Evaluating the relationship between blubber lipids and fatty acids across blubber thickness in beluga whales (*Delphinapterus leucas*) harvested for subsistence in the Inuvialuit Settlement Region

by

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Abstract

Blubber is an adapted fat layer common to marine mammals that is used for buoyancy, thermoregulation, and energy storage. Its thickness ranges widely between species, from 2.5 cm in harbour porpoises (*Phocoena phocoena*), up to 50 cm in bowhead whales (*Balaena mysticetus*). Blubber is composed of various lipids and fatty acids and is not homogenous in structure or function throughout its depth. Depletion of energy stores from environmental or nutritional stress affects blubber thickness and thus decreases its functionality and the fitness and survival of the animal. Recently, beluga whales (*Delphinapterus leucas*) from the Eastern Beaufort Sea (EBS) population have been observed with thinner blubber. Changes in blubber thickness and composition may reflect changes in prey availability and quality, or infection/injury of an individual, which may impact individual growth and reproduction rates, and overall population health. The composition and stratification of fatty acids and total lipid percent of EBS beluga blubber was examined and compared between individuals of differing blubber thicknesses. The influence of environmental temperature on fatty acid composition, as well as how biological covariates influence total lipid percent in the blubber and muscle tissue was also explored. Stratification of fatty acids was present, and the inner, middle, and outer blubber layers contained different proportions of fatty acid types as a function of blubber thickness. The innermost blubber layer contained more dietary fatty acids and fatty acids with higher melting points such as polyunsaturated fatty acids (PUFAs) and long-chain monounsaturated fatty acids (LCMUFAs), while the outermost blubber layer contained non-dietary fatty acids and fatty acids with lower melting points such as the short-chain monounsaturated fatty acids (SCMUFAs). The outer layers of blubber contained greater delta-9

desaturation values, demonstrating the impact that environmental temperature has on the fatty acid composition of the blubber. Whales with thinner blubber contained more SCMUFAs and fewer LCMUFAs than whales with thicker blubber, providing evidence of lower feeding rates in this subset of whales and demonstrating the altered distribution of fatty acids with lower melting points in thinner blubber in order to address environmental temperature effects. Total lipid percent was not influenced by body length, harvest location, standardized blubber thickness (body condition), or age, and was greatest in the middle layer of blubber. A significant, negative relationship was found between total lipid percent in the muscle tissue and outer blubber lipid percent, and a positive, significant relationship was found between total lipid percent in the muscle and total protein percent in the muscle. A shift in prey species distributions due to climate change is occurring in the Arctic, and thus a greater occurrence of thin beluga blubber and loss of the lipid-rich middle layer may occur. An overall reduction in thickness may render belugas vulnerable to increases in energy expenditure to maintain core body temperatures. Knowledge of beluga blubber, what factors affect its composition, and what compositional changes occur in thinner blubber is necessary to best inform conservation and management practices.

Dedication

This work is dedicated to my baby boy.

May you grow up in a world where the air is pure, the seas are alive, and the forest is full of song. I hope you find peace and belonging in the beauty of nature, and comfort in the rhythm of the tides.

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Contributions of Authors

The following are the author contributions for Chapters 2 and 3:

Lisa Kulchycki (thesis author) – conceptualization, methodology, project administration, data curation, investigation, writing (original draft)

Lisa Loseto – conceptualization, funding acquisition, methodology, supervision, writing (review and editing)

Marie Noel – methodology, investigation

Bruno Rosenberg - investigation

Megan Wardekker – investigation, lab support

Gail Davoren – writing (review and editing)

Cortney Watt – writing (review and editing)

Zouzou Kuzyk – writing (review and editing)

Thesis Format

This thesis is organized in a grouped manuscript format. Chapter 1 provides a broad introduction to the topic and outlines background information necessary to contextualize the studies. Chapters 2 and 3 are structured as individual manuscripts, complete with their own abstracts, introduction, methods, results, discussion, conclusion, acknowledgements, and reference sections. Chapter 4 provides a general conclusion of the studies from Chapter 2 and 3, and includes the significance and limitations of the studies, as well as suggestions for future research.

