the conditional coefficient of determination for 2 linear mixed-effects models investigating whether dietary biomarkers are associated with blubber cortisol concentrations in 4 Canadian beluga whale populations. Both models include population and sex and fixed effects and year as a random effect. Model 1 includes carbon and nitrogen stable isotope ratios ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$), while Model 2 includes PC1 (reflecting fatty acid profile)

Factor	N	df	<i>F</i>	<i>p</i>	<i>R</i> ²
Model 1	297				0.12
Population		3, 210.8	5.5	0.001	
Sex		1, 295.6	11.2	<0.001	
$\delta^{13}\text{C}$		1, 233.4	0.3	0.58	
$\delta^{15}\text{N}$		1, 291.5	0.9	0.35	
Model 2	207				0.16
Population		2, 207	6.7	0.001	
Sex		1, 207	14.4	<0.001	
PC1		1, 207	0.6	0.44	

($1.81 \pm 0.48 \text{ ng g}^{-1}$; Watt et al. 2021b). Note that the studies by Loseto et al. (2018) and Watt et al. (2021b) measured cortisol using different techniques (high-performance liquid chromatography tandem mass spectrometer for belugas and liquid chromatography-mass spectrometry for narwhals, rather than RIA) and were sampled over shorter time periods. Blubber as a matrix for measuring cortisol in cetaceans has a recent history of use, and thus different methods have been developed, which makes comparisons between studies challenging.

Analysis of dietary biomarkers suggested significant differences in diet among the 4 populations. Blubber fatty acid analysis represents the diet of the previous few weeks, while beluga whale skin stable isotope analysis is expected to reveal the diet of the previous 2–3 mo (St. Aubin et al. 1990, Kirsch et al. 2000, Iverson et al. 2004, Budge et al. 2006). Since our samples were collected between July and September, together they likely represent late spring and summer diet. EBS whales had significantly lower $\delta^{13}\text{C}$ and higher $\delta^{15}\text{N}$ than the other 3 populations, suggesting they may be feeding on more offshore prey and may occupy the highest trophic position of the 4 populations (Newsome et al. 2010). EBS is geographically distant from the other 3 populations, located in the Western Canadian Arctic near the Pacific Ocean, while the other 3 populations are found in the Eastern Arctic near the Atlantic Ocean. Levels of $\delta^{13}\text{C}$ vary geographically by latitude and longitude in the Canadian Arctic and subarctic, as well as among Arctic shelves, rivers, and basins, and $\delta^{13}\text{C}$ is more depleted in zooplankton (depending on

species) and particulate organic carbon in the Beaufort Sea compared to the Hudson Bay (Schell et al. 1998, Pomerleau et al. 2016, de la Vega et al. 2019). Values of $\delta^{15}\text{N}$ also vary by longitude and latitude, though to a lesser degree (Schell et al. 1998, Graham et al. 2010, Pomerleau et al. 2016). Further, EBS beluga whales undergo the longest migration of the 4 populations, traveling over 2000 km between the Beaufort and Bering seas (Luque & Ferguson 2010). Therefore, geographic variation in stable isotope ratios across the Arctic Ocean may also contribute to differences in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ among populations (Graham et al. 2010). Although our analysis suggests differences in diet among the 4 populations, results should be interpreted with caution, particularly comparing the EBS population to the other 3 populations, as differences in baseline stable isotope ratios have not been accounted for. However, baseline $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ do not vary significantly across the eastern Arctic for the HA, CS, and WHB populations (Graham et al. 2010).

EBS and HA had smaller SEA_c , while CS and WHB had the largest SEA_c among the populations. A broad isotopic niche suggests that either all individuals in the group have a generalist diet, or that there may be some dietary specialization within the population. It is difficult to discern with these data which is the case, though there is support in the literature for within-population dietary specialization (e.g. Loseto et al. 2006) and that beluga whales are typically generalist predators (e.g. Yurkowski et al. 2016). Future studies may wish to further investigate within-population dietary differences. Overlap of SEA_c suggests similarities in diet among the different groups and a broad dietary niche in all groups. SEA_B also showed overlap between most groups, though with some differentiation among populations (WHB had the highest 50% credibility intervals, while HA appears to have the lowest). Overall, females showed slightly greater SEA_B than males, though again there was considerable overlap.

