Seasonal estuary use of beluga whales

by

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Abstract

Beluga whales are year-round residents of the Arctic and top predators in the ecosystem. Many beluga whale populations are migratory and spend winters in polynyas and breaks in the ice to avoid entrapment. Once the ice in begins to break up and melt, beluga whale populations migrate to the same Arctic estuaries year after year. Possible uses of estuaries include feeding, aiding in skin sloughing, protection of calves from predators, and other warm water benefits. However, much is still unknown about the use of these habitats both spatially and ecologically. I investigated estuary use by Cumberland Sound beluga whales, through their distribution. Their summering area covers northern fiords of Cumberland Sound, including Clearwater Fiord where there is a large estuary. While the whales are known the frequent the fiords, the specific distribution and core areas were unknown. Using very high resolution satellite imagery, I established critical habitat within the summering region. I also provided an updated estuary abundance estimate for this small beluga whale population. Second, I explored estuary use of Hudson Bay beluga whales by investigating the primary feeding season. Migratory species often have one season where they eat the majority of their food for the year and build fat reserves, which provides energy and supports thermoregulation. This fat can be used to investigate feeding ecology of whales. Despite being the largest beluga population, fundamental ecological knowledge of Hudson Bay beluga whales, such as the primary feeding season is unknown. I used lipid content and adipocyte size throughout the blubber of Hudson Bay beluga whales to identify when they were consuming the majority of their food. I determined that there was no consistent primary feeding season, and therefore both summering and wintering areas were used for feeding. By exploring the beluga whales’ use of seasonal habitat through distribution and foraging ecology, my thesis illuminates fundamental ecological knowledge about two beluga whale populations.

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Contribution of Authors

Chapter 2: Amanda Belanger and Cortney Watt designed the concept. Amanda Belanger led the analyses with Bryanna Sherbo. Amanda Belanger wrote the manuscript with revisions from Cortney Watt, Bryanna Sherbo, and Jim Roth.

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1 Introduction

Estuaries are bodies of water where fresh and salt water mix, which allows the passage of nutrients between the freshwater and oceanic systems. Freshwater often contributes abundant nutrients and sediment to the marine environment (Lamberti et al., 2010). There are many estuaries throughout the Arctic used by various species. Anadromous fish such as Arctic char use estuaries as migratory zones before spawning in freshwater rivers and lakes (Moore et al., 2016). Arctic char may also aggregate in estuaries during summer (Moore et al., 2016). Other fish, such as capelin and lake whitefish will use estuaries as nurseries and occasionally for foraging (Stewart & Lockhart, 2005). Estuaries are also used by marine mammals such as seals and beluga whales. For example, the Churchill River estuary is a frequent haul-out site of harbour seals (*Phoca vitulina*) (Bajzak et al., 2013). During the ice-free period, the seals remain close to the estuary and shore, which may provide opportunities for foraging (Bajzak et al., 2013). Beluga whales (*Delphinapterus leucas*) also seasonally use estuaries.

Beluga whales are a top predator with a generalist diet (Breton-Honeyman et al., 2016) and only have three known predators: killer whales (*Orcinus orca*), polar bears (*Ursus maritimus*), and humans (O'Corry-Crowe, 2009). Beluga whales are found throughout the Canadian Arctic and subarctic year-round and are an important component of Inuit diet and culture. Hunters harvest the beluga and often share the muktuk (skin and blubber) and meat with community members (Kilabuk, 1998). Several beluga populations are migratory and spend their summers in estuaries, exhibiting annual site fidelity (COSEWIC, 2020). These summering areas remain consistent within a population through matrilineal lineages (Turgeon et al., 2012; Colbeck et al., 2013). Shallow waters in estuaries may provide protection from killer whales (Richard and Stewart, 2008; Smith et al., 2017).

In addition, the warmer waters of estuaries may aid in calf thermoregulation (Corkeron and Connor, 1999) and proliferation of skin cells during the beluga whales' annual skin molt (St. Aubin et al., 1990a; Pitman et al., 2020). Despite these many hypotheses, it is relatively unknown why beluga whales migrate in and out of estuaries, and if there are high-use areas within and around estuaries. My thesis focuses on the seasonal use of estuaries by beluga whales from the Cumberland Sound and Hudson Bay populations. I aim to investigate if foraging is a main reason the whales migrate to estuaries for Hudson Bay whales, and whether there are consistent areas Cumberland Sound whales use across years.

Cumberland Sound beluga whales are one of the smallest populations with approximately 1200 individuals (Watt et al., 2021; Biddlecombe and Watt, 2022). This population is listed under Committee on the Status of Endangered Wildlife in Canada (COSEWIC) as Endangered (COSEWIC, 2021). While Cumberland Sound beluga whales are migratory, the migration is on a smaller scale than other populations and the whales are believed to remain within Cumberland Sound year round (Richard and Stewart, 2008). Throughout the summer, these whales inhabit in the northern fiords of Cumberland Sound, such as Clearwater Fiord where there is an estuary. However, the specific areas of high use within the fiord have not been determined. Furthermore, it is unclear whether other fiords within the summering region are important. In chapter 2, I explored the distribution of Cumberland Sound beluga whales in their seasonal habitat, using Kernel density estimates (Whorton, 1989,).

Hudson Bay beluga whales can be separated into two populations known as Western and Eastern Hudson Bay (WHB and EHB). James Bay beluga whales remain within James Bay year-round (Postma et al., 2012) and are unlikely to be part of the samples in this thesis. The WHB population is the largest in the Canadian Arctic, with an estimate of over 54,000 individuals and is considered Not at Risk by COSEWIC (Matthews et al., 2017; COSEWIC, 2020, 2021). These beluga whales winter in Hudson Strait and migrate along the west and east coast of Hudson Bay to reach their core summer area, which covers the Churchill, Nelson, and Seal River estuaries (Smith, 2007;

Luque and Ferguson, 2010). The EHB population is much smaller with under 4000 individuals, and is considered Threatened (COSEWIC, 2020). The EHB whales share similar migration routes and regions to WHB whales but summer along the eastern coast of Hudson Bay. The second chapter of my thesis focuses on WHB whales, but there may be EHB beluga whales within my dataset due to the overlap between the two populations around the Belcher Islands (Smith, 2007), where the whales in this study were harvested by local Inuit. In chapter 3, I investigated seasonal use of estuaries in Hudson Bay beluga whales by studying their foraging ecology. The objective was to identify the primary feeding season of Hudson Bay beluga whales to examine whether feeding was a main use of estuaries.

As beluga whales exhibit different behaviour and select different habitats across seasons, it is important to understand their ecology in individual seasons. Understanding cross-seasonal ecology can lead to more effective and informed management decisions. Each chapter is written as a standalone publication and therefore some of the information in this introduction may be repeated.

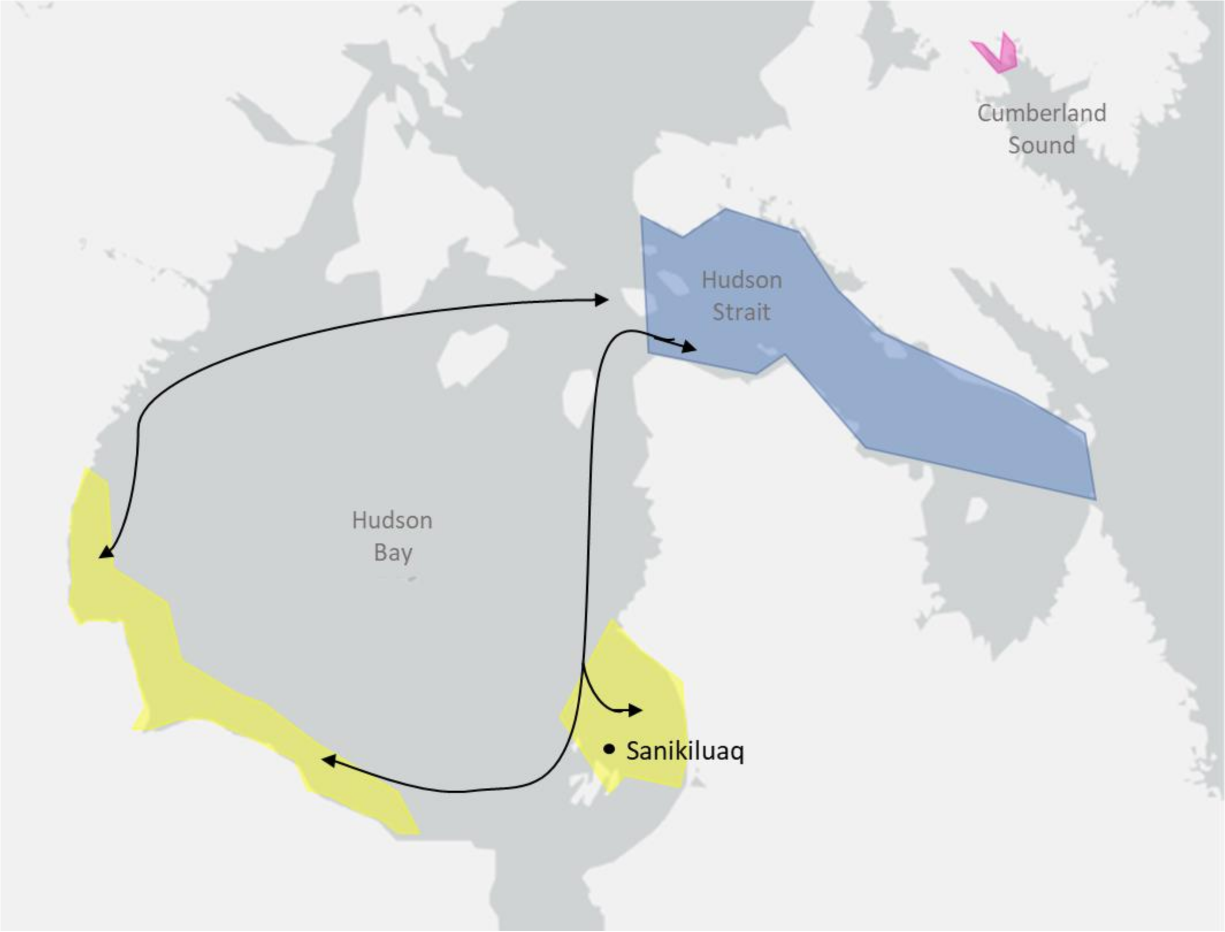

Figure 1.1. Seasonal ranges of Hudson Bay and Cumberland Sound beluga whales. The pink depicts the summering fiords of Cumberland Sound. Yellow is the summering range of Western and Eastern Hudson Bay beluga whales. Blue is the winter range of Hudson Bay beluga whales in Hudson Strait. The black lines depict the general migration routes of Hudson Bay beluga whales. Sanikiluaq in Belcher Islands is the site of beluga harvest for chapter 3.

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Chapter 2

2 Use of satellite imagery to estimate distribution and abundance of Cumberland Sound beluga whales reveals frequent use of a glacial river estuary

Abstract

Limiting disturbance in critical habitats is an important part of ensuring the well-being and sustainability of populations at risk, such as Cumberland Sound beluga whales (*Delphinapterus leucas).* Using non-disruptive Very High Resolution (VHR) satellite imagery, an emerging tool in cetacean monitoring, we aimed to estimate summer abundance and identify critical habitat for Cumberland Sound beluga whales. Specifically we looked in fiords that comprise their summer distribution, such as Clearwater Fiord where there is a large estuary, an important habitat to many beluga populations. Satellite images of the area were collected in 2020 and 2021, at 30 cm resolution, and in 2022 at 50 cm resolution. Clearwater Fiord abundance estimates were corrected for whales that were too deep to be identified in the images. The estimates were significantly lower in 2021 (197 whales, 95%CI: 180-216) and 2022 (194 whales, 95%CI: 172-218) compared to 2020 (393 whales, 95%CI: 366-422), possibly due to differences in distribution. Other fiords were only imaged in 2021 and 2022, resulting in average corrected abundance estimates for all fiords of 462 (95%CI: 425-502) and 252 (95%CI: 226-280) beluga whales, respectively. Down-sampling of 30 cm images to 50 cm resulted in up to 45% fewer whales detected. We evaluated beluga whale distribution using Kernel density, and identified critical habitats as areas consistently part of the beluga whale core distribution across years. The only critical habitat identified within the summer distribution was in Clearwater Fiord, in or near the estuary freshwater plume and in a bay to the west of the plume. The identified critical habitats should be areas of consideration in the continued discussion on the protection and sustainability of the Cumberland Sound beluga whale population.

2.1 Introduction

Identifying critical habitat is an important step in protecting key areas and ensuring the well-being of a species (Hoyt, 2011). Protection of critical habitat is particularly important for species and populations at risk, such as several populations of Arctic cetaceans. Hoyt (2011) defines critical habitat for cetaceans as an area regularly used by the species of interest that is essential to their continued survival and well-being. Critical areas can be identified through the frequent display of particular behaviours such as foraging or the presence of important resources like prey (e.g. McGuire et al., 2020; Panigada et al., 2017). Critical habitats can also be identified as high-use areas and have been defined using satellite tag movements (Baird et al., 2012) or areas of high concentrations of the target species (Awbery et al., 2022).

Estuaries, water bodies where the riverine fresh water and ocean salt water meet, specifically those that create a large visible plume, have been identified as critical or important habitat for several beluga whale (*Delphinapterus leucas*) populations (e.g., Moore et al., 2000; Smith et al., 2017; Whalen et al., 2020). Many of these Arctic estuaries are warmer than the surrounding ocean water, which may provide thermoregulation and skin sloughing benefits to whales (St. Aubin et al., 1990; Pitman et al., 2020; Whalen et al., 2020). Beluga whales are believed to use large estuaries as calving or nursing grounds and for skin proliferation (St. Aubin et al., 1990) and have site fidelity for these summering areas (COSEWIC, 2020). Smith et al. (2017) hypothesized that beluga whales may remain closest to where fresh and salt water mix to regulate skin proliferation, by controlling exposure to each water type. Beluga distribution may vary annually on a smaller scale around the mouth of the river that feeds the estuary, based on factors such as flowrate (Smith et al., 2017).

Cumberland Sound beluga whales are one such population that seasonally returns to an estuary. From July through September, these whales congregate in fiords at the far end of Cumberland Sound and regularly return to Clearwater Fiord and its estuary (Figure 2.1) (Richard and

Orr, 1986; Kilabuk, 1998; Richard and Stewart, 2008; Richard, 2013; Watt et al., 2021a). Millut bay, which forms the estuary, has been identified as an important part of the beluga whale's summering ground, based on acoustic monitoring (Booy et al., 2021). Cumberland Sound beluga whales are one of the smallest beluga populations in the world, having been heavily commercially hunted in the past, and continued hunting of this endangered population is likely unsustainable (COSEWIC, 2020; Watt et al., 2021a, 2021b). The latest aerial survey in 2017 resulted in a population estimate of 1381 (95%CI: 1270-1502) whales (Watt et al., 2021a). With climate change, the changing environment can have a negative impact on beluga whales and using environmental covariates, Biddlecombe and Watt (2021) provided an adjusted population estimate of 1245 (95%CI: 564−2715) whales for 2018 (Biddlecombe and Watt, 2022). This population is currently listed by the Committee on the Status of Endangered Wildlife in Canada as Endangered and under the Species at Risk Act as Threatened (COSEWIC, 2021; Species at Risk Act, 2023). Continued monitoring of the abundance and distribution of this endangered population are essential for management decisions (COSEWIC, 2020, 2021; Watt et al., 2021a).

An emerging tool in marine mammal research and monitoring that is particularly beneficial in remote locations, such as the Arctic, is Very High Resolution (VHR) satellite imagery (Höschle et al., 2021). Unlike many other survey methods, satellites guarantee a non-invasive and non-disruptive method of surveying whales that does not alter their behaviour (Charry et al., 2021; Hammond et al., 2021). A very large area can be captured in one satellite image, which mitigates over or undercounting whales as a result of their fine-scale movements. While groups of whales can still be missed if coverage with the satellite is insufficient, this method does alleviate missing whales that may otherwise be hidden by coastlines and islands, which can occur in aerial surveys (Richard, 2013). The use of satellite imagery to monitor cetaceans is increasing and is most often used for large whale species such as humpbacks, blue whales, fin whales, and right whales (Cubaynes et al., 2019;

Hodul et al., 2023). However, VHR satellites such as Worldview-2 and -3, which provide images at 50 cm, 40 cm, and 30 cm resolution (a measure of pixel size), have been identified as a feasible method to study medium-sized cetaceans like beluga whales (Charry et al., 2021; Fretwell et al., 2023; Watt et al., 2023). Although satellite imagery has been used to estimate beluga whale abundance (Watt et al., 2023), to our knowledge, it has yet to be used to evaluate cetacean distribution or identify critical habitat.

The objectives of this study were to evaluate summer abundance and distribution of Cumberland Sound beluga whales and identify critical habitat in Clearwater, Kangilo, Kangerk, and Shark Fiords (Figure 2.1) using satellite imagery collected in 2020, 2021, and 2022. Our study is the first examination of the fine-scale distribution of these beluga whales within the northern fiords of Cumberland Sound. In this study, we considered critical habitat as areas that are consistently part of the core distribution for beluga whales each summer. We expected that the estuary within Clearwater Fiord would be identified as critical habitat, but explored whether other areas and smaller estuaries may offer critical habitat as well. As a result of the short temporal span of our study (three years) and the large confidence intervals of abundance estimates (Watt et al., 2021a), we did not expect to detect significant changes in abundance over time.