Chapter 1. General Introduction

1.1 Climate change in the Arctic

Global warming due to anthropogenic climate change is exerting a particularly large effect on Arctic ecosystems, with warming occurring up to four times faster than the global average (Rantanen et al., 2022). This has led to warmer waters, a decrease in sea ice, an increase in ice-free days (Huntington et al., 2020) and a projection of the Arctic Ocean to be summer sea-ice-free by the 2030s (Wang & Overland, 2012). A decline in sea ice extent and thickness has created new migratory corridors for subarctic and temperate species (Stafford et al., 2022), allowing for an earlier Arctic arrival, a longer stay, the ability to migrate further north (Stafford et al., 2022), and an increase in abundance of representatives of these species in polar regions (Falardeau et al., 2014). For example, an increase of Pacific sand lance (*Ammodytes hexapterus*) was recently found in the Beaufort Sea, which has the potential to displace Arctic cod (*Borerogadus saida*) as the dominant forage fish species, as observed more than a decade ago in Hudson Bay (Falardeau et al., 2014; Gaston et al., 2012). Arctic cod are a prominent fish species in Arctic marine food webs and play a major role in the transfer of energy from zooplankton to higher trophic level species (Welch et al., 1992). They have been the most abundant fish in the Canadian Beaufort Sea (Benoit et al., 2008) and constitute a large part of the diet of the region's marine mammals, such as beluga whales (*Delphinapterus leucas*) (Loseto et al., 2009). Biodiversity of marine ecosystems will be affected as changes in water temperature and nutrient availability impact primary producers and food web composition (Michel et al., 2012; Kortsch et al., 2015). High trophic level predators are some of the most impacted by climate change (Laidre et al., 2015). Species with highly specialized diets such as polar bears (*Ursus maritimus*) who primarily feed on ice-dependent seals are at the highest risk, while generalist predators who feed

on a wider variety of prey, such as beluga whales are considered at moderate risk to the effects of climate change (Laidre et al., 2015).

1.1. Blubber in marine mammals

Blubber is an adapted layer of fat that played a fundamental role in the ability of Arctic marine mammals to move from their ancestral, terrestrial state into various oceanic species (Liwanag et al., 2012). Blubber is used as the main energy store, comprising up to 40% of the body mass in toothed whales (Cornick et al., 2016; Lockyer, 1991), allowing marine mammals to survive and maintain physiological homeostasis during times of food scarcity (Strandberg et al., 2008). It also plays a large structural role, contributing to body shape, as well as aiding in thermoregulation, streamlining, and buoyancy (Strandberg et al., 2008; Larrat & Lair, 2021; Macmillan et al., 2023). Blubber is located just below the skin and is primarily composed of lipid-filled cells called adipocytes, that are reinforced by collagen and elastic fibres (Iverson, 2009). In mammals, the number of adipocytes is typically set in early life and will enlarge or shrink with lipid accumulation or depletion throughout the individual's life (Struntz et al., 2004). Lipids are energy-dense, containing more than double the calories per gram that protein and carbohydrates contain (Moghadasian & Shahidi, 2017; Kwon et al., 2020), making them a preferred choice for energy reserves.

1.2. Lipids and Fatty Acids

Lipids refer to a group of macromolecules that are primarily composed of carbon, hydrogen, and oxygen (Moghadasian & Shahidi, 2017). Along with carbohydrates, proteins, and water, they are a major component of biological materials and have many important roles in various processes (Muro et al., 2014). They are fundamental to every cell, and are a key component of the plasma membrane, nuclear membrane, endoplasmic reticulum, and more (Dowhan & Bogdanov, 2002). They also carry fat-soluble vitamins throughout the body and are typically hydrophobic in nature (Moghadasian & Shahidi, 2017). Different lipids are required for various functions, and can have structural or signalling roles; therefore, tissue types require different compositions of lipids (Muro et al., 2014).

Fatty acids are the primary components of lipids, forming approximately 95% of the structure of a lipid molecule (Moghadasian & Shahidi, 2017). The types and positioning of fatty acids in a lipid molecule will determine the physical and chemical properties of the lipid (Moghadasian & Shahidi, 2017). Fatty acids can contain any length of carbons in a chain; however, most naturally occurring fatty acids have an even-number-length chain between four and 22 carbon atoms, with 18 carbons being the most common (Gunstone & Harwood, 2007). Fatty acids are usually considered to be 'long-chain' if they contain more than 18 carbon atoms in the chain (Abedi & Sahari, 2014), and 'short-chain' if they contain two to six carbon atoms in the chain (Layden et al., 2013). While there are over 1000 known fatty acids, only approximately 20 are widely found in nature (Gunstone & Harwood, 2007).