Fatty acid analysis also revealed differences among CS, EBS, and WHB. Although the 3 populations were not clearly clustered on the PCA biplot, WHB coordinates differed from EBS and CS along the PC1 axis, suggesting WHB has a significantly different fatty acid composition compared to the other 2 populations. These results are similar to previous studies that found the fatty acid profile of WHB beluga whales from 1983–1987 to be representative of a primarily capelin diet, differing from CS and HA whose profiles suggested a cod-based diet (Kelley et al. 2010). Therefore, although the diets of beluga whales

in all 3 populations are shifting towards greater proportions of capelin (Kelley et al. 2010, Yurkowski et al. 2018, Choy et al. 2020), the dietary fatty acid composition of WHB still differs from the more northern populations.

The PCA was influenced primarily by polyunsaturated fatty acids. Omega-6 fatty acids, such as 22:2n6 and 22:5n6, have been attributed to capelin and bottom-feeding fish (Loseto et al. 2009, Kelley et al. 2010). Loseto et al. (2009) also found the fatty acid 22:4n3 to be of high importance in beluga whale prey items, and although it appears to be associated with estuarial fish species in their PCA analysis, the dietary source is unknown. The fatty acid 16:4n3 is found in polar marine microalga (Graeve et al. 2002, Boelen et al. 2013, Morales-Sánchez et al. 2020), suggesting that beluga whale prey may be indirectly feeding upon these algae (i.e. ingesting prey that have been eating microalga). Greater sea ice at high latitudes likely also results in a greater contribution of sea ice algae to beluga whale diet (Brown et al. 2017). The very long-chain monounsaturated fatty acid 22:1n7 is the only fatty acid of the top 5 contributors associated with cod (Kelley et al. 2010). This fatty acid is decreasing significantly over time in the diet of CS beluga whales, potentially due to the increasing availability and consumption of capelin in CS (Watt et al. 2016). Whereas omega-6 fatty acids were important here, previous studies have found 20:1 and 22:1 monounsaturates that are associated with Arctic cod to be more prevalent in beluga whale diet (Loseto et al. 2009, Kelley et al. 2010, Choy et al. 2020). The greater influence of omega-6 fatty acids in this study may support the observed shift to capelin as an important prey species (Watt et al. 2016, Yurkowski et al. 2018, Choy et al. 2020).

Both stable isotope ratios and dietary fatty acids differed between sexes, indicating different dietary niches between males and females. Females had higher $\delta^{13}\text{C}$ but lower $\delta^{15}\text{N}$ signatures than males on average, suggesting females may forage on a higher proportion of near-shore prey and at a lower trophic level than males. These observations are consistent with sexual segregation in diet observed in fatty acids in the EBS and stable isotopes in CS, the High Arctic, and West Greenland (Marcoux et al. 2012, Szpak et al. 2020, Choy et al. 2020, Louis et al. 2021). Males tend to consume more cod and other high trophic level prey, such as Greenland halibut, while females consume higher proportions of capelin (Marcoux et al. 2012, Choy et al. 2020). There are several hypotheses for why males forage at a higher trophic level than females. Beluga whales are sexually dimor-

phic, and males are on average larger than females (Stewart & Stewart 1989). Large males tend to select off-shore, closed- or mixed-ice habitats, and thus may have greater access to energy-rich Arctic cod and other benthic prey (Loseto et al. 2006). Females also have a prolonged period of maternal investment, with a 14 mo gestation period followed by approximately 2 yr of nursing (Sergeant 1973, Heide-Jørgensen & Teilmann 1994, Matthews & Ferguson 2015). Females with calves tend to select shallow open water or ice-edge habitat, potentially to avoid predation (Loseto et al. 2006). They are also limited by the lower diving capabilities of calves and spend more time at the surface than females without calves (Heide-Jørgensen et al. 2001, Noren & Suydam 2016). Therefore, they likely have different energetic requirements and their access to prey is potentially more limited than large males (Szpak et al. 2020, Louis et al. 2021). Although fatty acid profiles differed by populations and sex, there were no graphically discernable groupings on the PCA biplot related to these factors. We could also not discern any obvious groupings related to length or year. However, individuals appear to be clustered into 3 main groups along the PC1 axis, suggesting that other characteristics may be driving variation in blubber fatty acid composition. Beluga whales are known to segregate among habitats based on other factors not measured here, such as reproductive status (Loseto et al. 2006). Future studies may wish to investigate on what basis these clusters are forming.