2.2 Methods

2.2.1 Satellite Images & Whale Detection

Satellite images of northern Cumberland Sound (N 66°25, W 67°20), specifically Clearwater, Kangilo, Kangerk, and Shark Fiords (Figure 2.1), were collected on September 4 and 7, 2021, and August 17, 2022 covering a maximum extent of 3124 km^2 (1281 km^2 of water). Additional images of Clearwater Fiord were collected August 21 and 30, 2020. The 2020 and 2021 images were taken with Maxar's WorldView-3 satellite at 30 cm resolution and the 2022 images were from a WorldView-2 satellite at 50 cm resolution. Collection time was within a few weeks each summer and varied based on tasking availability of the satellite company and cloud cover of the area. All images used were cloud free, except August 21, 2020 which had some scattered clouds. Whale detections were possible through the clouds. Pan-sharpening was not conducted, as not all dates had a matching multi-spectral image.

For each date, multiple images were taken in strips by the satellite within 30 seconds to cover the whole area. The 2021 images were covered in three strips approximately 60 km by 16 km. The 2022 image was covered by two 75 km by 40 km strips. Overlap in images was systematically removed to avoid duplication in counts For each date, we selected either the right or left side consistently across all strips. The version (all left or all right) selected was the one with the most overall whales detected. The single band panchromatic satellite images were read in ArcMap (Version 10.8.1) at 1:800 or 1:1000, using a 250 m by 250 m grid for a systematic search. During image analysis, readers could zoom in or out to look at features and contextual cues. Objects of interest were marked using a two-level confidence system of possible whale (50-80% confident) and confident whale (>80% confident). Identifications of whales as confident instead of possible observations were made based on known formations, shapes, and group patterns observed in highly confident whale identifications. For example, two whales will often swim next to each other forming a “v” shape or in pod formations (Figure 2.2). A confident classification over a possible classification was more likely if other confidently identified beluga whales were present nearby, as they are a contextual cue. Once an image was read by the primary reader, the second reader would examine the marked points indicating agreement or disagreement. Points of disagreement were then discussed by the readers until a consensus was reached on whether the point of interest should be considered a confident whale or not. Locations of confidently identified whales are displayed in Appendix A figures. Seals are also observable in satellite imagery, and harp seals (*Pagophilus groenlandicus*) in particular are found throughout Kangilo Fiord in large aggregations (personal observation), which could be misidentified as beluga whales. However, seals were distinguished from beluga whales by their smaller size and aggregations of seals were excluded. Narwhal (*Monodon monoceros*) may be occasional transients in the greater Cumberland Sound but are not a significant presence in the area (Dietz et al. 2008; Westdal et al. 2010).

The ability to detect whales in an image depends on water clarity, sea state, presence of surrounding sea ice, cloud cover, and light. Low light can reduce the amount of detail captured by the camera and excess light can cause reflection off the water's surface. To help eliminate false identifications, additional resources such as Google Maps and images from other days were sometimes used. For instance, if an object of interest was suspected of being a rock, additional images could be viewed to determine whether that object was present in the same location on another day.

2.2.2 Distribution

Utilization distribution maps for the beluga whales on each date are presented as 50% utilization distribution to represent the core area and 95% utilization distribution representing the overall distribution of the confidently identified beluga whales (MacLeod, 2013). Utilization distributions can be calculated using Kernel density estimates (KDE) and represent the probability of use throughout an area (Whorton, 1989, Keating and Cherry, 2009). In this case we are presenting a population's distribution based on the locations of observed individuals. KDEs are a proportional sum of kernel functions centered over each data point (Keating and Cherry, 2009). We calculated KDEs, with barriers, in ArcGIS Pro (Version 2.8.8). The barrier shape files include land, islands, and large rocks. ArcGIS uses an algorithm following Silverman's rule of thumb (Silverman, 1986) to calculate the fixed bandwidth. A cell size of 15, and a geodesic method was used for all KDEs. KDEs were calculated for the entire surveyed area of northern Cumberland Sound in 2021 and 2022. KDEs were also separately calculated for all dates in Clearwater Fiord only. As Clearwater Fiord is a smaller area, the scale of the KDE changes. This KDE provides a finer resolution of beluga whale distribution within the fiord, which allows identification of specific high-density areas. Finally, to examine how the lower resolution (50 cm) image in 2022 may impact whale detectability, the 2020 images were down-sampled from 30 cm to 50 cm resolution, using nearest neighbour, and re-read by the same observer months later to compare the number of confidently identified whales.

2.2.3 Abundance Estimates

Abundance estimates using confidently identified whales were calculated for Clearwater Fiord in all years. Abundance estimates (N_C) were calculated for all fiords (Kangerk, Kangilo, and Clearwater) in 2021 and 2022. Due to the difference in area across dates, only whales found in the area extent of the smallest image (September 7) were included in abundance estimates (Figure 2.1). Shark Fiord is outside this image extent. To produce an abundance estimate, we needed to correct surface counts from the satellite imagery for whales that were too deep to be seen (referred to as availability bias). Based on Stewart et al. (unpublished data), beluga whales are visible in satellite imagery up to 2 m deep in clear water, and only at the surface in turbid water. Each near-surface estimate ($N_{surface}$) was corrected for the instantaneous availability bias (C_a) calculated by Watt et al. (2021a) using average weighted time spent above a certain depth, obtained from 2006 satellite tag dive data in Clearwater Fiord (Orr et al., 2001; Richard and Stewart, 2008; Watt et al., 2021a). An availability bias correction factor of 2.06 (CV = 0.056), representing time above 2 m of depth, was applied to beluga counts outside the estuarine plume. Due to the opaqueness of the Millut Bay estuary plume (Figure 2.3), we assumed whales in this area were only seen at the surface in the satellite imagery. Therefore, an availability bias correction factor of 4.46 (CV= 0.117), representing time above 1 m of depth, was used to correct beluga counts within the plume. The corrected estimates (N_C) were calculated using:

$$\boldsymbol{N_C = C_a * N_{surface}}$$

Annual average estimates were calculated using a weighted average based on variance:

$$\boldsymbol{Weight\ of\ N_{C_i}\ in\ \bar{N}_C = \frac{\widehat{var}(N_{C_i})^{-1}}{\sum \widehat{var}(N_C)^{-1}}}$$

Confidence intervals (CI) for average estimates ($\bar{N}_C$) were calculated following Buckland et al (2001):

$$\boldsymbol{(CI_{lower}, CI_{upper}) = (\bar{N}_C / C, \bar{N}_C * C)}$$

where

$$\boldsymbol{C = \exp\left[1.96 * \sqrt{\log_e\left[1 + \frac{\widehat{var}(\bar{N}_C)}{\bar{N}_C^{\,2}}\right]}\right]}$$

Since the satellite images provide full-coverage of the region of analysis, and readers came to consensus on all detections, the coefficient of variation of the availability bias correction factor is the only variance in the abundance estimate.

2.3 Results

2.3.1 Distribution

Beluga whales were observed in Clearwater and Kangilo Fiords in all images that covered those respective areas. The distribution of beluga whales was most consistent within Clearwater Fiord, where whales were always identified within the estuary, in or near the freshwater plume. The freshwater plume shape and size differed from day to day, ranging from approximately 4 to 22 km^2 (Table 1; Figure 2.4). The core area of the beluga distribution within Clearwater Fiord overlapped with the estuary plume on all dates except August 30, 2020 (Figure 2.4). On both 2020 dates, part of the core distribution was east of the estuary, in Shilmilik Bay (Figure 2.4). The whales were more tightly aggregated on August 30, with a stronger presence in Shilmilik bay, compared to August 21, 2020 (Table 1). On some days, belugas were present towards the entrance of the fiord, but this area was never within the 50% utilization area. September 4, 2021 had the smallest 95% utilization distribution in Clearwater Fiord (1.67 km^2; Table 1). Three days later on September 7, the belugas were more spread out throughout the estuary, with a 95% utilization distribution of 14 km^2 (Table 1).

Only one beluga was identified within Shark Fiord in 2022 (Figure A7) and this area was not part of the 95% utilization distribution. While whales were present in Kangilo Fiord in all images collected, the distribution changed greatly from day to day and across years, with no consistent core areas (Figure 2.5). The majority of the whales observed within Kangilo Fiord were in small groups and widely dispersed. Kangerk Fiord, found near the entrance to Clearwater Fiord, was part of the 50% utilization distribution of whales on September 4, 2021 but not on September 7, 2021 (Figure 2.5).

The two 2020 images down-sampled to a 50 cm resolution had 15% and 45% fewer belugas confidently observed than in the original 30 cm image. However, utilization distributions remained relatively similar, only with smaller core areas in the down-samples images. In the down sampled images, the August 21 50% utilization distribution was 3.4 km^2 compared to the original 3.6 km^2 and August 30 was 1.8 km^2 compared to the 2.1 km^2 in the 30 cm resolution.

2.3.2 Abundance Estimates

The highest number of beluga whales observed near-surface in Clearwater Fiord was 202 (unadjusted count) on August 30, 2020 (Table 2). The average corrected abundance estimate for Clearwater Fiord was 393 (CV = 0.036; 95%CI: 366-422) beluga whales in 2020, 197 (CV = 0.047;

95%CI: 180-216) in 2021, and 194 (CV = 0.06; 95%CI: 172-218) in 2022. For all fiords combined, the average corrected estimate was 462 (CV = 0.042; 95%CI: 425-502) beluga whales in 2021 and 252 (CV = 0.054; 95%CI: 226-280; Table 2) beluga whales in 2022 (Table 2).

2.4 Discussion

Beluga whales have been present in Clearwater Fiord and Kangilo Fiord for decades and continue to return during summer (Kilabuk, 1998; Watt et al., 2021a). While there is the recurring presence of whales within Kangilo and Kangerk Fiords, it does not appear to be centralized in a specific location. On the other hand, beluga whales were present in the central part of Clearwater Fiord on all surveyed dates and we identified this area as critical habitat. Within our study, whales were mainly observed in and around the Millut Bay estuary plume, with the core distribution extending into Shilmilik Bay in 2020. Beluga whales were not identified in Ranger River, which appeared quite shallow in the satellite images.

2.4.1 Clearwater Fiord

The preference for the two bays in Clearwater Fiord has been previously observed. Hydrophones in Shilmilik Bay recorded five times more calls than the entrance of the fiord, and Millut Bay had 19 times more calls (Booy et al., 2021). The 2014 and 2017 aerial surveys also found beluga whales primarily spread out across Shilmilik Bay and the estuary (Marcoux et al., 2016; Watt et al., 2021a). The August 2017 aerial surveys show movement to and from each area over eight days (four days of observation; Watt et al., 2021a). A similar pattern can be seen in the August 1999 aerial survey. From August 6 to 8, 1999, beluga whales were observed in highest abundance progressively from Shilmilik Bay to the estuary (Richard and Stewart, 2008). The similarity in distribution of beluga whales across time and survey methods further supports the importance of this area as critical habitat for this population, and also demonstrates that satellite imagery is a comparable tool to identify beluga whale distribution.

Whale distribution between the estuary and Shilmilik Bay can change within days. Average beluga speeds have been measured at 3.7 km/h and 3.98 km/h (Lydersen et al., 2001; Richard et al., 2001). At this speed, belugas can reasonably travel the 4 to 6 km between the two bays in under two hours and may make a return trip within the same day. What motivates these changes in distribution across the bays is currently unknown. The transition to Shilmilik Bay does not appear to be directly influenced by the size and shape of the estuary plume (Figure 2.4). However, it is possible that underwater currents from the river influx, not visible at the surface, flow into the downstream Shilmilik Bay, which may influence beluga whale distribution, if the river plume is important. Tide cycles can influence beluga whale movement, changing salinity and access to shallower areas(Ezer et al., 2008; Ezer et al., 2013; Smith et al., 2017), which could influence whale movement between the two bays. However, both 2020 and 2021 images, with beluga whales observed primarily in Shilmilik and the estuary, respectively, were collected at low tide (Table 1). The upper portion of Millut Bay is relatively shallow (Richard and Stewart, 2008); however, fine scale bathymetry is not available. Time of day also does not appear to influence beluga distribution within Clearwater Fiord. The 2014 surveys were primarily conducted in the morning and the beluga observations were spread out over the two bays (Marcoux et al., 2016). The 2017 surveys, which had a strong presence of beluga within Shilmilik Bay, were conducted in the afternoon. However, our images were all collected between noon and 1PM EDT and showed concentrated distribution in the estuary on both days in 2021 (Table 1). In addition, Booy et al. (2021) did not find any correlation between peak vocalization patterns and tide or time of day in either bay.

2.4.2 Estuaries

Estuaries, such as that in Clearwater Fiord, are an important part of summer distribution for many beluga whale populations, including Western Hudson Bay, Cook Inlet, and Eastern Beaufort Sea beluga whales (Goetz et al., 2012; Smith et al., 2017; Whalen et al., 2020). Many estuaries are considered calving grounds. Calves are undetectable at a 30 cm resolution in satellite images, and therefore calf presence throughout the fiords could not be assessed in this study. Calves may be observable in higher resolutions such as 10 or 15 cm, but the technology is not currently commercially available. Brodie (1971) observed young calves in Clearwater Fiord, and Inuit recognize this area as a primary calving ground (Kilabuk, 1998). Warm estuarine waters may help slough skin and aid in calf thermoregulation (Pitman et al., 2020; St. Aubin et al., 1990; Watts et al., 1991; Whalen et al., 2020). However, the Clearwater Fiord estuary is fed by Ranger River, which is said to be much colder than the surrounding salt water (Richard and Stewart, 2008) because the river input is actually glacial water from the Penny Ice Cap. Shilmilik Bay has a small river offshoot from Ranger River, rather than the main input into Millut Bay that creates the large estuary, and therefore may be warmer than the plume and may contribute to beluga movement between the bays.

Despite the apparent cooler waters of Clearwater Fiord compared to other estuaries, Inuit indicate whales enter Clearwater Fiord with a yellow coating, commonly present on beluga in spring (St. Aubin et al., 1990b), and use the waters to molt, returning to their white coloration (Kilabuk, 1998). In estuaries in the Eastern Beaufort Sea, belugas preferentially selected areas of rough substrates, which may be beneficial for exfoliation (Whalen et al., 2020). Those beluga whales in the estuary were also found to concentrate in shallow waters, which may be another preferred environmental characteristic potentially beneficial for calving (Fraker et al., 1979; Whalen et al., 2020). Most of Clearwater Fiord is relatively deep, with parts reaching 190 m in depth (Richard and Stewart, 2008); however, more detailed bathymetry mapping is needed to compare with beluga distribution. While warm water is described as preferential for beluga whales, such as in Scharffenberg et al. (2019), these authors were unable to separate the effects of salinity and water temperature on beluga distribution, since freshwater was the warmest. Direct investigation into the temperature differences among fresh, brackish, and salt water across estuaries would provide additional insight into the benefit of warm water. The presence of fresh water influx may be more or equally important for beluga, possibly for skin proliferation. The abundant nutrients provided by freshwater (Lamberti et al., 2010) and increased primary productivity can lead to an abundance of prey (Reichert et al., 2010; Antell and Saupe, 2021), potentially influencing the belugas' movement between the two bays. Changes in environmental variables such as temperature may also affect how tightly the whales are aggregated.

While there are other river inputs throughout the study area, the Millut Bay estuary is the only one with a substantial riverine plume. No beluga whales were observed near Isuituq River, at the western end of Clearwater Fiord, which does not have direct glacial input. No whales were observed within the small estuary at the most northern end of Kangilo Fiord. Only one group and three lone whales were observed on September 7, 2021, in the farthest part of the fiord, near but not in the small estuary (Figure A7). This estuary has narrow access with slight rapids, which may contribute to its lack of use by the whales, and the water input is from Kipisa Lake (Tallman and Marcoux, 2021), which also does not have direct glacial water input. Whales were observed in this area during the 2014 and 2017 aerial surveys in groups under 10 individuals (Watt et al., 2021a). While there is repeated presence of belugas in this area, it is not in large numbers nor part of the core areas in our KDE.

2.4.3 Abundance Estimates

Our Clearwater Fiord abundance estimates are lower than previous years of 603 beluga whales in 2014 and 1286 in 2017 (Watt et al., 2021a), and 2021 and 2022 have significantly lower abundance estimates than 2020. Unfortunately, we cannot evaluate whether this higher abundance in Clearwater Fiord in 2020 resulted in a lower abundance in the neighboring fiords as those areas were not imaged. While the abundance estimate for all fiords is much lower in 2022 compared to 2021, 2022 only represents a minimum abundance estimate, since whale detectability in 50 cm resolution images is lower than in 30 cm images. Based on the down-sampled images at 50 cm resolution, the lower counts in 2022 can, in part, be attributed to lower image resolution. The difference in the percentage of missed whales between the two down-sampled images may be due to cloud cover and areas of low light. The down-sampled image with 45% of confidently identified whales missed had dark shadows cast by the thicker clouds, in addition to a thin cloud cover over most of the image, potentially making it more difficult to detect lower resolution beluga whales. Beluga whales are typically under one meter in width, meaning a 50 cm resolution image only has one or two pixels in width per beluga, compared to three or four in a 30 cm image. The two pixel width was observed in both the original 50 cm image from 2022 and the down-sampled images from 2020. The reduction in resolution affects the shape of the beluga in the image and can impact a reader's ability to detect or confirm it as a whale. A 30 cm or finer resolution is preferred for detection and monitoring of beluga whales using satellite imagery.