Fatty acids may be saturated or unsaturated, depending on the presence of carbon-carbon double bonds in the carbon chain (Moghadasian & Shahidi, 2017). Saturated fatty acids (SFA)

contain no double bonds between carbon atoms, and the remaining bonds are connected to hydrogen atoms (Lunn & Theobald, 2006). Saturated fatty acids are less chemically reactive and have a higher melting point than unsaturated fatty acids and are therefore typically solid at room temperature (Moghadasian & Shahidi, 2017; Knothe & Dunn, 2009). Their melting point further increases with increasing chain length (Moghadasian and Shahidi, 2017; Knothe & Dunn, 2009). Straight-chain compounds with 12, 14, 16, or 18 carbon atoms are the most abundantly found SFAs in animal tissues (Gunstone & Harwood, 2007), while palmitic acid (16:0) is the most common natural saturated fatty acid, making up approximately 20-30% of animal lipids (Carta et al., 2017). Saturated fatty acids partially compose cell membranes and can all be produced within the body, and therefore all saturated fatty acids found in marine mammal blubber are not required to be obtained from diet (Gershuni, 2018).

Unsaturated fatty acids are fatty acids that contain at least one double bond in the carbon chain (Lunn & Theobald, 2006). If they contain one double bond, they are termed monounsaturated fatty acids (MUFAs), and if they contain two or more double bonds, they are called polyunsaturated fatty acids (PUFAs) (Das, 2006). Monounsaturated fatty acids are often liquid at room temperature and will solidify around 4°C (Moghadasian & Shahidi, 2017). Some MUFAs can be synthesized endogenously; however, long-chain MUFAs need to be obtained from food or derived from precursor fatty acids (Iverson et al., 2004; Grahl-Nielsen et al., 2005). One of the major nutritionally important MUFAs is oleic acid (18:1n-9), which has been shown to have many positive health benefits in mammals (Moghadasian & Shahidi, 2017; Pravst, 2014). Similarly, some PUFAs can be endogenously synthesized via precursor fatty acids and desaturase enzymes (e.g., γ-linolenic acid (C18:3n6) from linoleic acid (C18:2n6) and delta-6 desaturase enzyme (Das, 2006)), but the majority of PUFAs come directly from diet

(Moghadasian & Shahidi, 2017). PUFAs with a chain length of 20 carbons or more that contain at least three double bonds are considered highly unsaturated fatty acids (Kothapalli et al., 2023). Highly unsaturated fatty acids (HUFAs) such as arachidonic acid (20:4n-6), and the omega-3 fatty acids (fatty acids with a double bond on the third carbon atom from the terminal methyl group) are commonly discussed as nutritionally important fatty acids; the most important of these include alpha-linolenic acid (ALA) (18:3n-3), eicosapentaenoic acid (EPA) (20:5n-3), and docosahexaenoic acid (DHA) (22:6n-3) (Nakamura & Nara, 2002; Moghadasian & Shahidi, 2017). All three of these fatty acids are often considered essential, meaning they must be obtained by diet (Das, 2006). However, as previously mentioned, mammals are capable of synthesizing minor amounts of some HUFAs, such as EPA and DHA via precursor PUFAs linoleic acid (LA) (18:2n-6) and ALA (Das, 2006; Nakamura & Nara, 2002). LA and ALA cannot be synthesized within the body and are therefore true essential fatty acids that can only be acquired by diet (Das, 2006). Omega-3 and omega-6 PUFAs are powerful intra and intercellular mediators in cell signalling as well as cell membrane fluidity, which is especially important in cold Arctic habitats where tissue integrity is at risk due to the sub-zero temperatures (Trumble & Kanatous, 2012). They are necessary for growth and development, as well as for physiological processes such as vision and brain functioning, skin integrity, and eicosanoid signaling, and thus a sufficient amount of these fatty acids is required for the maintenance of good health and prevention of disease (Nakamura & Nara, 2002; Moghadasian & Shahidi, 2017). The limitations of mammals with respect to fatty acid synthesis and modification mean that any intraspecific differences in fatty acid signatures are thought to be associated with diet and foraging differences (Thiemann et al., 2008).