Blubber cortisol has been proposed to provide information about the physiological state of marine mammals due to its role in the storage and mobilization of fat stores to provide energy when food is limited (Kershaw & Hall 2016). In pinnipeds, periods of fasting due to lactation or molt are associated with elevated levels of circulating (Crocker et al. 2014) and blubber cortisol (Kershaw & Hall 2016). Although we do not know the quantity of food consumed by these beluga whales, limited prey availability or limited access to primary prey may be a source of physiological stress on beluga whales, with negative impacts on individual and population health. Despite the variation in diet among populations, dietary differences do not appear to correspond with differences in cortisol among populations. Neither stable isotopes nor dietary fatty acids were associated with cortisol levels, suggesting that diet does not have a major influence on blubber cortisol concentrations. CS had marginally elevated cortisol concentrations, yet dietary biomarkers did not suggest any major differences from the other 4 populations. EBS had the

most distinct stable isotope profile while WHB had the most distinct dietary fatty acid composition, but both had similar cortisol concentrations. EBS is considered not at risk by COSEWIC, and WHB was recently downgraded in status from special concern after the period of study to not at risk when the previous Designatable Unit was split into WHB and James Bay (COSEWIC 2016, 2021). Although EBS and WHB are the largest beluga whale populations in the world, we did not find evidence that dietary changes are prompting a population health concern. Trophic level was associated with glucocorticoids in Leach's storm petrels *Oceanodroma leucorhoa*, where feather corticosterone was negatively correlated with trophic level (Fairhurst et al. 2015). Contrarily, in this study, stable isotope analysis suggested that EBS beluga whales are foraging at the highest trophic level, yet do not have significantly lower cortisol than any population other than CS, nor was $\delta^{15}\text{N}$ a predictor of blubber cortisol. Therefore, cortisol may not be associated with trophic position in beluga whales, or the effect is insignificant.

Further, it is hypothesized that the shift in primary prey from Arctic cod to capelin may potentially have energetic consequences for beluga whales (Pedro et al. 2019). Despite both providing similar concentrations of high-quality lipids and occupying a similar trophic level, capelin are smaller than cod and have lower lipid content and thus may not provide the same energy content per catch (Marcoux et al. 2012, Hop & Gjørseter 2013, Watt et al. 2016, Pedro et al. 2019, 2020, Choy et al. 2020). If there is an energetic impact, it does not appear to influence blubber cortisol in WHB beluga whales, who consume the most capelin (Kelley et al. 2010) yet have similar blubber cortisol levels to the other populations. Beluga whales are considered generalist predators, though diet depends on location, and there is some dietary specialization at the population level (Quakenbush et al. 2015, Yurkowski et al. 2016). Although several marine mammals have exhibited changes in cortisol levels in response to changes in diet and prey availability (Ayres et al. 2012, Crain et al. 2021, Guo et al. 2022), beluga whales appear to be flexible as new sources of prey become available (Watt et al. 2016, Yurkowski et al. 2018, Choy et al. 2020).

Population was associated with blubber cortisol concentrations in the model. Aside from physiological stress from food web changes, psychological stress from other environmental stressors may be contributing to the higher cortisol levels observed in CS beluga whales. Environmental changes in the Arctic have led to increased risk of predation (i.e. from

killer whales, whose presence disrupts the behaviour of narwhal and bowhead whales; Higdon & Ferguson 2009, Higdon et al. 2012, Breed et al. 2017, Matthews et al. 2020), as well as parasitism and disease (Burek et al. 2008). Anthropogenic threats include increased vessel traffic (Wright et al. 2007, Pizzolato et al. 2016, Halliday et al. 2017, Dawson et al. 2018), increased exposure to contaminants (Muir & de Wit 2010), and subsistence hunting pressure. Exposure to contaminants has led to a wide range of negative health effects in Arctic biota, and contaminant levels in CS beluga whales are among the highest in Canada (DFO 2002, Smythe et al. 2018, Dietz et al. 2019). Finally, beluga whales are an important resource for many northern communities, and with proper management, subsistence hunting does not pose a threat to healthy populations. However, the current harvest rate in CS is not considered sustainable, and a reduced quota is likely necessary to allow the recovery of the CS population (DFO 2019). Unfortunately, these factors are not addressed by the data presented here, and further studies are needed to investigate whether these factors are affecting CS beluga whales.