While our abundance estimates do not include the whole population of Cumberland Sound, as whales can be observed throughout the western strata (the south-west half of Cumberland Sound) in summer (Watt et al., 2021a), they do provide a new estimate for the northern fiords and Clearwater Fiord in particular. Our estimate is likely conservative, as whales in the estuary plume can only be seen at the surface and likely not 1 m deep, and the dive data resolution is insufficient to evaluate time at surface. Continued exploration of the comparison between aerial and satellite surveys is important for establishing accurate abundance estimates. Despite these limitations, the satellite imagery abundance estimates from Clearwater Fiord may suggest a decline in beluga whales since the most recent aerial survey estimates in 2014 and 2017, which estimated 603 and 1286 whales in the fiord respectively (Watt et al., 2021a).

2.4.4 Conclusion

While Cumberland Sound beluga whales have a continued presence in both Kangilo and Clearwater Fiord, no specific area within Kangilo, Kangerk, or Shark Fiords could be considered critical habitat that is used consistently by the beluga whales. Richard and Stewart (2008) also saw the primary congregation of beluga in Clearwater Fiord. Within Clearwater Fiord, we identified the estuary and its large plume as critical habitat. While beluga whales were not present in Shilmilik Bay across the years of our study, we suggest this bay may act as critical habitat based on the repeated presence of beluga whales within the bay in 2020 and past aerial surveys. The comparison of beluga distribution across time in Shilmilik Bay using previous surveys to illuminate trends shows the importance of long-term studies when researching animals. Long-term data are important for identifying patterns that may not be evident in short temporal spans. Our results also demonstrate that VHR satellite imagery is an effective tool for monitoring beluga whale abundance and distribution. It is clear that Clearwater Fiord bays and its estuary are consistently used by beluga whales across time. However, the specific use of these areas is still unknown. One study has used VHR imagery to infer beluga behaviour by evaluating the positioning and orientation of beluga whales (Fretwell et al., 2023). In our study, based on basic visual examination of the beluga whales, there was no consistent directionality or positioning of whales, which may suggest that multiple behaviours are occurring in this critical habitat. Further investigation on the drivers of the movement between these two bays could provide insight into estuary and habitat use. Finally, continued minimal boat traffic and noise

(Booy et al., 2021) and locally enforced laws that ban hunting in the fiord (Kilabuk, 1998) particularly in these critical habitats, may prove beneficial to the belugas' wellbeing and survival.

2.5 References

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2.6 Figures

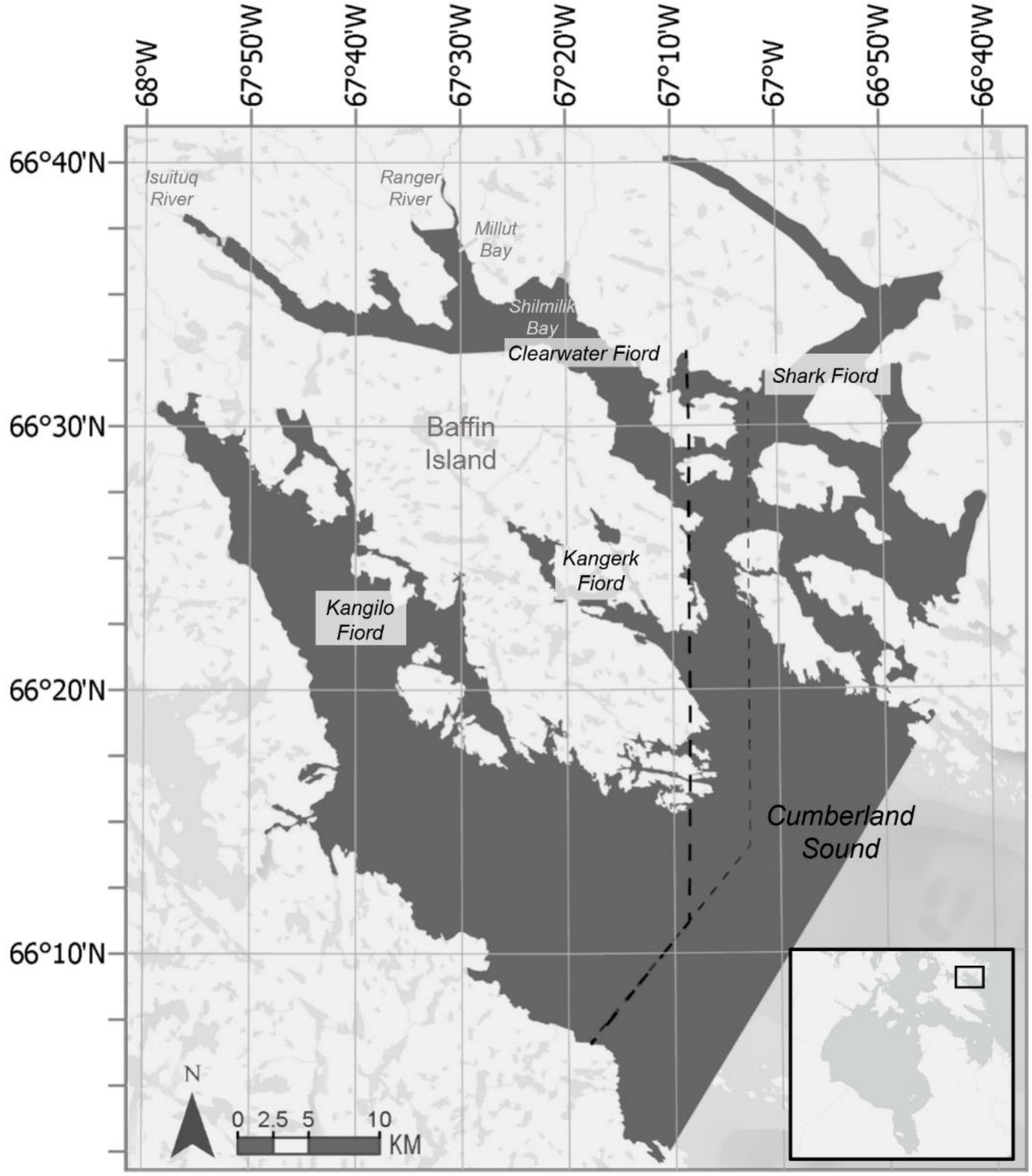

Figure 2.1. Map of northern Cumberland Sound, Baffin Island, Nunavut. Four fiords, Kangilo, Kangerk, Shark, and Clearwater, are indicated. Rivers and bays are labelled in grey. The dark grey shaded area depicts the maximum extent of the satellite images captured in 2022, with 1281 km^2 of water covered. The thick dashed line depicts the border of the area used in abundance estimates based on the extent of September 7, 2021 images. The thin dashed line is the image extent for September 4, 2021.

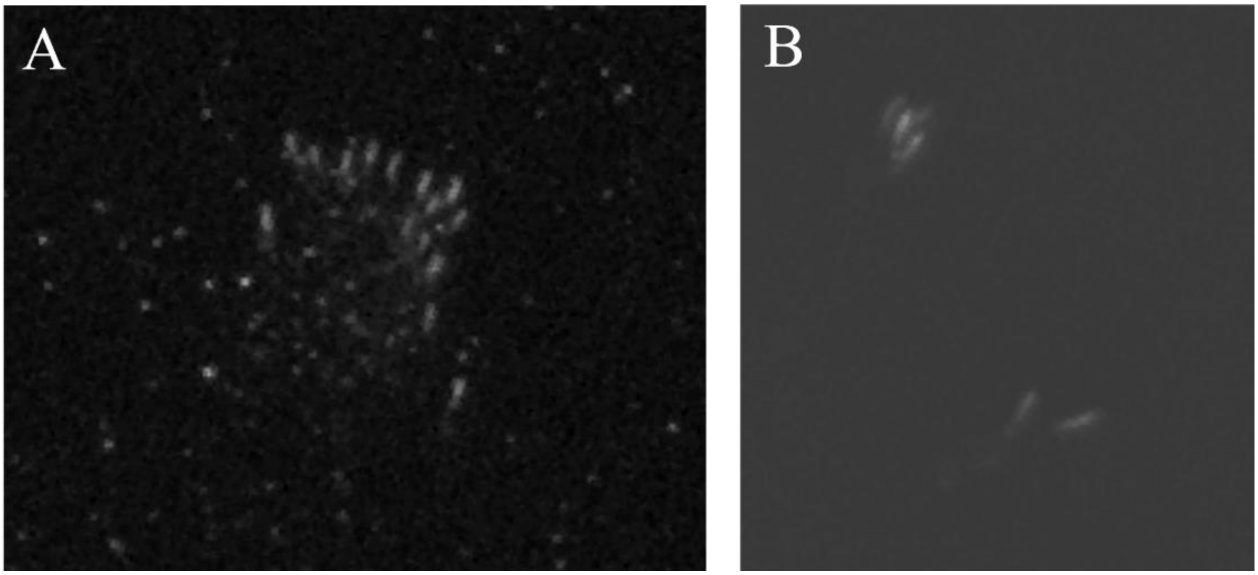

Figure 2.2. Examples of pod formations in panchromatic 30 cm WorldView-3 satellite images of beluga whales taken on Sept 7, 2021 in Kangilo Fiord, Nunavut (A) and Aug 30, 2020 in Clearwater Fiord, Nunavut (B) (republished under an end user license agreement with Maxar Technologies, original copyright 2021 and 2020, respectively).

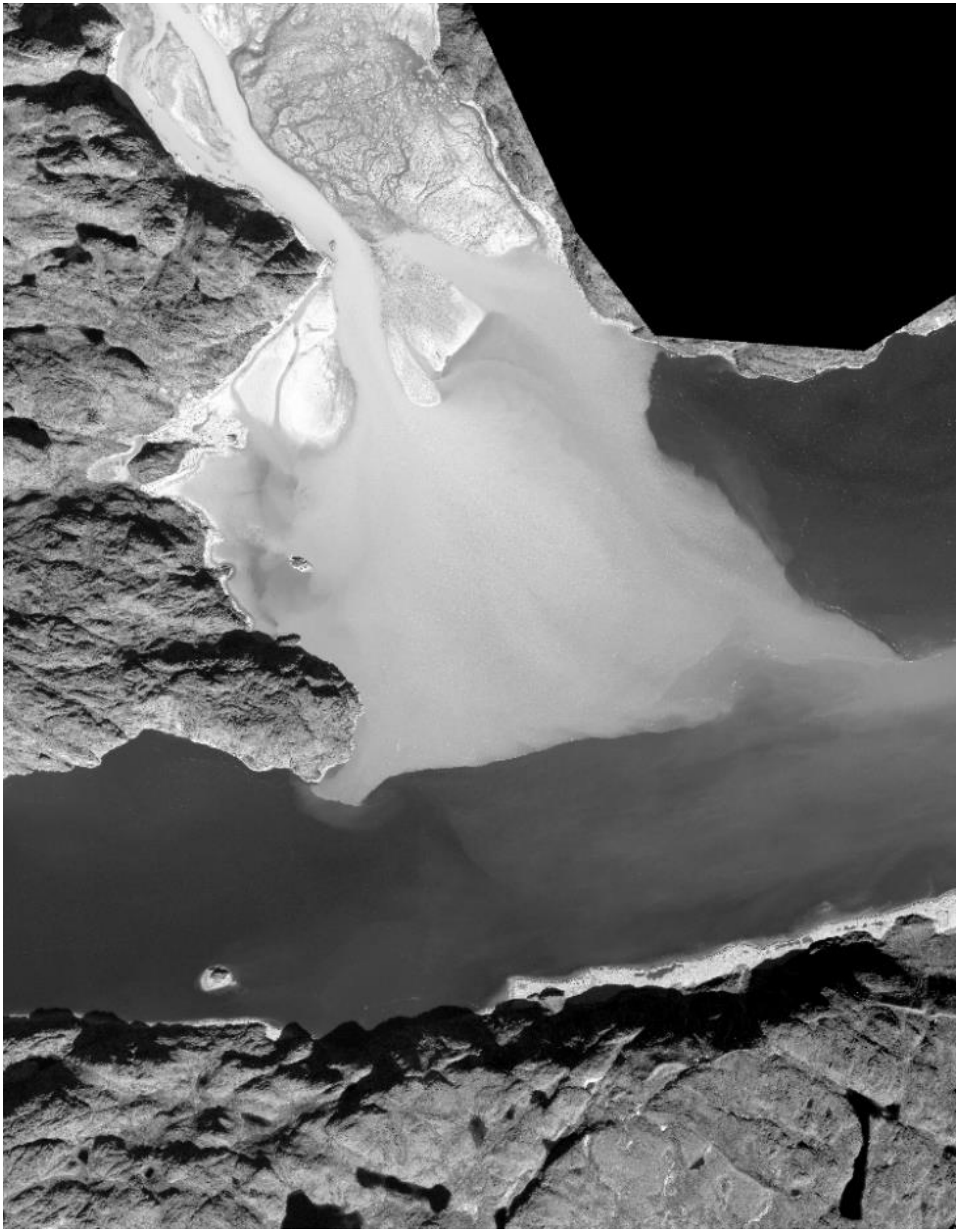

Figure 2.3. Clearwater Fiord estuary plume (light grey), Sept 7, 2021 (republished under an end user license agreement with Maxar Technologies, original copyright 2021).

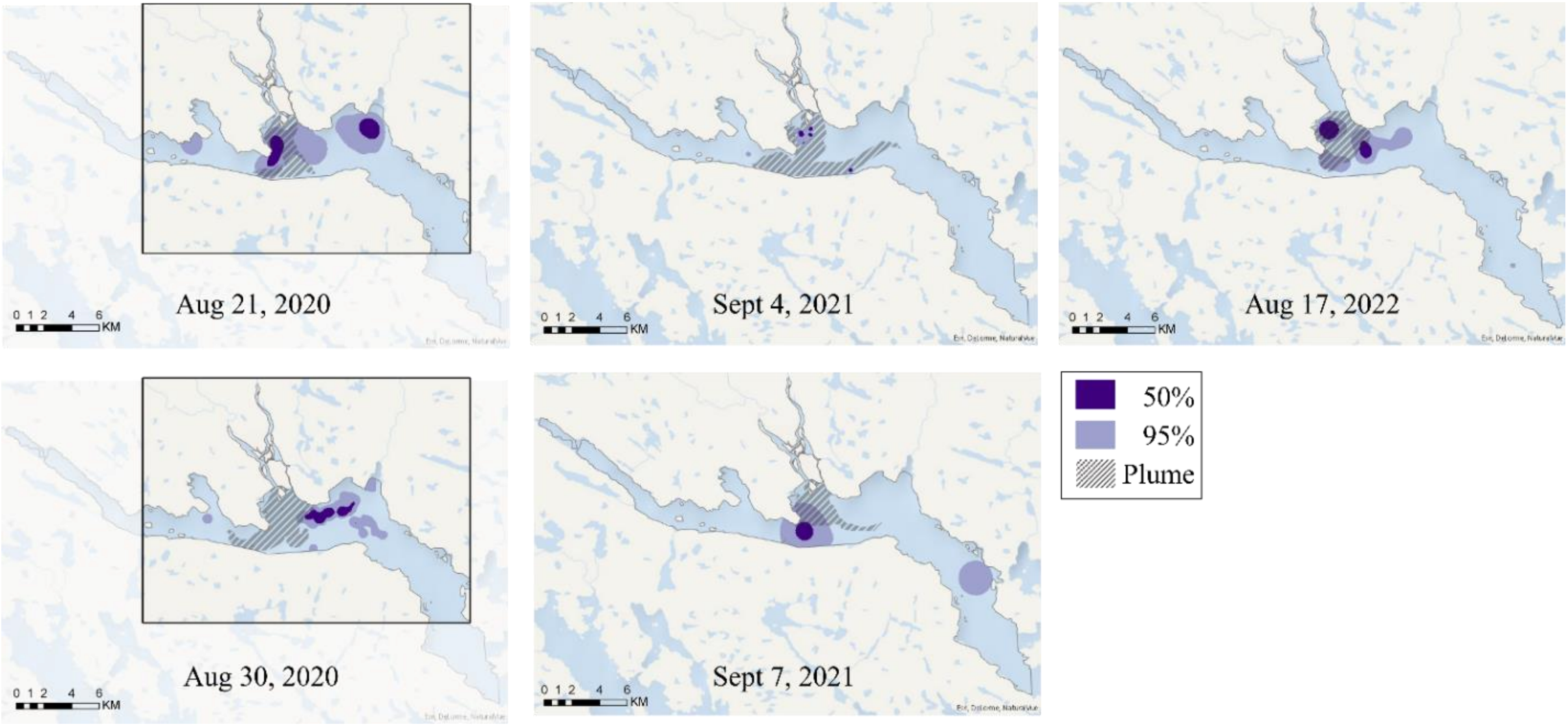

Figure 2.4. Kernel density estimates, based on satellite images, showing the 50% (dark purple) and 95% (light purple) utilization distribution of beluga whales in Clearwater Fiord. The extent of the 2020 images are bordered. The 2022 image was captured at high tide where the small islands at the mouth of Ranger River are covered by water.