1.3. Blubber stratification and thermal properties

1.3.1 Stratification

Vertical stratification of blubber lipids has been previously documented in various marine mammal species. Total lipid percent throughout the depth of blubber has been shown to vary in some species (fin whales (*Balaenoptera physalus*), Aguilar & Borrell, 1990; sperm whales (*Physeter macrocephalus*), Jackson et al., 2022), while remaining stable in others (Pacific walrus (*Odobenus rosmarus divergens*), Jay et al., 2021), and stratification of blubber fatty acids has been reported in ringed seal (*Pusa hispida*) (Strandberg et al., 2008), minke whale (*Balaenoptera acutorostrata)* (Olsen and Grahl-Nielsen, 2003), bowheads (*Balaena mysticetus*) (Budge et al., 2008), Baikal seal (*Pusa sibirica*) (Grahl-Nielsen et al., 2005), as well as other marine mammal species. Some species, such as fin whales and harbour porpoises (*Phocoena phocoena*), contain at least two chemically distinct layers of blubber (Ackman et al., 1965; Koopman et al., 1996), while others, such as bottlenose dolphins (*Tursiops truncatus*) and ringed seals, possess three distinct layers (Samuel & Worthy, 2004; Strandberg et al., 2008). Similar stratification patterns throughout blubber layers have been consistently found across differing body sites in various species as well (Jay et al., 2021; Strandberg et al., 2008; Samuel & Worthy, 2004; Koopman et al., 1996), with an exception found in the caudal peduncle of harbour porpoise, hypothesized to be due to the specialized locomotory purpose of the body site (Koopman et al., 1996). The inner layer of blubber (that closest to the muscle and furthest from the skin) is the most metabolically active (Koopman et al., 2002) and will act as the primary site of lipid mobilization (Strandberg et al., 2008), that is, the release of fatty acids into the blood stream for use in energetic and metabolic demands, particularly during times of energy deficit (Contreras & Sordillo, 2011). Numerous long-chain MUFAs and dietary PUFAs are transferred from prey to this inner layer of

blubber and thus the composition of this layer is the most representative of what has been consumed (Koopman et al., 2002; Budge et al., 2006; Guerrero & Rogers, 2017; Choy et al., 2019). There is also evidence that genetics and/or metabolism plays a role in determining which fatty acids from the prey are deposited in the inner layer of blubber, as a number of studies have shown that the fatty acids within the inner layer do not directly match those of the prey items consumed (Olsen & Grahl-Nielsen 2003; Kirsch et al., 2000; Andersen et al., 2004). Many studies have also consistently shown that saturated fats tend to distribute more towards the inner layer as well, although these fats are not as representative of diet as they can be endogenously created (Olsen & Grahl-Nielsen, 2003; Strandberg et al., 2008; Waugh et al., 2014; Jackson et al., 2022).

The outer layer does not seem to reflect diet but is important in streamlining and buoyancy and acts as an insulator (Olsen & Grahl-Nielsen, 2003), and an abundance of short-chain MUFAs have been recorded here (Olsen & Grahl-Nielsen, 2003; Strandberg et al., 2008; Waugh et al., 2014). The middle blubber layer has often been excluded in studies examining marine mammal blubber, likely because its boundaries are not easily identifiable. However, the middle layer has been documented as containing a continuous gradient in fatty acid concentration throughout the layer, acting as a continuum for change from the inner to outer blubber layer (Olsen & Grahl-Nielsen 2003; Strandberg et al., 2008). Additionally, a study by Strandberg et al. (2008) found that the middle layer of blubber in ringed seals expanded the most when the seal reached a blubber thickness that exceeded 4 cm, and the blubber of thin seals (who had blubber less than or equal to 3 cm) was characterized by an absence of a middle layer. Stratification is present across many marine mammal species, most containing different fatty acid compositions between inner and outer layers, and the degree of stratification differs between species (Grahl-Nielsen et al.,

2005). Fatty acid stratification and composition in blubber is the result of biosynthesis, deposition, transport, and mobilization of lipids (Grahl-Nielsen et al., 2005).