Life history differences among populations may also result in population-specific cortisol concentrations. For example, CS beluga whales undergo the shortest migration between summering and wintering grounds (see Fig. 1). A longer migration may be beneficial for beluga whales, as the 2 Canadian populations with highest conservation concern, CS (threatened) and St. Lawrence Estuary (endangered), are those with the shortest migration (COSEWIC 2004, 2014). Similarly, the 4 populations occur along different latitudinal ranges and represent multiple phylogenetic clades (Skovrind et al. 2021). It is possible that these geographic and behavioural differences have resulted in differing baseline levels of blubber cortisol among populations, with CS having slightly higher blubber cortisol concentrations on average than the other 3 populations. We did not find a significant change in blubber cortisol over the study period, which supports CS beluga whales potentially having higher intrinsic blubber cortisol concentrations and potentially a greater allostatic load resulting in an elevated baseline cortisol concentration. Elevated baseline cortisol levels may warrant conservation attention, since this may more quickly lead to population health consequences.

Sex was the other significant factor predicting blubber cortisol concentrations, and overall, females had higher cortisol concentrations than males. Although results in the literature are mixed, cortisol has been found to differ between sexes in several marine mam-

mals. In beluga whales, Trana et al. (2016) found no difference between sexes in the EBS population, while Loseto et al. (2018) found sex differences in the same population for the innermost blubber layer only. Both studies were restricted by a small sample of females due to hunter selection of males and avoidance of females with calves (Harwood et al. 2002). We also had large differences in sample size both among populations and between sexes in this study, potentially influencing results. However, as we found here, when sexes differ, females typically have higher cortisol concentrations than males (Reeder & Kramer 2005). Female harbour seals had higher blubber cortisol than males in Scotland (Kershaw & Hall 2016), while female polar bears *Ursus maritimus* had higher plasma cortisol than males in Svalbard, Norway, but not hair cortisol in East Greenland (Oskam et al. 2004, Bechshøft et al. 2013). Reproductive status can also influence glucocorticoids concentrations in marine mammals. Pregnant female North Atlantic right whales had higher fecal glucocorticoid concentrations, and female polar bears had higher hair cortisol concentrations than other sex and reproductive categories (Hunt et al. 2006, Macbeth et al. 2012). Therefore, the influence of pregnancy and calving may be partially responsible for this difference, since the annual subsistence hunt typically corresponds with the calving season (Brodie 1971). Males also display seasonal and intra-annual differences in cortisol associated with reproductive patterns (Hunt et al. 2018).

Although we did not find a significant relationship between cortisol and year, qualitative trends suggest that in some populations cortisol may be increasing as changes in the Arctic have progressed over the last 3 decades. These patterns may be influenced by sample availability over the years since we have few samples and large gaps for some populations in the earlier years of the study (e.g. HA). Climate-related changes in the Arctic are expected to have a negative impact on beluga whales and other marine mammals (Burek et al. 2008, Laidre et al. 2008). However, beluga whales are predicted to be moderately sensitive to climate change (Laidre et al. 2008). Thus far, it appears that beluga whales have been able to adapt well to shifts in prey availability (Watt et al. 2016, Yurkowski et al. 2018, Choy et al. 2020) and more generally to long-term trends in environmental change (Skovrind et al. 2021).

Marine mammals are increasingly being subjected to anthropogenic and climate-related changes in their environment, including shifts in diet. Blubber cortisol, which may be used to understand the long-

term effects of stressors in marine mammals, has the potential to be used as a tool for monitoring population health (Atkinson et al. 2015, Loseto et al. 2018). Generally, blubber samples obtained via biopsy dart are easier to collect than blood or fecal samples and are less likely to reflect the short-term stress of capture (Kellar et al. 2006, Mingramm et al. 2020). Analyzing multiple dietary biomarkers alongside hormone levels in the same individual can provide a more holistic view of diet and health (Fleming et al. 2018). Since biopsies of muktuk can provide information for both diet and hormone concentrations, blubber samples are a valuable tissue for evaluating population health and measuring changes over time. Although our results suggest that diet is not contributing to elevated levels of blubber cortisol in beluga whales, we observed here that the threatened population, CS, had slightly higher blubber cortisol levels than the other measured populations. Elevated cortisol is often interpreted in ecology as a stress response (Romero 2004), and although we do not have baseline cortisol measurements for comparison, these results align with the COSEWIC (2004) assessment that this population is under threat. Beluga whales play an important role in CS, both in the ecosystem and as a resource for local communities, and this population does not appear to be recovering from overexploitation. Continued monitoring of this population and others will be key for quantifying the long-term impacts of elevated blubber cortisol levels in CS beluga whales, and determining how changing environmental conditions affect this important marine predator.

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