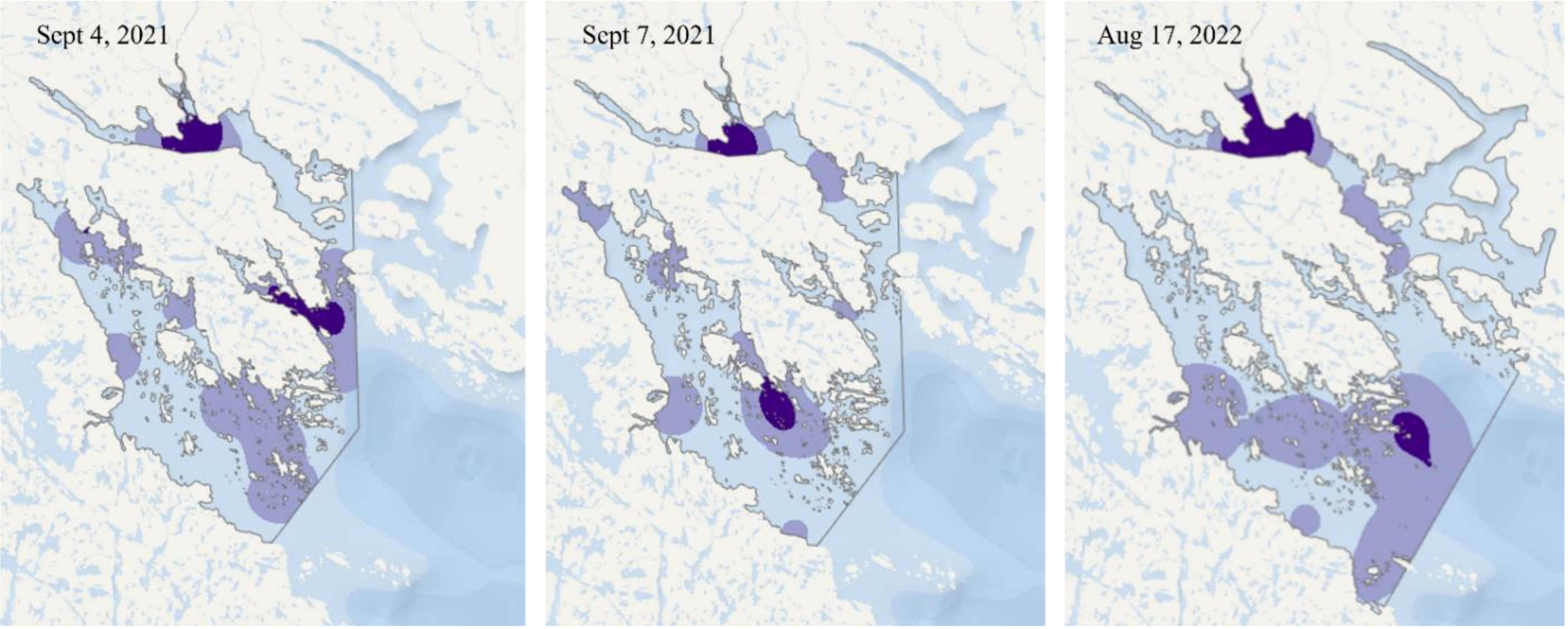

Figure 2.5. Kernel density estimates of beluga whales, based on satellite images, showing the 50% (dark purple) and 95% (light purple) utilization distribution throughout the northern fiords of Cumberland Sound (Clearwater, Kangilo, Kangerk, and Shark Fiords). Note that 2022 has ~500 km^2 larger extent, as shown by the outline.

2.7 Tables

Table 2.1. Approximate extent (km^2) of the estuary plumes and Kernel density estimate utilization distributions (UD) for each date imaged.

Date	Plume Area	95% UD of Clearwater Fiord	50% UD of Clearwater Fiord	95% UD of all fiords	50% UD of all fiords
Aug 21, 2020	11	23	3.6	-	-
Aug 30, 2020	14	10	2.1	-	-
Sept 4, 2021	22	1.7	0.3	318	46
Sept 7, 2021	4	14	1.4	208	36
Aug 17, 2022	11	13	2.4	510	41

Note: 2022 covers approximately $500km^2$ greater area, and, in this case, includes Shark fiord for ‘all fiords’.

Table 2.2. Estimates of beluga whale abundance in Clearwater Fiord, and a ‘total’ estimate when combined with Kangilo and Kangerk Fiords. Image collection time is presented in local time zone (EDT). Tide height in Clearwater Fiord is estimated by the Government of Canada (station 04040, https://tides.gc.ca). Tide is split into seven categories (low ebb, low peak, low flood, mid, high flood, high peak, high ebb). N_S is the observed count at the surface and N_C is the corrected estimate after applying a correction factor for availability bias (C_a = 2.06 for fiords and C_a = 4.46 for the estuary plume; Watt et al., 2021a). The bold values are the abundance estimates with their coefficient of variation and confidence interval.

				Clearwater Fiord							Kangilo & Kangerk Fiords		Total (all fiords)			
				Fiord		Estuary Plume		Total								
Date	Time	Tide Level	Tide Height	N_S	N_C	N_S	N_C	N_C	CV	CI	N_S	N_C	N_S	N_C	CV	CI
Aug 21, 2020	12:00	Low - Ebb	1.64m	154	317	9	40	357			-	-	-	-		
Aug 30, 2020	12:45	Low - Flood	2.71m	189	389	13	58	447			-	-	-	-		
2020 Estimate								**393**	**0.036**	**366-422**						
Sept 4, 2021	12:00	Low - Peak	1.99m	4	8	52	232	240			94*	194	150*	434		
Sept 7, 2021	12:27	Low - Peak	0.35m	82	169	5	22	191			142	293	229	484		
2021 Estimate								**197**	**0.047**	**180-216**				**462**	**0.042**	**425-502**
Aug 17, 2022	12:21	High - Ebb	4.24m	53	109	19	85	194			28*	58	100*	252		
2022 Estimate**								**194**	**0.060**	**172-218**				**252**	**0.054**	**226-280**

*27 observations of whales on a single image from Sept 4, 2021 and 49 observations from Aug 17, 2022 that extended beyond the scope of the surveyed area for abundance were excluded.

** 2022 represents a minimum abundance estimate due to its lower resolution of 50 cm and consequently lower whale detectability

Chapter 3

3 Seasonal and annual variation in fat content indicate no consistent primary feeding season in Hudson Bay beluga whales

Abstract

Feeding in migratory species is often highly seasonal. During the primary feeding season, marine mammals often accumulate fat, mainly in the form of blubber. Hudson Bay beluga whales (*Delphinapterus leucas*) exhibit a distinct migratory behaviour, wintering in the dynamic waters of Hudson Strait to avoid entrapment in sea ice and spend summers in Hudson Bay estuaries, which may provide favorable conditions for skin sloughing, nursing, and feeding. Despite the ecological significance of feeding, the timing of the primary feeding season for Hudson Bay beluga whales is currently debatable. We aimed to determine the primary feeding season, which is important for identifying primary feeding areas and the role feeding plays in beluga whale migration. Beluga whale blubber samples were collected from whales hunted along their migration route, during Inuit subsistence hunts from 2015 to 2021 (excl. 2018). We analyzed the blubber for lipid content and adipocyte size of three blubber sections (outer, middle, and inner). Sampling occurred in spring (June) and fall (October), representing feeding behaviour in winter and summer, respectively. The season with the highest fat content differed across years, with higher lipid content in spring samples in some years. While adipocyte size did not differ seasonally, minima were observed in 2017 and 2019. Additionally, fat stores differed significantly across blubber sections, with the highest lipid content and largest adipocytes in the middle section, followed by outer and inner sections, respectively. The observed seasonal variation indicates there is no consistent season in which Hudson Bay beluga whales predominantly accumulate fat. Consequently, access to food may not be the primary driver of their migration to and from the wintering or summering grounds. The use of both winter and summering areas to build and maintain fat stores highlights the flexibility in feeding behaviour and possibly indicates ecological resilience.

3.1 Introduction

Seasonal migration can evolve when the movement to a different location provides seasonal benefits that increase fitness, such as increased access to food or mates (Avgar et al., 2014). The area with consistent seasonally abundant food allows intense feeding where individuals build energy stores for the seasons with reduced prey availability. Many migratory cetaceans are known to have a primary feeding season and area where they build up fat/energy stores in the form of blubber (Corkeron & Connor, 1999; Iverson, 2009; Oftedal, 1997; Pitman et al., 2020).

Beluga whale (*Delphinapterus leucas*) populations vary in their feeding habits and migration patterns where summering areas are consistent within a population (Colbeck et al., 2013; Hauser et al., 2014; Kelley et al., 2010; Turgeon et al., 2012). During summer, most beluga populations aggregate in estuaries (e.g., Moore et al., 2000; Smith et al., 2017), which have many possible uses such as nursing grounds, annual skin molting, and protection from predators (Corkeron & Connor, 1999; Pitman et al., 2020; Richard & Stewart, 2008; Smith et al., 2017; St. Aubin et al., 1990). With multiple possible benefits, the primary reason for migration to these estuaries is not well understood (Smith et al., 2017). Another possible use of estuaries is foraging (Smith et al., 2017). Cook Inlet, Alaska beluga whales depend on abundant prey in the estuary during summer, with blubber thickness increasing over the summer and maximum accumulation by the fall (Goetz et al., 2012; Huntington, 2000).

Hudson Bay beluga whales have been observed feeding in summer estuaries, rivers, and along the coast (Breton-Honeyman et al., 2016; Watts & Draper, 1986). Despite these summer foraging observations, Indigenous knowledge from Nunavik (Eastern Hudson Bay) communities indicates that Hudson Bay beluga whales have thicker blubber in spring and therefore likely feed and accumulate fat in the wintering area (Breton-Honeyman et al., 2016). Hudson Bay beluga whales winter in Hudson Strait, an area of moving sea ice that provides access to open water leads, thereby decreasing the risk of ice entrapment in sea ice (Smith et al., 2017). In addition, based on an approximately 14-month gestation, the whales are believed to mate in winter and early spring (Brodie, 1971). For the whales to undertake the energetic cost of migration, the benefit of both winter and summer areas must be substantial. The estimated cost of transport for beluga whales is 1.4±0.1 J kg^{-1} m^{-1} which is higher than expected for their size (John et al., 2024). The relative importance of estuaries as feeding grounds and the primary feeding season have yet to be identified for Hudson Bay beluga whales.

Beluga whales are generalist feeders and feed on a wide variety of prey such as Arctic and polar cod (*Boreogadus saida* and *Arctogadus glacialis*), capelin (*Mallotus villosus*), sculpin (*Myoxocephalus scorpius*), Greenland halibut (*Reinhardtius hippoglossoides*), and shrimps (e.g., *Pandalus borealis*), with variation across populations (Breton-Honeyman et al., 2016; Dahl et al., 2000; Kelley et al., 2010; Loseto et al., 2009; Marcoux et al., 2012; Yurkowski et al., 2017). While many prey are available, beluga whales prefer energy-dense prey and often consume one primary prey species more than others based on the relative prey abundance (Choy et al., 2020, Loseto et al., 2009; Watt et al., 2016). For Hudson Bay beluga whales, Kelley et al. (2010) reported a switch from Arctic cod to capelin in their diet. Capelin abundance has increased in the Arctic, with their distribution shifting north as temperatures increased, and sea ice has reduced (Rose, 2005).

As whales eat, the fatty acids of their prey assimilate into the blubber as triglycerides (Krahn et al., 2004). The lipids are stored within fat cells known as adipocytes, which expand as fat accumulates and increases blubber thickness. Beluga blubber is a stratified tissue where the inner section is the most metabolically active and representative of, but not identical to, the beluga's diet from the past few weeks or months based on estimated blubber turnover rate (Guerrero & Rogers, 2017; Choy et al., 2019; Koopman, 2007; Iverson et al., 2004; Kirsch et al., 2000). The middle section is believed to act as part of the energy store and often contains the most lipids (Gómez-Campos et al., 2015; Strandberg, 2008; Struntz et al., 2004). For beluga whales, Krahn et al. (2004)

reported higher lipid content in the middle blubber section, but their study had a very small sample size (n=3). The outer section is believed to be more stable and primarily function as insulation (Koopman, 2007), but changes in lipid concentrations can occur over time (Bernier-Graveline et al., 2021). Adipocyte sizes also vary across the blubber depth of marine mammals and are typically largest in the middle section (Koopman et al., 2002; Struntz et al., 2004), but histological analysis of blubber sections has not been conducted for beluga whales, to our knowledge. Lipid content, adipocytes, and blubber thickness can be used as indices of fat accumulation (Koopman et al., 2002; Struntz et al., 2004) and compared seasonally to identify the primary feeding season.

The main objective of this study was to determine the primary feeding season of Hudson Bay beluga whales using blubber seasonally sampled from Inuit subsistence hunts in Sanikiluaq, NU. The primary feeding season is defined as the season in which the beluga whales gain the majority of their fat consistently every year. Our study tests the hypothesis that Hudson Bay beluga whales build fat reserves in the summer and, therefore, use estuaries and coastal waters for the majority of their feeding. Within our study, full blubber depth of individual samples was seldom available, so lipid content and average adipocyte size were chosen as chemical and physical measurements of fat content. Lipid droplet size can also be used to evaluate fat content, but it is highly correlated with adipocyte size (Ferguson et al., 2020), and therefore, was not considered. If summer is the primary feeding season, adipocytes will be larger and percent lipid content will be higher in the samples collected in fall every year. To evaluate and account for differences across blubber sections, we subsampled from the inner, middle, and outer sections of beluga whale blubber.

3.2 Methods

3.2.1 Sample Selection

Fifty-three Hudson Bay beluga whale blubber samples were collected from Indigenous subsistence hunts in Sanikiluaq on the Belcher Islands (Table B1) in spring and fall (before and after summering in the estuaries) in the years of 2015-2021 (excluding 2018). Spring samples were collected primarily in June (May 26 to July 1) and fall samples were collected primarily in October (September 8 to November 16; Figure B1). All available fall samples were used (n=24, 20 males, 4 females) and spring samples were selected to best match fall sample size for each year and sex (n=29, 19 males, 10 females). Sex was determined genetically at the Freshwater Institute (Fisheries and Oceans Canada; FWI) with DNA extracted from skin tissue (Qiagen DNeasy Kit) and analyzed following PCR procedures described in Rosel (2003).

To ensure no claves were included in the data set, we used body size to estimate age. Thirty-three males in the study had been previously aged using teeth (analyzed by Matson's Laboratory). The size of the beluga whales used in this study (measured by Inuit hunters from rostrum to fluke notch), at a particular age (Figure B2) are similar to the Hudson Bay beluga whale growth curve created by Luque & Ferguson (2010). Their growth curve can be used to estimate age classes for the whales in our study that did not have ages available. Individuals below 200 cm in length were not included in the dataset.

3.2.2 Subsampling for lipid extraction

The longest section of the best quality blubber (light pink, not red or freezer burnt) was chosen to subsample, as reduced sample quality can impact measurements of the index of interest (Trana et al. 2014). Choosing the longest part allowed the best approximation to the actual inner section of the blubber from each individual. Blubber was measured from skin to muscle (or the end of the blubber) with a ruler to the nearest 0.1 cm. Next, the blubber was separated into inner, middle, and outer sections, with outer closest to the skin, inner furthest from the skin, and middle midway between the two (Figure 3.1). An approximately 0.5 g cube was subsampled for each section where each subsample was no more than 1 cm long (oriented skin to muscle). The blubber had to be approximately 3 cm to obtain three separate sections, with the smallest three section sample being 2.8 cm in length. Smaller samples were cut into one (outer) or two (outer and middle) sections accordingly.

3.2.3 Histology – Adipocyte Size

Only samples with sufficient blubber for three sections were chosen for this analysis (n=43; spring: 15 males, 6 females; fall: 17 males, 5 females; Table B2). The sections were sampled in the same locations as those for lipid extraction. Samples were 0.4 x 1.5 x 2 cm in size (Figure 3.1). The samples were placed in falcon tubes with 50 ml of 10% neutral buffered formalin, placed on a shaker, and fixed at room temperature for a minimum of 48 hours. After being fixed in formalin, the samples were transferred to 50 ml of 70% ethanol for shipping. The samples were sent to The Centre for Phenogenomics (Toronto, ON) for histological analysis. The samples were processed, embedded, cut, stained, scanned, and analyzed by the lab. Samples were embedded using the maximum surface area, cut to their standard 4 µm, stained using an H&E stain, and scanned with a Hamamatsu Nanozoomer at 20x magnification. Images were analyzed using an algorithm following Ferguson et al., (2022). The analysis area was either 1794913 or 1796336 µm^2 covering an average of 345 cells (± 114 SD; range: 168-641). The algorithm measured the area of each cell within the analysis area, from which an average was calculated for each sample (Figure B3). For slides where large cells were not properly separated by the program, we manually remeasured the incorrectly outlined cells using ImageJ (version 1.53e).