1.3.2 Thermal properties of blubber

Water temperature likely plays a role in the thickness of blubber (Iverson, 2009), as well as in blubber stratification, as cold water species have been shown to exhibit thicker blubber as well as a greater fatty acid stratification throughout their blubber depth compared to temperate or tropical species (Samuel & Worthy, 2004; Koopman, 2007). A major role of blubber is to act as an insulator, maintaining consistent body temperature in an aquatic environment where heat loss occurs much faster than in air (Iverson, 2009). To act as a functional insulator, the fatty acids need to remain fluid to maintain a proper thermal gradient from the body core to the skin (Pond, 1998; Liwanag et al., 2012). The skin of marine mammals is only slightly warmer than the surrounding water temperature (Irving & Hart, 1957; Hart & Irving, 1959; Melero et al., 2015; Favilla et al., 2021), and therefore, to prevent solidification of blubber lipids, fatty acids with a lower melting point are needed to maintain fluidity (Strandberg et al., 2008). As a result, the outer layers of blubber would be expected to have a greater concentration of unsaturated fatty acids, which tend to have lower melting points than saturated fatty acids, whereas inner layers that are closer to the warm body core will have greater amounts of fatty acids with higher melting points (Strandberg et al., 2008). The effect of environmental temperature is thought to reach a blubber depth of 21-24 mm from the skin (Hart and Irving, 1959); therefore, a reduction in blubber thickness may result in compositional changes if the environmental temperature is able to permeate the full depth of blubber.

1.4. Body condition

Blubber is often used in dietary studies and can also be used to gain information about population health by examining cortisol levels or exposure to lipophilic toxins (Thiemann et al., 2008; Budge et al., 2008; Ellisor et al., 2013; Trana et al., 2015; Loseto et al., 2018). Blubber is also often used in the inference of an individuals' body condition, i.e., the amount of energy stores found in an individual (Macmillan et al., 2023). As no standardized approach currently exists to assess body condition in marine mammals, blubber thickness, total lipid percent, body girth, or more recently, scaled mass index is commonly used (Larrat & Lair, 2021; Macmillan et al., 2023; Sherrill et al., 2024). Blubber thickness is also used by Inuit, traditional harvesters of marine mammals, as an important health indicator and an important metric for regular monitoring as it signifies seasonal change (Inuuvik Community Corporation et al., 2006; Ostertag et al., 2018; Macmillan et al., 2023). However, blubber thickness alone may not be an accurate representation of an animal's body condition. Lipid content has been shown to vary independently from blubber thickness in some cetaceans (Evans et al., 2003; Kershaw et al., 2019), and other organs, such as the liver, pancreas, and skeletal muscle also hold lipid stores that can be drawn upon during energy depletion (Olsen et al., 2021). Furthermore, some catabolism of muscle protein during energy deficit has been recorded in marine mammals, which suggests that there is not always a direct link between body condition and blubber thickness (Bernaldo de Quiros et al., 2024). Fat and protein catabolism from internal tissues can lead to a decrease in body circumference and therefore a relatively increased blubber thickness in poorer body condition animals (Larrat & Lair, 2021), giving the illusion of a healthy body condition. Research aimed at learning how energy is preferentially mobilized from different body sites will aid in the assessment of marine mammal body condition as some researchers believe that

alternative energy storage sites will be used before lipid stores from the blubber to prioritize maintenance of blubber thickness (Macmillan et al., 2023).

Fatty acids are not commonly used in body condition analysis; however, a relationship between blubber fatty acid composition and body condition may be present based on the preferential mobilization of fatty acids into the blood stream. Lipid mobilization within blubber is a selective process as certain fatty acids are prioritised over others depending on the molecular structure of the lipid (Bernier-Graveline et al., 2021). Mobilization of fatty acids into the blood stream increases with an increasing number of double bonds and decreases with an increase in chain length (Raclot, 2003). This means that highly unsaturated or short-chain fatty acids are more readily mobilized for energy use than saturated or long-chain fatty acids. Eicosapentaenoic acid (EPA, C20:5n3), for example, has been shown to quickly mobilize during energy depletion (Iverson et al., 1995). This has been documented in humpback whales (*Megaptera novaeangliae*), where dietary polyunsaturated fatty acids were reported as being the first fatty acids to be mobilized in whales who were undergoing a period of deteriorating body condition during a period of stress (Waugh et al., 2012). Similarly, in a study of varying condition St Lawrence Estuary beluga whales by Bernier-Graveline et al. (2021), three dietary PUFAs, arachidonic acid (AA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) were found in higher concentrations in the blubber of good condition whales compared to those in poor or moderate condition. It would therefore be expected that all poor body condition marine mammals would have less abundant short-chain and polyunsaturated fatty acids than good body condition animals. Polyunsaturated fatty acid metabolism uses less oxygen than monounsaturated fatty acid and saturated fatty acid metabolism and yields less ATP per same fatty acid length (Trumble & Kanatous, 2012). This is important as diving mammals need to

conserve oxygen during breath-holding while hunting prey under the water (Trumble & Kanatous, 2012). They rely entirely on internal oxygen stores and therefore maximum dive duration is correlated with oxygen rate utilization and any other factors affecting oxygen consumption rate (Trumble & Kanatous, 2012). A decline in body condition in belugas may be associated with a decrease in physiological parameters of oxygen stores and can contribute to a vicious cycle in which lower oxygen storage capacity due to decreased lipid stores can further limit diving ability, leading to a further decline in body condition via reduced prey consumption (Choy et al., 2019; Choy et al., 2020).

1.5. Eastern Beaufort Sea Beluga whales

Marine mammals greatly impact ecosystem health and resiliency through their predation, which promotes biodiversity, as well as through their diving and migratory behaviours, which contribute to sediment and nutrient distribution and deposition (Prasad, 2024). Marine mammals are often used in the study of Arctic ecosystems, as their health is thought to act as a good indicator of the health of their environment. Beluga whales greatly influence ecosystem structure (Loseto et al., 2009), and act as an indicator species for the Arctic marine ecosystem (Choy et al., 2017). Belugas are a toothed whale belonging to the Monodontidae family (Stewart & Stewart, 1989), are the most common Arctic odontocete (Laidre et al., 2015) and are near circumpolar in distribution. They range in body size from 3.5 to 5.5 metres (O'Corry-Crowe, 2009), and feed on a wide range of prey, but stable isotope analysis and stomach analyses have shown that Arctic cod is one of the most preferred and abundant prey items in some beluga populations (Matley et al., 2015; Quakenbush et al., 2015).

One of the world's largest beluga whale populations is the Eastern Beaufort Sea (EBS) population, estimated as containing 38,451 individuals (ranging from 20,735 to 71,304 (95% CI) (Marcoux et al., 2025)). EBS belugas spend their summers in the Canadian Arctic Ocean, and thus, two marine protected areas (MPAs) have been established in the Inuvialuit Settlement Region (ISR) in the Western Canadian Arctic by Fisheries and Oceans Canada (DFO) under Canada's Oceans Act (1997) (Loseto et al., 2018) for the conservation of the EBS beluga. The Tarium Niryutait Marine Protected Area (TN MPA) was created in 2010 and was the first MPA in the Canadian Arctic. The TN MPA is comprised of three separate parcels in the Mackenzie Estuary and was created to conserve the EBS population of beluga whales and their supporting habitat. A second MPA, the Anguniaqvia Niqiqyuam MPA (AN MPA, designated in 2016), was established with an objective to conserve multiple marine species including the EBS beluga, and consequently the sustainable harvest of belugas by the Inuvialuit. The two MPAs allow for a connection between the two areas, which is necessary as EBS belugas are highly migratory with diverse habitat use (Loseto et al., 2018).