3.2.4 Lipid Content & Fatty Acids

A variation of the Folch et al. (1957) fatty acid protocol was implemented to extract lipids and fatty acid methyl esters (FAME) from the blubber (here-after, the use of blubber ‘sample’ refers to the subsample rather than the entire sample provided by the hunters). Blubber samples were placed in a 2:1 chloroform/methanol mixture with 0.01% butylated hydroxytoluene (BHT) for a minimum of 24 hours. The blubber was compressed to extract the lipids, and remaining tissue was removed. Next 2.6 ml of NaCl was added to the blubber and vortexed. The lower phase containing the chloroform lipids was extracted and placed in anhydrous sodium sulfate to remove excess water. The remaining solvent was evaporated using a nitrogen stream in a silver ball bath at 25-30°C. The final weight of the lipid was recorded and used to calculate percent lipid content of the initial wet sample.

Transesterification was conducted to obtain FAME for fatty acid analysis. We added 1.5 ml of methylene chloride with 0.01% BHT and 3.0 ml of a Hilditch solution (100 ml dry methanol : 1.5 ml sulfuric acid) to the lipid sample and then heated it for one hour at 100°C. Hexane and water were added to the solution and the top section was subsequently extracted and placed in sodium sulfate. The final solvent was evaporated under nitrogen in the silver ball bath. Hexane was added to the FAME and diluted to approximately 0.2 mg/ml. FAME samples were analyzed using an Agilent Technologies 7890A GC system. Supelco (37 component FAME mix) and Nucheck (54 component mix GLC-463) standards were used for fatty acid analysis, and FAME were identified via retention time and known standard mixtures by the Biotracers lab at FWI. Fifteen samples were duplicated to calculate precision and the average standard deviation across the 80 fatty acids was 0.018%.

3.2.5 Statistical analyses

For lipid percentage and average adipocyte size, we used AICc model selection (bbmle; Bolker 2022) to compare a set of candidate mixed-effect models describing their relationship to season (spring, fall), year (as a continuous variable), blubber section (inner, middle, outer), and sex (male, female), with beluga ID as a random effect. The interaction of year and season was included based on trends observed in data exploration. No multicollinearity was observed between the response variables, with all adjusted generalized variance-inflation factors under 1.18. Lipid content candidate models had a beta distribution with a logit link, since it is percentage data (glmmTMB; Brooks et al., 2017). Model diagnostics were verified using the DHARMa package (Hartig, 2022), in addition to normality of residuals. Candidate models for average adipocyte size were fit with a Gaussian distribution and an identity link, since it is normally distributed continuous data. The final model was visually evaluated for homogeneity of variance, normality of residuals, and normality of random effects. Normality of random effects was also statistically evaluated using Shapiro's test. 'Tests in Linear Mixed Effects Models' (lmerTest; Kuznetsova et al., 2017) was used to statistically evaluate the average adipocyte size model. Post-hoc tests of the models to evaluate differences between blubber sections were conducted using the emmeans package (Lenth, 2024), with a Bonferroni correction. Differences between years were evaluated using ANOVA with Bonferroni corrected pairwise comparisons for adipocyte size, and Kruskal-Wallace and Bonferroni corrected Dunn's post-hoc tests for lipid content. Lipid percent was logit transformed prior to the test and separated by season.

To determine whether changes in fat content were related to changes in prey consumption, we evaluated differences in beluga whale dietary fatty acids across seasons and years. Only the inner section of blubber (n=45) was used because it best represents diet. Specifically, 31 dietary fatty acids identified by Iverson et al., (2004) (Table B3) were plotted using Principal Component Analysis (PCA). Prior to analysis, the appropriate transformations were conducted on the fatty acid data, as suggested by Budge et al (2006). First, all values of zero were assigned an arbitrarily small number (0.00001). Then, each value was divided by the value of the reference fatty acid C18:0. This fatty acid was used because it is not indicative of diet and is typically well measured (Budge et al., 2006). All values were then log transformed to obtain the final log-ratio values.

The first three principal components of the PCA, which explained 86% of the variability of the data, were used as the response variables in a type II, factorial Multivariate Analysis of Variance (MANOVA). The MANOVA was used to evaluate differences in beluga dietary fatty acids across seasons (2 level factor variable: spring and fall) and years (6 level factor variable: 2015-2021, excl 2018). The third principal component only explained 8% of the variability but was included, because the first principal component primarily separates six individuals from the main group. Pillai's trace was used to evaluate significance because it is the most robust (Olson, 1974). The MANOVA assumptions of normality (qqplot), multicollinearity of the dependent variables (correlation test), homogeneity of variance (Levene's test), and homogeneity of variance-covariance matrices (Box's M test; $\alpha = 0.001$) were sufficiently met. Two outliers based on Mahalanobis distance were removed. ANOVAs were conducted for the significant variables.

3.3 Results

Fat accumulation differed seasonally and annually in Hudson Bay beluga whales. The candidate model that best explained blubber lipid content (Table 3.1 & 3.2) was:

$$\begin{aligned} & Percent\ Lipid\ Content \\ & \quad = exp(1.74 + 0.09 \times Season_{Spring} + 0.36 \times Section_{Middle} \\ & \quad + 0.2 \times Section_{Outer} - 2.67 \times Year + 1.26 \times Year^{2} \\ & \quad + 3.92\,(Year \times Season_{Spring}) - 0.96\,\left(Year \times Season_{Spring}\right) + BelugaID) \end{aligned}$$

(eqn 1)

The difference in lipid content of beluga whales between seasons changed over time ($\chi^2 = 22.1$, $p < 0.001$; Figure 3.2). The trend indicated an increase of lipid content in spring (June) and a decrease in fall (October), with variation between years. Kruskal-Wallace and post-hoc comparisons of years, for each season indicated annual differences in spring ($\chi^2 = 18.77$, $p = 0.002$) and fall ($\chi^2 = 20.04$, $p = 0.001$). Spring 2016 was significantly lower than spring 2019 ($p = 0.022$) and 2021 ($p = 0.004$). Fall 2019 was significantly lower than fall 2015 ($p = 0.001$) and 2016 ($p = 0.041$). Within the model, the blubber sections (inner, middle, outer) differed significantly ($\chi^2 = 26.9$, $p < 0.001$), with the middle section being higher than outer ($p = 0.040$) and inner ($p < 0.001$), and the outer section higher than inner ($p = 0.032$; Figure 3.3); there was no interaction with season.

The top model for average adipocyte size included year as a polynomial, blubber section, season, an interaction term between section and season, and beluga ID as a random effect. Post-hoc comparisons of the season and section interaction term only indicated significant differences between middle and inner adipocyte sizes in fall ($p = 0.005$). A simpler model had a ΔAIC of 0.5, where adipocyte size did not differ by season but was affected by year (Table 3.3 & 3.4; $R^2_c = 0.39$):

$$\begin{aligned} Average\ Adipocyte\ Size &= 4380 + 786 \times Section_{Middle} + 368 \times Section_{Outer} - 5631 \times Year \\ &\quad + 7814 \times Year^{2} + Beluga\ ID \end{aligned} \tag{eqn 2}$$

Adipocytes were largest in the first year of our study period, 2015 (mean ± SD: 5640 ± 1651 μm^2), and smallest in 2017 and 2019 (3316 ± 802 μm^2 and 3493 ± 1229 μm^2; Figure 3.4). The variation in adipocyte size over time was best explained using a quadratic polynomial ($\chi^2 = 36.7$, $p < 0.001$) with no seasonal effect. Adipocyte sizes were similar in 2015, 2016, and 2021, with 2017 being significantly lower than 2015 ($F_{5,123} = 9.18$, $p = 0.006$) and 2019 significantly lower than all three years ($p < 0.001$, $p = 0.007$, $p = 0.007$). Similar to lipid content, blubber sections differed significantly ($\chi^2 = 7.68$, $p = 0.021$). The middle section had the largest adipocytes and significantly differed from the inner section ($p = 0.022$). The outer section adipocytes were intermediate in size but did not significantly differ from the middle ($p = 0.447$) or inner sections ($p = 0.610$; Figure 3.3).

The MANOVA showed significant differences of season ($F_{3,36}$ = 4.27, p = 0.011) but not year ($F_{15,114}$ = 1.62, p = 0.079), with a significant difference between seasons on PC3 ($F_{1,43}$ = 6.26, p = 0.016) for beluga dietary fatty acids in the inner section (Figure 3.5). PC1 was driven by C22:4n6 (Adrenic acid) and this fatty acid was only present within the five individuals.

3.4 Discussion

3.4.1 Primary Feeding Season

The accumulation of fat in Hudson Bay beluga whales varied across seasons and years. Overall, our results indicated that there was no primary feeding season where the beluga whales were consistently accumulating more fat. The variation in seasonal lipid content across years suggests the beluga whales use both summer and winter for feeding but that the winter was more important in building fat reserves in some years. The fatty acid composition differed by season on the third principal component of the PCA and therefore differences in prey might contribute to differences in fat content between seasons, with the winter having some higher fat content prey than the summer. However, the difference between seasons was not the primary differentiation of fatty acid composition of the beluga whales. There were also no annual differences in fatty acid composition indicating annual fluctuations in fat content were likely not due to a change in the fatty acid content of prey. Beluga whales appear flexible in their consumption of food and use both seasons to feed.

The use of summer and winter for feeding suggests that access to food is not a primary driver of beluga whale migration to and from the estuaries. However, at least in some years, substantial feeding takes place in or around estuaries during summer. Along Eastern Hudson Bay, beluga whales are known to feed in the rivers and bays (Breton-Honeyman et al., 2016). The whales have been recorded feeding off the coast of Churchill where they would often dive to the sea bed, typically less than 50 m deep (Martin et al., 2001). More detailed studies about the feeding frequency of beluga whales in the rivers are required to identify the whales' primary feeding locations within the summering areas. Our study suggests feeding is important in summer habitat, in addition to the other benefits estuaries provide such as skin proliferation and calf thermoregulation (Corkeron & Connor, 1999; Pitman et al., 2020; St. Aubin et al., 1990).

If substantial prey are available along the Hudson Bay coasts, migration to the Hudson Strait for winter may be primarily driven by the advancement of ice into Hudson Bay. Chukchi Sea (North Pacific) beluga whales left their summering areas later in the year in response to late winter sea ice (Hauser et al., 2017). In addition, the movement into the wintering area of Bering Strait for the Chukchi and Eastern Beaufort Sea beluga whales was positively correlated with freeze-up timing (Hauser et al., 2017). Hudson Bay beluga whales may display similar adaptations to changes in sea ice, particularly if food is abundant in the summering areas. Eastern Hudson Bay beluga whale migration timing was associated with sea surface temperatures, where warmer temperatures led to delayed migration out of the estuaries (Bailleul et al., 2012).

Feeding in wintering areas is supported by Inuit Knowledge. Hunters estimated that the beluga whales harvested along the eastern Hudson Bay migration route lose 5 cm of blubber over the course of the summer, with a median of 11 cm of blubber during the spring migration (Breton-Honeyman et al., 2016). Hunters also reported that the hunted beluga whales floated in the spring due to the increased blubber thickness but sank in the fall (Breton-Honeyman et al., 2016).

In addition to feeding occurring in both summer and winter, these beluga whales may also feed during their migration, but probably to a lesser degree, as the majority of their time would be consumed by travelling (Storrie et al., 2023). Storrie et al. (2023) described Eastern Beaufort Sea beluga whales as having a nomadic migration strategy. The belugas exhibited many foraging dives during their migration and may follow food while maintaining the relative direction toward their next seasonal area. Hudson Bay beluga whales may eat along their migration as well. Beluga whales leaving Churchill, MB exhibited foraging-type dives in deeper water at the start of their migration (Martin et al., 2001).

The last two years of our study period had small sample sizes with three of fewer individuals per season, each year. Therefore, the trends in 2020 and 2021 may be driven by this limited sample size, and spring lipid content may in fact be more stable over time.

3.4.2 Annual Trends

Blubber adipocyte size did not show any seasonal differences but did have significant annual trends. The reduction of fat in certain years is likely not due to foraging on prey with different fatty acid composition, as the fatty acid profiles of beluga whales did not differ across years. Instead, variations in beluga fat content may be due to environmental factors and cycles. For instance, the Arctic Oscillation Index (AO) represents differences in surface pressure between the Arctic and mid-latitudes, reflecting a strength in the polar vortex. The AO is often correlated with anomalous surface air temperature and river discharge (Thompson & Wallace 1998). These environmental fluctuations can affect primary production, in turn affecting consumers and higher trophic levels.

One of the years with the smallest adipocyte sizes and beginning of the increased separation between spring and fall lipid content was 2017. There may have been changes at the end of 2016 or early 2017 that caused changes in prey availability. In summer 2017, Hudson Bay saw a reduction in summer sea ice and increased river runoff (Environment and Climate Change Canada data; https://wateroffice.ec.gc.ca/search/historical_e.html). This year could be considered a wet year such as 2005 (Smith et al., 2017), seeing a peak of over 6000 m^3/s of water in the Nelson River (Stations 05UF006 and 05UF007) and over 2500 m^3/s in the Churchill River (Station 06FD001). Interestingly, very similar environmental patterns occurred in 2020 but we did not identify decreases in beluga fat content that year. The primary difference in 2020 was the anomalously high AO, which was associated with lower temperatures in the Eastern Canadian Arctic/Subarctic (National Weather

Service, 2023; Lawrence et al., 2020). The high AO may have changed the impacts on the environment and did not result in lower prey abundance in 2020.

Sea ice may also affect availability of the primary and supplemental prey. A reduction in sea ice would cause a reduction in ice-associated algae that feeds amphipods. In turn, ice associated species such as Arctic cod would be negatively affected (Hop & Gjøsæter, 2013). In contrast, capelin is an open water pelagic species, associated with phytoplankton blooms. A reduction of ice allows increased sunlight to enter the water column which promotes the growth of phytoplankton. The phytoplankton is eaten by zooplankton such as copepods and krill, both consumed by capelin (Hop & Gjøsæter, 2013). Capelin stocks can be quite variable from year to year (Carscadden et al., 2001; Hop and Gjøsæter, 2013). This pelagic fish is particularly sensitive to changes in temperature and can have significant changes in distribution and spawning time at lower temperatures (Carscadden et al., 2001). Furthermore, a mismatch between capelin spawning and phytoplankton blooms affected by early sea ice melt and changes in sea surface temperature can affect the survival of capelin (Mullowney et al., 2016). Capelin presence in ringed seal stomachs was higher in years with later ice break-up (Young & Ferguson, 2014) and similar trends may be present for beluga whales. The interactions between these environmental processes and their effects on the ocean food web are complex and require an in-depth investigation into their influence on primary production and the link to beluga whale fat accumulation.

3.4.3 Fat Measures

Interestingly, there was disparity between the lipid content and adipocyte size. We identified annual trends in adipocyte size but no differences in season, whereas lipid content showed variations between seasons across years. Both adipocyte size and lipid content have been linked to body condition (Castrillon & Bengtson Nash, 2020; Struntz et al., 2004), but the relationship between adipocyte size and lipid content is not consistent across studies. Adipocyte size predicted feeding season in humpback whales *(Megaptera novaeangliae*), but lipid content did not (Castrillion 2017). However, the humpback whale blubber samples were collected from the outer section using biopsy darts, which are not effective in representing lipid content (Castrillion 2017, Christiansen 2013, Ryan 2022). Our samples were collected from subsistence harvest rather than biopsies, which may reduce the chance of lipid loss (Krahn et al., 2004). Bowhead whale (*Balaena mysticetus*) adipocyte sizes were smaller in stranded whales compared to harvested whales, but the chemically extracted lipid content did not differ (Ferguson et al., 2020). Ferguson et al. (2020) suggested that lipid content may be more sensitive to variations in analysis or sample storage than adipocyte size. Blubber samples thaw very quickly, and lipids may leave the subsample prior to analysis. Chemical lipid extraction can also vary based on the procedure used (Castrillon & Bengtson Nash, 2020). Our beluga whale lipid content range (69-99%) extends higher than that of Krahn et al., (2004; 68-85%), and is similar to those measured in stranded St-Lawrence beluga whale carcasses (70-100%, with one individual at 48%) (Martineau et al., 1987).

Lipid content is associated with body condition in many dolphin species. Emaciated bottlenose dolphins (*Tursiops truncates*) had reduced lipid content and adipocyte size (Struntz et al., 2004). Lipid content was also associated with body condition in striped dolphins (*Stenella coeruleoalba*) (Gómez-Campos et al., 2011), but the lipid content varied much more than the lipid levels observed in beluga whales. The striped dolphins had a mean difference of over 30% lipid content between healthy and emaciated whales, whereas our entire lipid percent range only spans 30%. Even small margins of error may have a greater impact on our results due to the smaller range of possible lipid content.

Analysis of lipid content may be more useful for comparing large changes in fat content, such as the comparison between healthy and emaciated whales. Adipocyte size might have less procedural variation and might represent finer scale differences in blubber. However, there are many possible ways that the tissue samples could be affected prior to imaging. Artefacts, artificial alterations to the sample, may occur during procedures such as fixation or staining. Fixation can cause shrinkage of cells (Struntz 2004; Chatterjee, 2014) and inadequate fixation could lead to further shrinking if lipids leave the adipocytes when the blubber melts. In addition, the repeated freezing and thawing of samples during subsampling may cause loss of lipids which could alter cell shape and size. During preparation, handling of the samples with too much force can also cause damage to the tissue and cells (Chatterjee, 2014; Taqi et al., 2018). In good quality samples, storage time does not appear to impact blubber composition (Lind et al., 2012; Trana et al., 2015), but physical attributes such as cell size and shape may be affected. In addition, the use of frozen samples over fresh samples may results in ruptured cells from ice crystals (Roe et al., 2012).

An additional measure that is used to evaluate fat content is blubber thickness (Castrillon & Bengtson Nash, 2020). Unfortunately, we did not have full blubber samples to measure thickness. The relation of lipid content and adipocyte size to blubber thickness should be further explored to understand how lipid content, adipocyte size, and blubber depth are related. Further understanding could provide insight into the reliability of our measures and how the annual and seasonal fluctuations observed within our study affect the overall body condition and health of individuals. Finally, our results support the notion that the middle blubber section acts as a fat storage as it had the highest lipid content and adipocyte size.

3.4.4 Conclusion

Overall, lipid content and adipocyte size indicate there is no consistent primary feeding season where significantly more fat is accumulated. There are fluctuations across years in the amount of fat being accumulated, with 2017 and 2019 being lower than other years. There may have been a decline in prey consumption, particularly in summer, in those years. Since one season does not consistently contribute to the accumulation of fat more than the other, access to prey is likely not a primary driver of the annual beluga whale migration.

3.5 References

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3.6 Figures

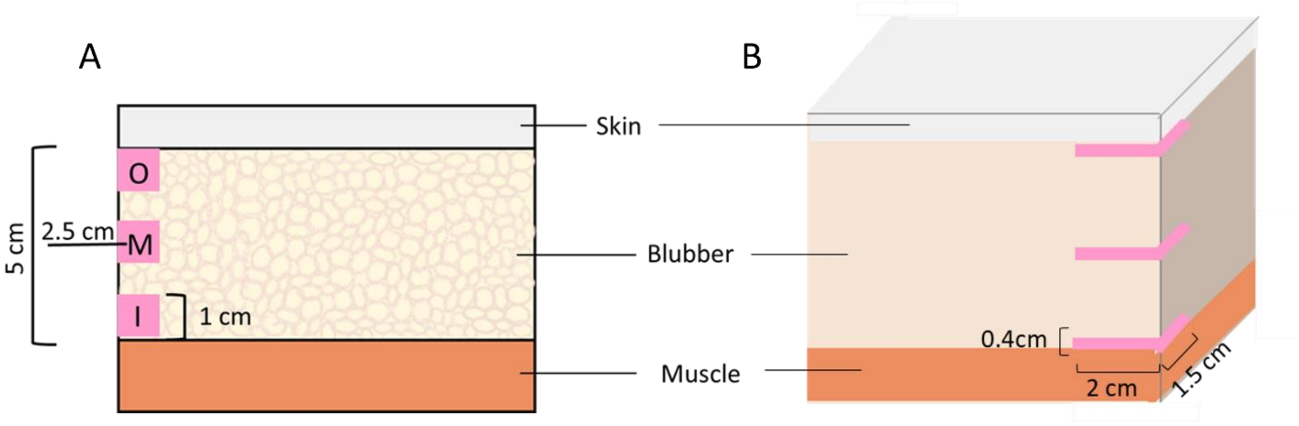

Figure 3.1. Example of sampling methods of blubber sections (O = outer, M= middle, I = Inner) for lipid content (A) and histological analysis (B). The middle section was cut around the center point of the blubber. Each lipid content subsample was an approximately 1 cm^3. The histology subsamples were approximately 0.4 x 2 x 1.5 cm.

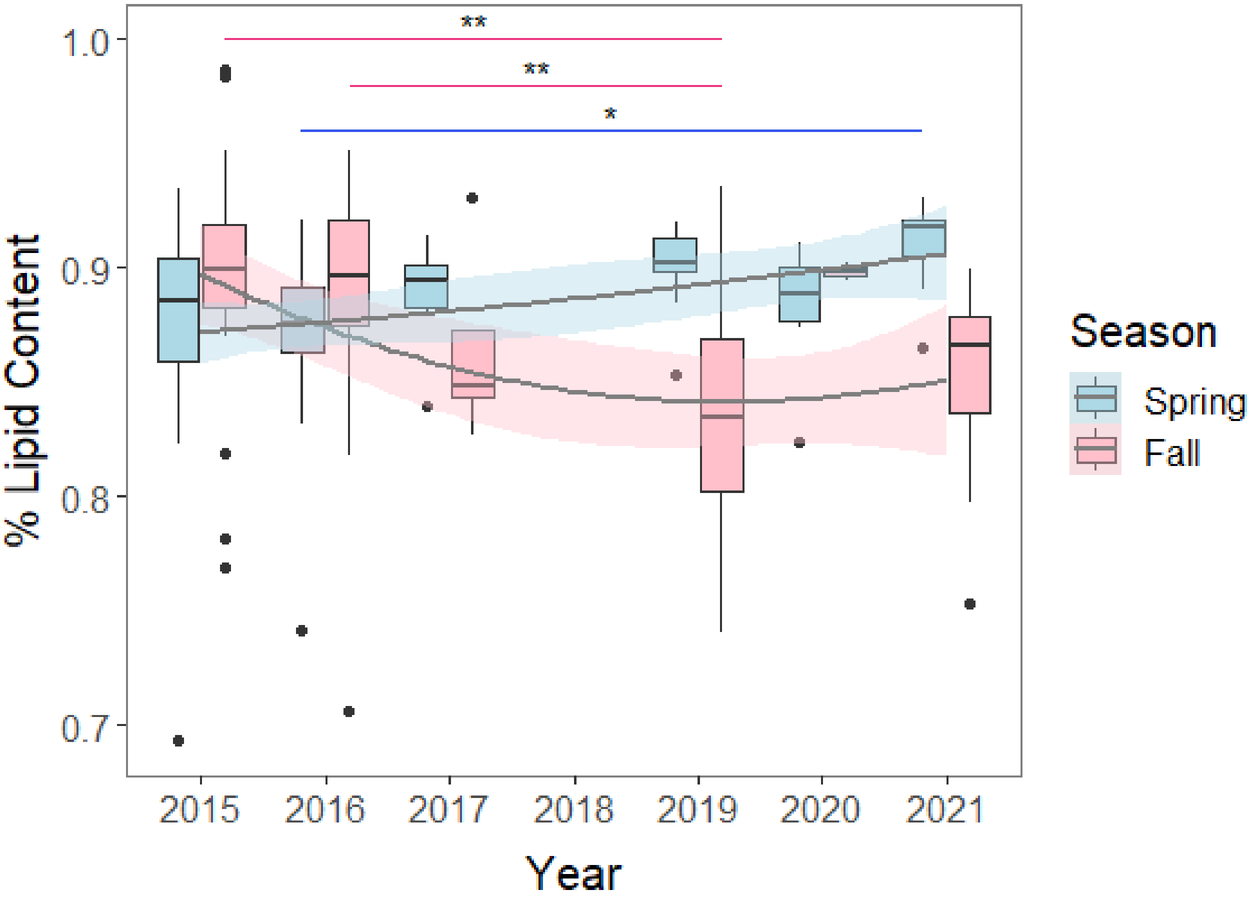

Figure 3.2. Annual and seasonal variation in percent lipid content in blubber, from Hudson Bay beluga whales sampled in spring (June) and fall (October). All blubber sections (inner, middle, outer) are included. Significance lines indicate significant differences between years for individual seasons (spring = blue, fall = pink; * $p < 0.05$, ** $p < 0.01$).

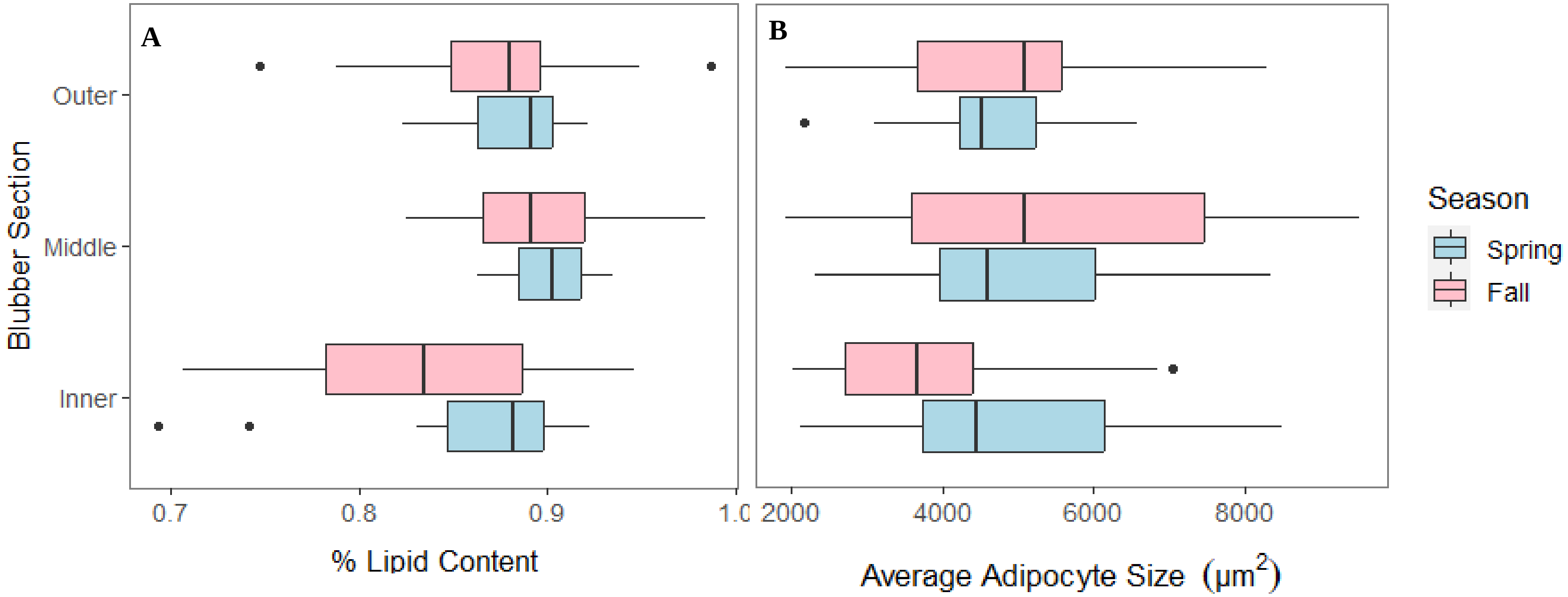

Figure 3.3. Comparison of A) percent lipid content and B) adipocyte size (µm^2) of blubber sections of Hudson Bay beluga whales across spring (June) and fall (October).

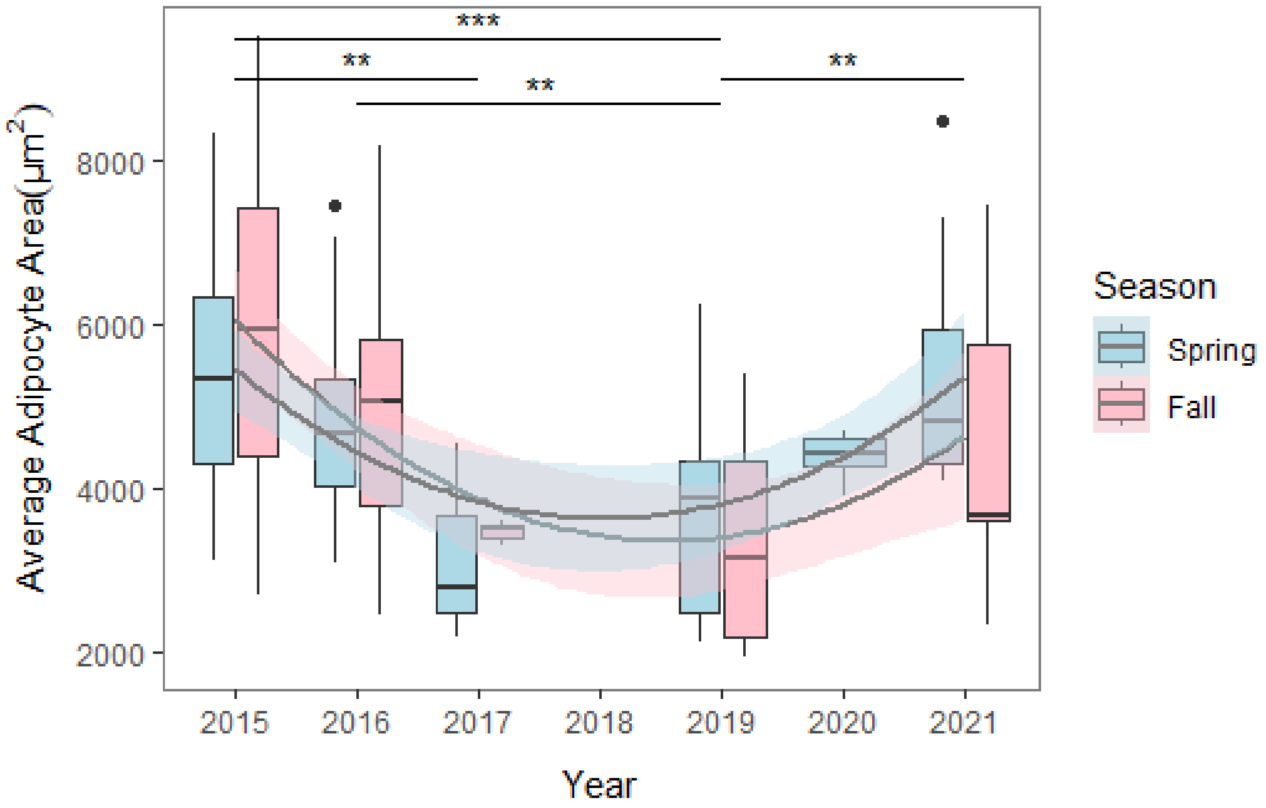

Figure 3.4. Annual variation in average adipocyte size (μm^2) in blubber, from Hudson Bay beluga whales sampled in spring (June) and fall (October). All blubber sections (inner, middle, outer) are included. Significance lines indicate significant differences between years with seasons combined (** $p < 0.01$, *** $p < 0.001$).

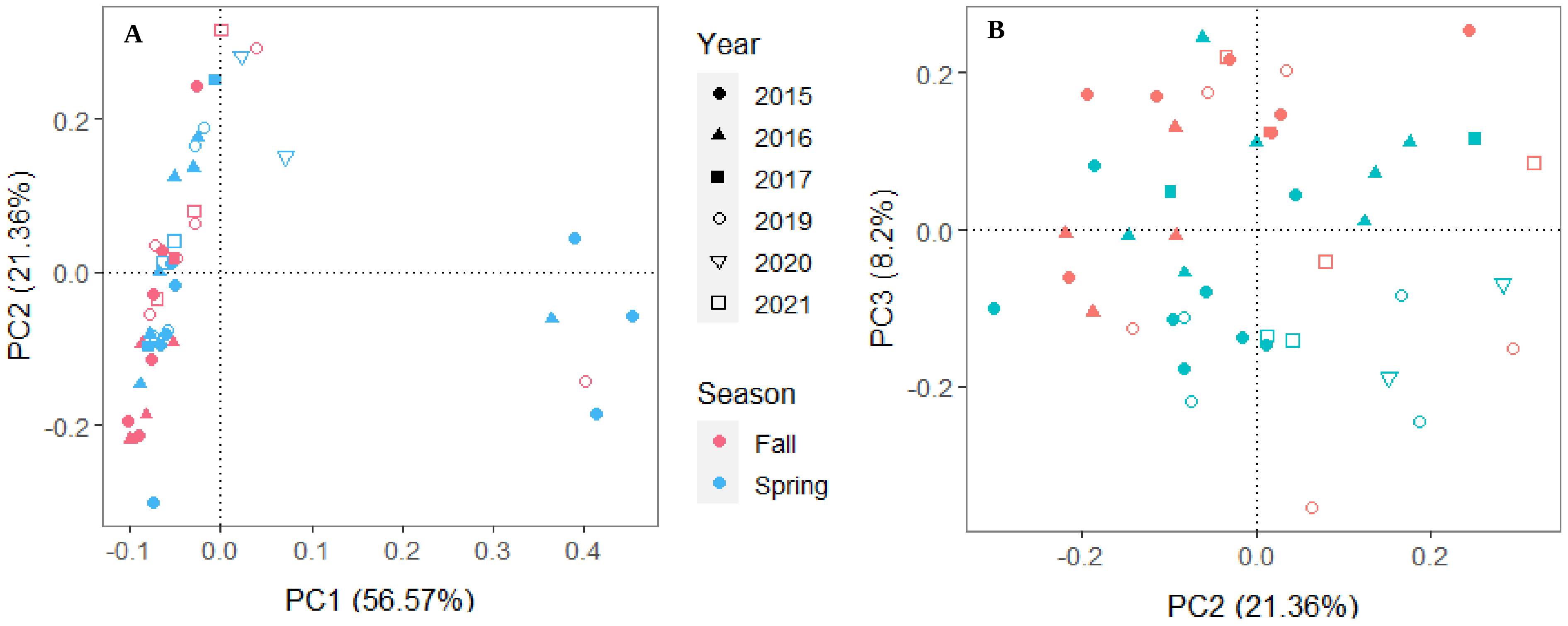

Figure 3.5. PCA of dietary fatty acids from the inner blubber section of Hudson Bay beluga whales in spring (June) and fall (October) from 2015-2021 (excl 2018). A) Principal components 1 and 2. B) Principal components 2 and 3.