The EBS belugas migrate between the Bering Sea and Canadian waters, arriving during late May to early June in the Mackenzie Estuary, the Amundsen Gulf, Viscount Melville Sound, and the Eastern Beaufort Slope in the Canadian Beaufort Sea, where they spend their summer months (Storrie et al., 2022; Richard et al., 2001; Mayette et al., 2023). By December they will return to the Bering Sea to spend the winter (Storrie et al., 2023). Habitat and range of beluga is heavily influenced by sea-ice conditions and habitat selection has been linked to age and sex of the belugas, with females and young males selecting open-water locations closer to shore, and males concentrating more in areas farther away from the mainland that have a greater sea ice concentration (Loseto et al., 2006). Preferred diet may also contribute to differences in habitat

selection (Loseto et al., 2009). In sexually dimorphic marine mammal species, larger individuals have a greater capacity to dive deeper and for longer durations than smaller individuals, allowing for the opportunity to capture higher quality or quantity of prey (Storrie et al., 2022). Adult male EBS belugas are about 25% larger than females (O'Corry-Crowe, 2009) and are thought to rely more heavily on large offshore Arctic cod than females and young males during the summer months (Loseto et al., 2009). While both male and female belugas feed on Arctic cod, offshore Arctic cod have been shown to contain different levels of C20 and C22 monounsaturated fatty acids than near-shore Arctic cod, suggesting that offshore and near-shore cod in the Beaufort Sea feed at different trophic levels (Loseto et al., 2009), providing different opportunities for energy gain from Arctic cod for male and female belugas.

During their seasonal migration, belugas are sustainably harvested as they pass through coastal communities and camps in the Inuvialuit Settlement Region in the Northwest Territories (Ostertag et al., 2018). The Inuvialuit are a group of Inuit people indigenous to the western Canadian Arctic. Beluga whales are thought to have been the primary food source for pre-contact Inuvialuit who hunted beluga in the Mackenzie Delta every summer (Friesen & Arnold, 1995). Still today, beluga is considered culturally significant and an important food resource for the Inuvialuit, who use traditional knowledge and practices in the harvest of the whales (Ostertag et al., 2018; FJMC, 2013). While all communities within the Inuvialuit Settlement Region hunt beluga, Tuktoyaktuk is the most active (Harwood et al., 2002). Tuktoyaktuk is located on Kugmallit Bay, adjacent to an important EBS beluga calving area, and therefore congregations of beluga are accessible to hunters with a day trip to a seasonal whaling camp by boat (Waugh et al., 2018). The mouth of the Mackenzie River around to Hendrickson Island contains the shallowest waters and is where the majority of hunting occurs (Waugh et al., 2018). Tuktoyaktuk

hunters will often bring the whales to Hendrickson Island to butcher the animal, before bringing it back to Tuktoyaktuk for processing (Ostertag et al., 2018). Hunting in this area mainly occurs during the month of July and lasts four to six weeks (Harwood et al., 2002). Hunter-based beluga monitoring programs have taken place in the Mackenzie Delta since 1973, where number of whales harvested was recorded, and since 1980, body length, fluke width, sex, and age of whales have also been documented (Harwood et al., 2002).

In Paulatuk, a small community located in Darnley Bay that is only accessible by air (Arnold et al., 2011), belugas are harvested more opportunistically than in Tuktoyaktuk and were not hunted more consistently and in larger numbers until the 1990s, when beluga migration patterns changed and they began to migrate closer to Paulatuk (Ostertag et al., 2018). For this reason, harvesters in Paulatuk may be less selective in choosing whales to harvest than in other communities (Ostertag et al., 2018). Hunting in Paulatuk takes place after the belugas have left the Mackenzie estuary, typically in late July or August, when the whales move to offshore areas to feed (Harwood et al., 2002). Data collection from beluga hunting in Paulatuk began in 1989 and mirrors data that is collected by community members in Tuktoyaktuk (Harwood et al., 2002).

EBS belugas are currently managed via a long-term collaborative research and health monitoring program through joint efforts by the federal government and the Fisheries Joint Management Committee (FJMC), who work in cooperation with the Tuktoyaktuk and Paulatuk Hunters and Trappers Committees (HTCs) to manage conservation in the Inuvialuit Settlement Region. Every year, research teams work in tandem with Inuvialuit hunters at traditional whaling camps to obtain data measurements and samples of beluga tissue, which is then sent to the Freshwater Institute in Winnipeg for laboratory processing for collection of biological data.