3.7 Tables

Table 3.1. Model selection for percent lipid content. Section refers to blubber sections (inner, middle, outer), sex: male, female, season: spring (June), fall (October), year: 2015-2021 (excl 2018), beluga ID (random effect).

Model	Terms	df	ΔAICc
1	**Season + Section + Year^2+ Year^2*Season + ID**	10	0
2	Season + Sex + Section + Year^2 + Year^2*Season + ID	11	2.2
3	Season + Section + Year^2+ Year^2*Season + Season*Section + ID	12	2.3
4 (Linear)	Season + Section + Year+ Year*Season + ID	8	2.3
5	Season + Year^2+ Year^2*Season + ID	8	20.1
6	Section + Year^2+ ID	7	21.4
7	Season + Year^2+ Section + ID	8	21.2
8	Season + Section + ID	6	23.4
9	Season + Section + Year^2+ Sex + ID	9	22.1
10	Section + Sex + ID	6	24.3
11	Season + Section + Sex + ID	7	24.0
12 (Null Model)	ID	3	40.1
13	Season + Sex + ID	5	43.0

Table 3.2. Estimated polynomial regression parameters, standard errors (SE), z-values, and p-values f or the percent lipid content beta GLMM presented in eqn (1).

	Estimate	SE	Z-value	p-value
Intercept	1.736	0.060	28.747	< 0.001
$\text{Season}_{\text{Spring}}$	0.087	0.059	1.472	0.141
$\text{Section}_{\text{Middle}}$	0.357	0.073	5.167	<0.001
$\text{Section}_{\text{Outer}}$	0.199	0.070	2.837	0.005
Year	-2.665	0.531	-5.019	<0.001
Year^2	1.263	0.494	2.556	0.011
$\text{Year*Season}_{\text{Spring}}$	3.918	0.737	5.314	<0.001
$\text{Year}^2\text{*Season}_{\text{Spring}}$	-0.956	0.717	-1.333	0.183

Table 3.3. Model selection for adipocyte size (μm^2). Section refers to blubber sections (inner, middle, outer), sex: male, female, season: spring (June), fall (October), year: 2015-2021 (excl 2018), beluga ID (random effect).

Model	Terms	df	ΔAICc
1	**Section + Year^2+ ID**	7	0
2	Section + Year^2 + Season + Season*Section + ID	10	0.9
3	Season + Year^2+ Section + ID	8	2.2
4	Season + Section + Year^2+ Sex + ID	9	2.6
5	Season + Sex + Section + Year^2+ Year^2*Season + ID	11	2.8
6	Season + Section + Year^2+ Year^2*Season + ID	10	3.4
7	Season + Year^2+ Year^2*Season + ID	8	5.6
8 (Linear)	Section + Year+ ID	6	17.4
9	Section + Sex + ID	6	22.5
10	Season + Section + Sex + ID	7	24.5
11	Season + Section + ID	6	25.1
12 (Null Model)	ID	3	25.5
13	Season + Sex + ID	5	26.9

Table 3.4. Estimated polynomial regression parameters, standard errors (SE), z-values, and p-values for the adipocyte size gaussian GLMM presented in eqn (2).

	Estimate	SE	t-value	p-value
Intercept	4380	215	20.326	< 0.001
Year	-5631	1591	-3.541	< 0.001
Year^2	7814	1591	4.913	< 0.001
Section$_{\text{Middle}}$	786	284	2.769	0.007
Section$_{\text{Outer}}$	368	284	1.297	0.198

4 Conclusion

The objective of my thesis was to study seasonal estuary use for two Arctic beluga whale populations. I investigated the spatial use of estuaries for Cumberland Sound beluga whales. The identification critical habitat indicates areas in which minimal disturbance to the whales should occur to minimize impact on their wellbeing. For Hudson Bay beluga whales, I studied the ecological use of estuaries through the investigation of the primary feeding season, which provides foundational knowledge about the whales' ecology. My thesis provides further support on the importance of estuaries to beluga whales.

In Chapter 2, I identified the Clearwater Fiord estuary as a critical habitat and presented the importance of the neighboring bay 'Shilmilik Bay'. Based on the repeated core range of the beluga whales being within and around the freshwater plume, the estuary is important to Cumberland Sound beluga whales. These frequently used areas are ideal locations to test hypotheses about the conditions that create favorable environments for these beluga whales. Environmental criteria such as salinity, temperature, wind speed and direction, depth, and seabed morphology may influence beluga whale movements (Scharffenberg et al., 2019; Whalen et al., 2020). Further investigation of preferred specific environmental criteria could provide further insight into the use of the estuary. For instance, certain substrates may be more beneficial for skin molting (Whalen et al., 2020). Furthermore, Mackenzie River estuary beluga whales moved away from cold saline water influxes (Scharffenberg et al., 2019). Clearwater Fiord provides a unique opportunity to investigate the separate influences of salinity and temperature, if the freshwater feeding the estuary is colder than the fiord.

This is the first use of satellite imagery to study the distribution of cetaceans. I show that 30 cm resolution is preferred over 50 cm resolution when studying beluga whales, particularly for abundance estimation. However, the lower resolution has minimal impact on evaluating the distribution and core areas of the whales. Increased image resolution could allow accurate evaluation of morphometrics of the beluga whales such as body size and condition. Furthermore, it is currently very difficult to detect beluga whales in winter when there is significant ice. Higher resolution images would allow monitoring of beluga whales in all seasons, using this technology. In addition, obtaining multi-spectral imagery could increase the ability to detect beluga whales through pan-sharpening (Charry et al., 2021). I provided an updated abundance estimate for the Cumberland Sound beluga whale population, using satellite imagery. In comparison to the most recent aerial surveys (Watt et al., 2021) our estimate suggests a decline in the population. Ground truthing with drone and aerial surveys will increase our confidence of the abundance estimates from satellite imagery. In addition, the correction factors applied to the abundance counts were based on the ability to observe, within satellite imagery, a beluga cutout placed in lakes. This work is done as part of Jordan Stewart's thesis. Further work should be conducted on the availability bias by repeating this experiment in additional environments and conditions, to further explore how the depth at which the cutout is observable varies. Finally, as the use of satellite imagery for monitoring cetaceans increases, automated detection of belugas will improve efficiency of image analysis (Charry et al., 2021). Budget permitting, this could allow the increased frequency of, and area covered by satellite images.

In Chapter 3, I explored the seasonal use of estuaries based on foraging ecology. Being the most abundant beluga whale population, WHB beluga whales are a large research focus. However, there is still a lack of information on fundamental ecological questions, such as when they eat. My objective was to determine whether the beluga whales primarily eat in winter or in summer, in and around the estuaries. I concluded that there is no consistent primary feeding season and that the beluga whales are using both summer and winter seasons to accumulate fat. The accumulation of fat can vary across years and appears to have declined in the summer in some years. The reasons for fluctuations in fat content are unclear but are likely related to changes in prey abundance. Hudson Bay beluga whales have been observed feeding in estuaries in the surrounding bays and in their wintering grounds along Hudson Strait (Breton-Honeyman et al., 2016). My thesis supports that feeding is likely taking place in both locations. Continued monitoring of seasonal fat accumulation trends will inform on whether changes are occurring in food availability, and whether these are regular annual fluctuations.

Future research should investigate the environmental criteria that attract Cumberland Sound beluga whales to the Clearwater Fiord estuary and the neighboring bay, to further understand the use of estuaries. Clearwater Fiord provides an ideal location to test hypotheses on the benefits of warmer water typically provided by estuaries, because the Clearwater Fiord estuary is fed by glacial water and is believed to be colder than the surrounding salt water (Richard and Stewart, 2008). The prevalence of feeding behaviour within the estuary itself could also be explored for Cumberland Sound and Hudson Bay beluga whales. Beluga whales are important in the Arctic marine ecosystem and to local Inuit. This thesis addresses important seasonal ecological questions on beluga whales.

4.1 References

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Whalen D., Loseto L. L., Hornby C. A., Harwood L., Hansen-Craik K. (2020). Mapping and understanding the role of seabed morphology in relation to beluga whale (*Delphinapterus leucas*) hotspots and habitat use in the Mackenzie estuary, NT. *Estuaries Coasts* 43, 161–173. doi: 10.1007/s12237-019-00653-8

Appendix A

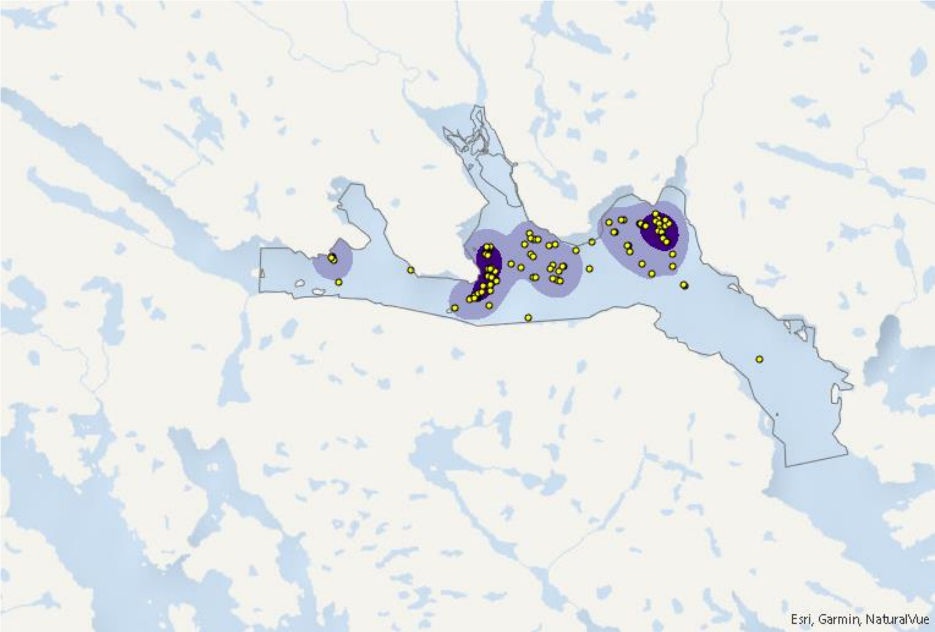

Figure A1. August 21, 2020 points overlayed on KDE utilization distribution maps in Clearwater Fiord.

Figure A2. August 30, 2020 points overlayed on KDE utilization distribution maps in Clearwater Fiord.

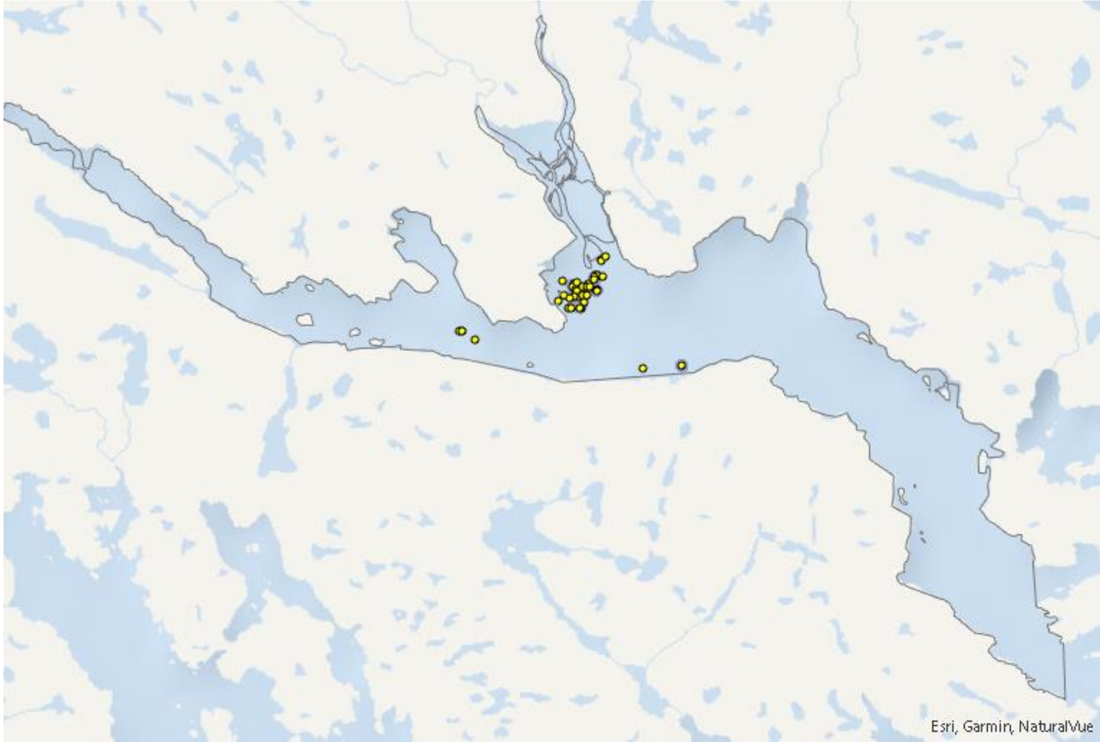

Figure A3. September 4, 2021 points overlayed on KDE utilization distribution maps in Clearwater Fiord.

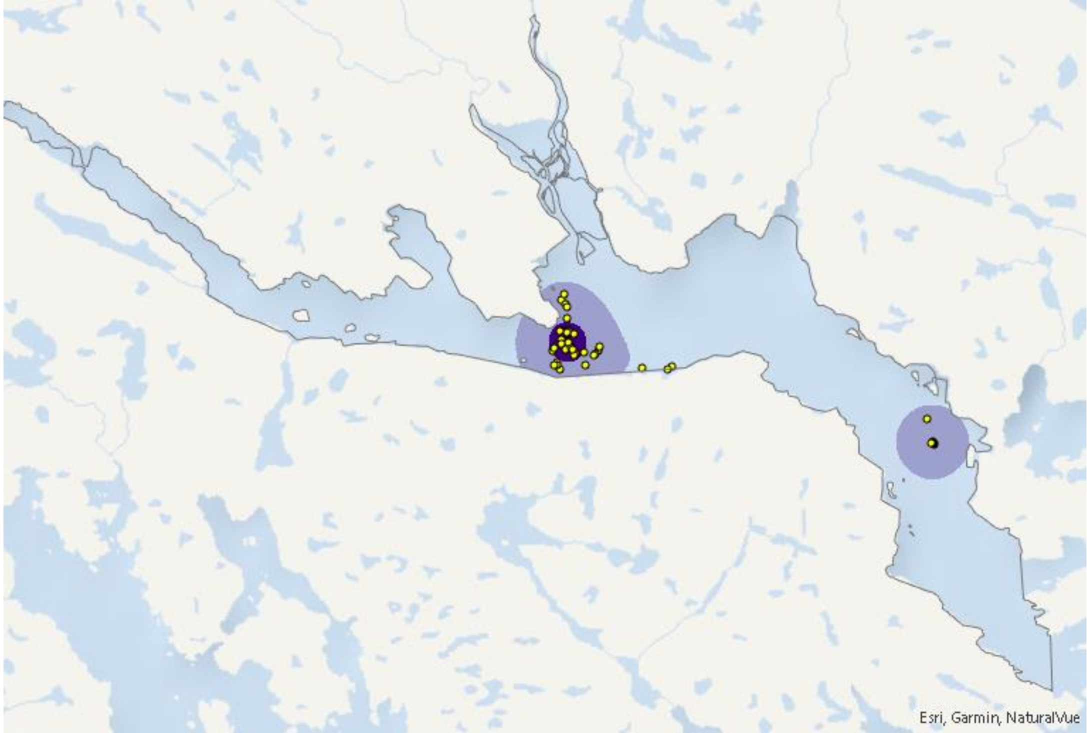

Figure A4. September 7, 2021 points overlayed on KDE utilization distribution maps in Clearwater Fiord.

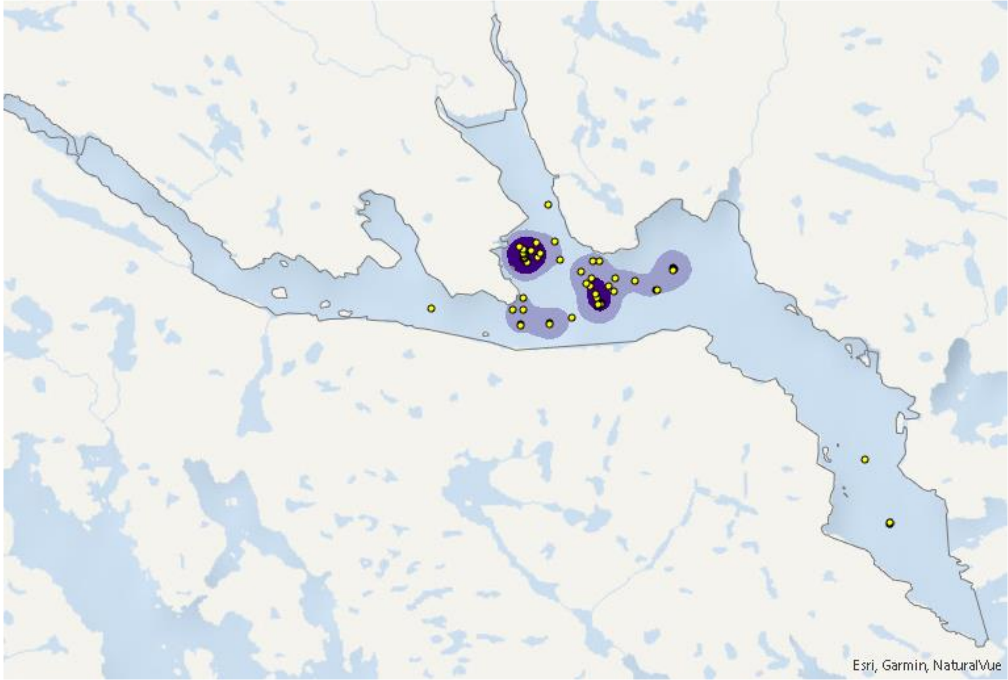

Figure A5. August 17, 2022 points overlayed on KDE utilization distribution map in Clearwater Fiord.

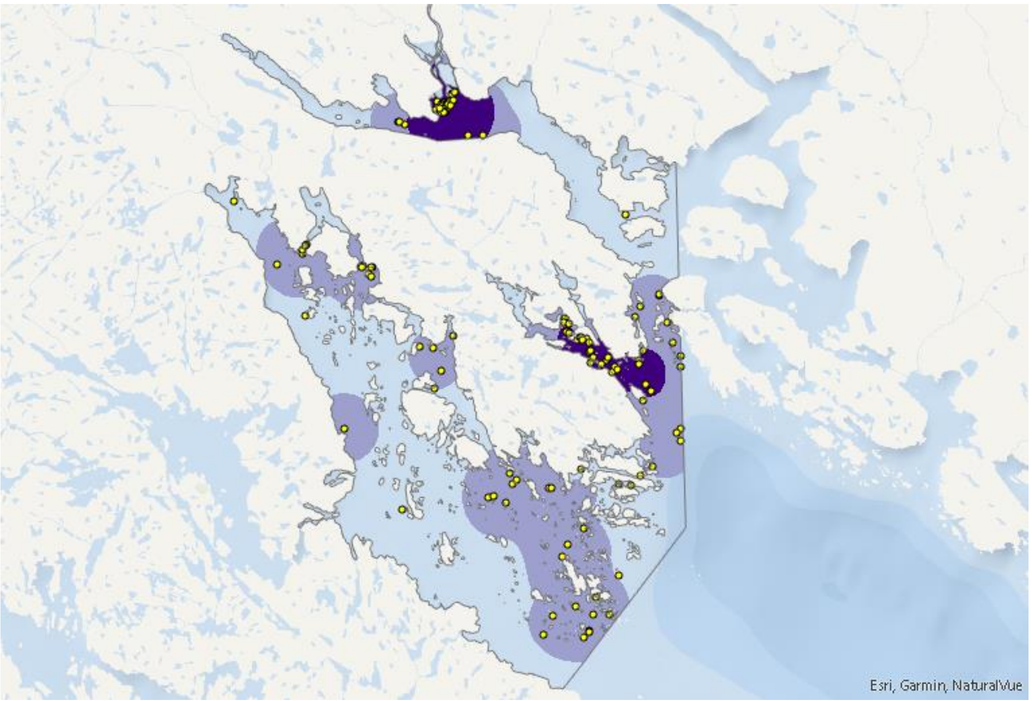

Figure A6. Septemeber 4, 2021 points overlayed on KDE utilization distribution map of Clearwater, Kangilo, and Kangerk Fiords.

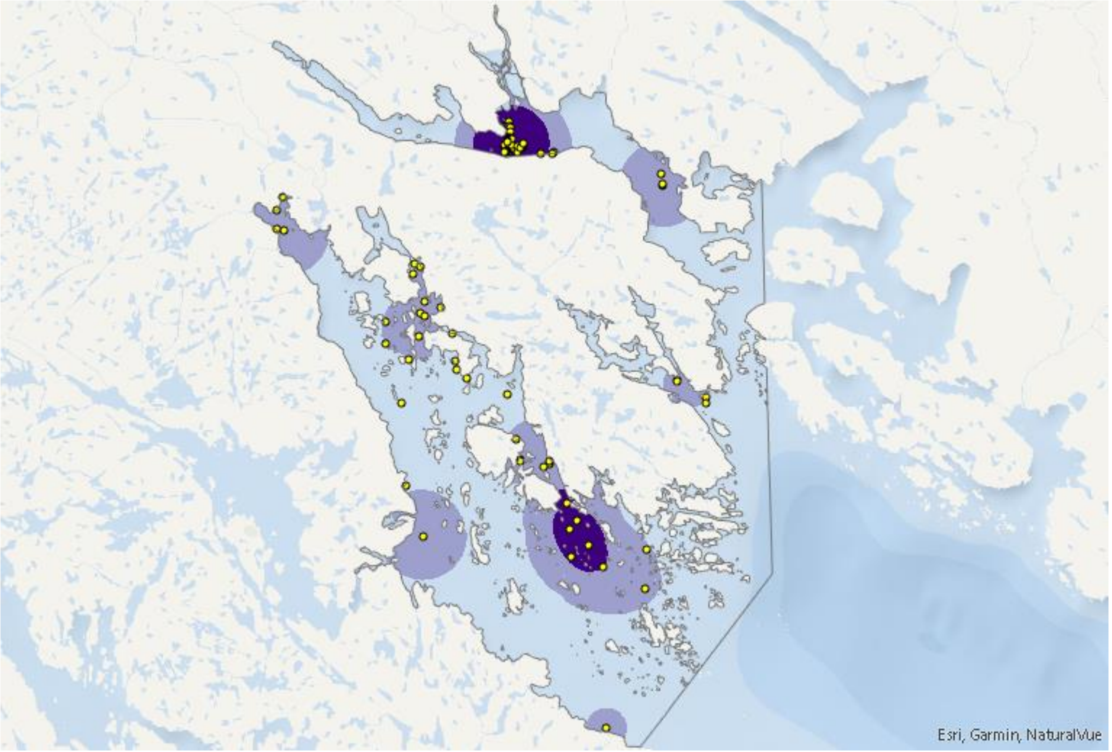

Figure A7. September 7, 2021 points overlayed on KDE utilization distribution map of Clearwater, Kangilo, and Kangerk Fiords.

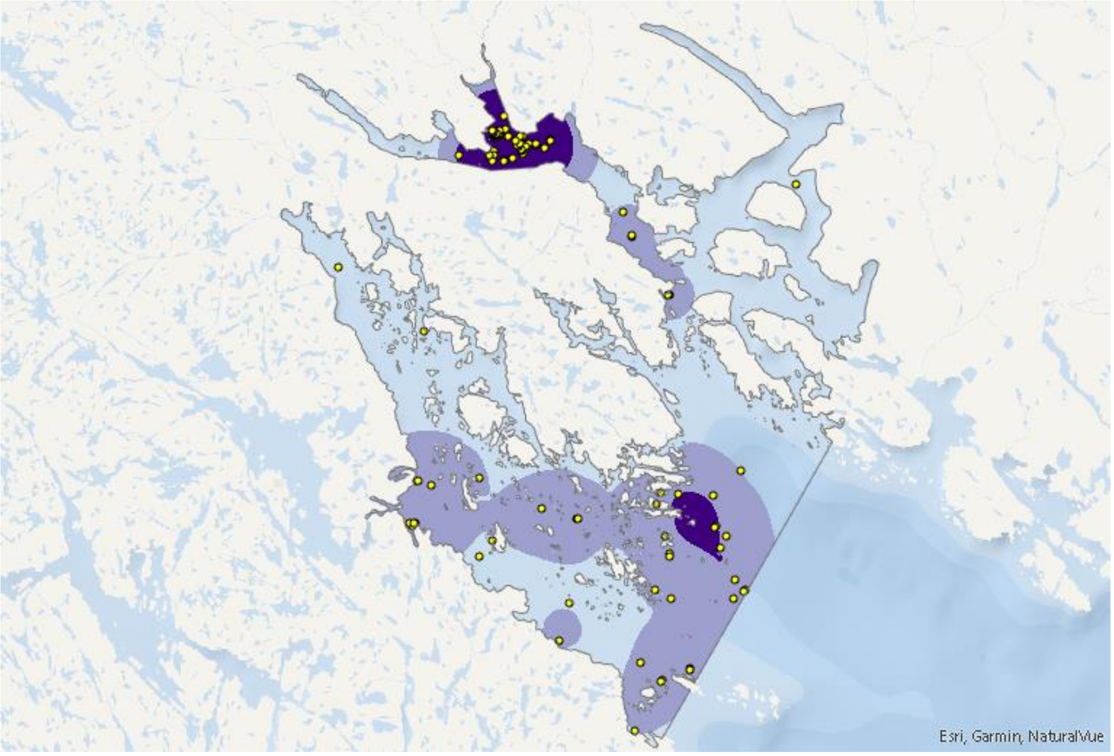

Figure A8. August 17, 2022 points overlayed on KDE utilization distribution maps of Clearwater, Kangilo, Kangerk, and Shark Fiords.

Appendix B

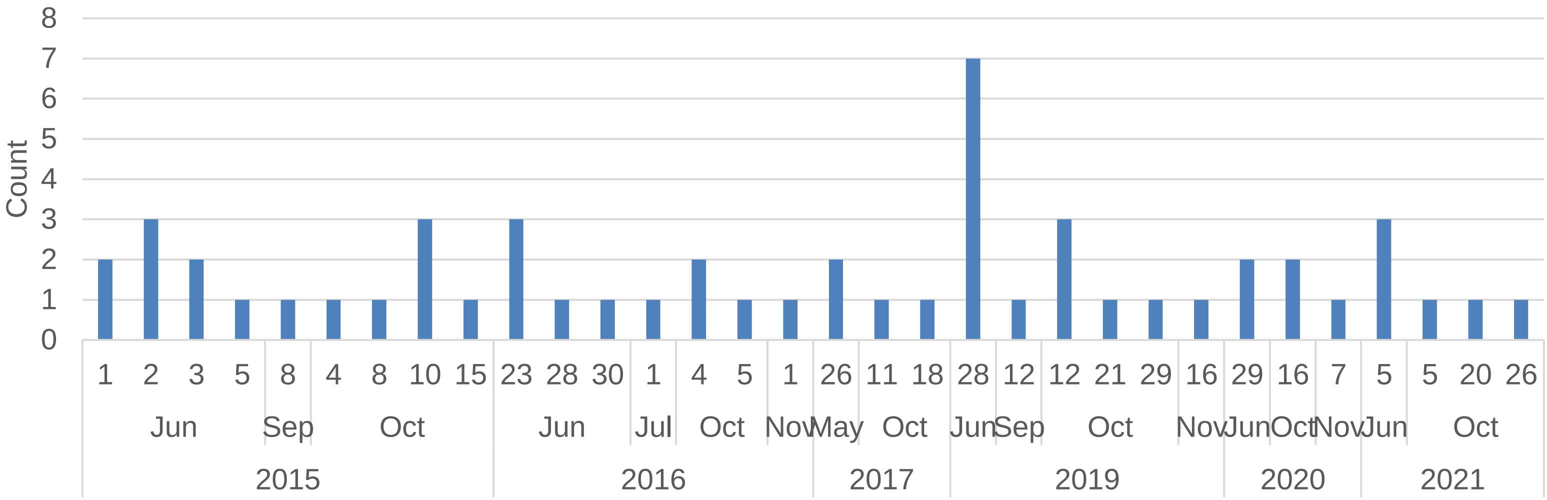

Figure B1. Breakdown of beluga harvest dates.

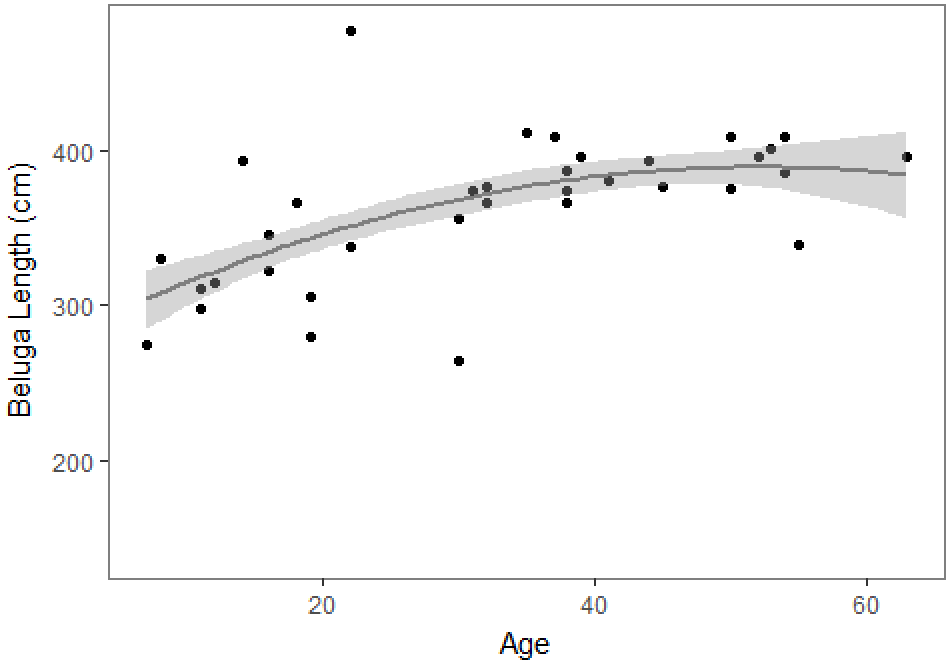

Figure B2. Age versus body length curve of Hudson Bay beluga whales.

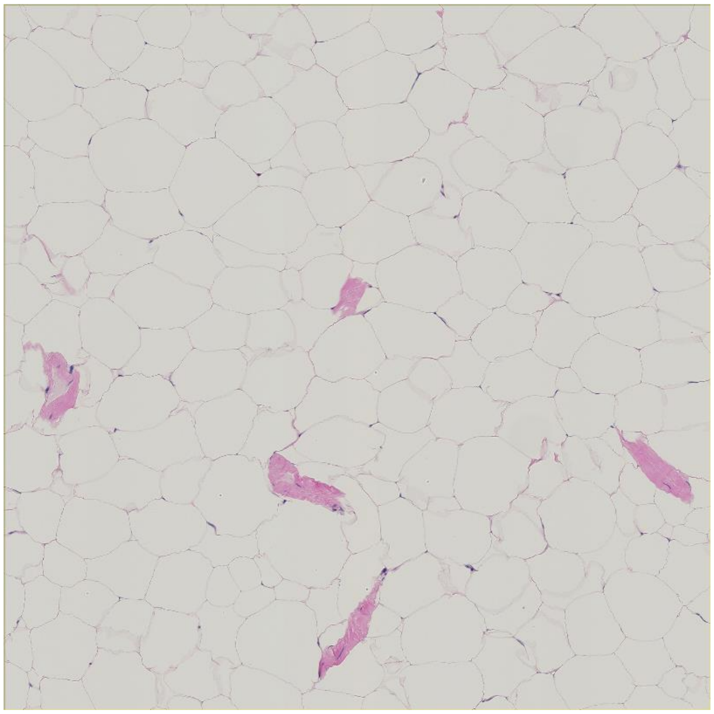

Figure B3. Example of image from histological analysis where beluga whale blubber adipocyte area was measured.

Table B1. Sample breakdown of beluga whales for percent lipid content models (n=53).

Year	Season	Males	Females	Total
2015	Spring	7	1	8
	Fall	6	1	7
2016	Spring	7	0	7
	Fall	4	0	4
2017	Spring	2	0	2
	Fall	2	0	2
2019	Spring	1	5	6
	Fall	6	1	7
2020	Spring	1	2	3
	Fall	1	0	1
2021	Spring	1	2	3
	Fall	1	2	3

Table B2. Sample breakdown of beluga whales for adipocyte size models (n=43).

Year	Season	Males	Females	Total
2015	Spring	7	1	8
	Fall	6	1	7
2016	Spring	4	0	4
	Fall	4	0	4
2017	Spring	1	0	1
	Fall	1	0	1
2019	Spring	1	3	4
	Fall	5	1	6
2020	Spring	1	1	2
	Fall	0	0	0
2021	Spring	1	2	3
	Fall	1	2	3

Table B3. List of fatty acids in beluga blubber that originate only from the diet, based on Iverson et al (2004).

Dietary fatty acids	16:2n6, 16:2n4, 16:3n4, 16:4n3, 16:4n1, 18:2n6c, 18:2n4, 18:3n6, 18:3n4, 18:3n3, 18:3n1, 18:4n3, 18:4n1, 20:1n11, 20:1n9, 20:1n7, 20:2n6, 20:3n6, 20:4n6, 20:3n3, 20:4n3, 20:5n3, 22:1n11, 22:1n9, 22:1n7, 22:2n6, 21:5n3, 22:4n6, 22:5n6, 22:4n3, 22:6n3.