A larger variability in blubber thickness in adult male belugas from the EBS population harvested in the TN MPA was observed throughout 2000-2007 and 2011-2014 (Harwood et al., 2014, Choy et al., 2017, Macmillan et al., 2023), and a decline in size-at-age of individuals in the EBS whale population has been observed over the last 20 years, hypothesized to be due to changing prey availability (Harwood et al., 2014). Inuvialuit hunters have described a seasonal shift in beluga body condition, noting that belugas harvested later in the summer (late July-August) are fatter than those harvested earlier (June) (Macmillan et al., 2023). Inuvialuit have sustainably harvested belugas for centuries and have developed a deep understanding of EBS beluga behaviour, migration, and health from information passed down through generations (Ostertag et al., 2018).

In 2018, harvesters reported that healthy whales have about 4 or 5 inches of blubber by mid-July, and whales that are thin this late in the season are probably old or sick (Ostertag et al., 2018). Size and age of the whale also influence blubber thickness, as young and small whales will have thinner blubber (Ostertag et al., 2018). While blubber thickness is an important aspect in determining beluga health, thin blubber is not immediately dismissed as belonging to a sick whale, as it may be exceptionally old or young (Ostertag et al., 2018). However, a whale with 0.5 inches or less of blubber will be considered unhealthy (Ostertag et al., 2018). Inuvialuit harvesters in Tuktoyaktuk have reported that over time beluga blubber thickness has declined (Ostertag et al., 2018). During the 2022 and 2023 FJMC meetings, both the Tuktoyaktuk and Paulatuk HTCs reported whale health as a primary concern, and a desire to understand the cause of thinning blubber was stated as a priority by the Paulatuk HTC. While it is unknown why the thinner whales were observed, relationships between the thickness of blubber and the vertical

stratification of lipids and fatty acids need to be characterized to consider the implications of the observed reduced blubber thickness.

1.6. Thesis Objectives

The overall objective for this thesis was to examine how lipids distribute in the blubber of beluga whales, particularly in the percentage of total lipids and the fatty acid composition, with an emphasis on examining the composition in relation to differing body conditions.

The objective of my first data chapter (Chapter 2) was to document the vertical stratification of fatty acids throughout beluga blubber and describe how composition varies throughout the blubber layers. Differences in fatty acid composition between individuals of differing blubber thickness were also explored, as well as the effects of environmental temperature on the fatty acid composition of the blubber.

The objective of my second data chapter (Chapter 3) was to examine how total percent of lipid changed throughout the full depth of blubber, and to evaluate whether a relationship existed between total lipid percent in the blubber and commonly measured variables such as age, blubber thickness, body length, and harvest location. Additionally, the relationship between these variables and total lipid percent in the skeletal muscle of beluga whales was explored.

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Chapter 2. Fatty acid composition and stratification patterns in the blubber of Eastern Beaufort Sea belugas (*Delphinapterus leucas*) of varying blubber thickness

2.1 Abstract

Increased observations of thin blubber in the Eastern Beaufort Sea population of beluga whales (*Delphinapterus leucas*) from subsistence harvesters in the Inuvialuit Settlement Region prompted this study of beluga whale blubber. The vertical stratification of 47 fatty acids throughout the full depth of beluga whale blubber from the Eastern Beaufort Sea population were documented in whales of varying blubber thicknesses. Analysis revealed the innermost, middle, and outermost layers were chemically distinct, with the inner layer containing more dietary fatty acids such as the polyunsaturated fatty acids (PUFAs) and long-chain monounsaturated fatty acids (LCMUFAs). The outer layer contained a greater proportion of short-chain monounsaturated fatty acids (SCMUFAs) and fatty acids with a lower melting point. The outer layer also exhibited evidence of greater rates of delta-9 desaturation, and thus, the endogenous transformation of fatty acids from higher to lower melting point, which consistently decreased towards the muscle until approximately 25-30 mm from the skin, demonstrating the scale of impact of environmental temperature on fatty acid composition. The middle layer acted as a transition layer between the innermost and outermost blubber layers. Proportions of fatty acids remained relatively constant between differing blubber thicknesses except for the SCMUFAs, which were found in greater abundance in whales with thin blubber, and LCMUFAs, which were found in larger proportion in whales with thick blubber. LCMUFAs are typically obtained by diet, and thus, a decrease in LCMUFAs in whales with thin blubber suggests lower rates of feeding, as well as supports the concept of environmental temperature influencing the composition of the full depth of thin whales' blubber due to the relatively higher melting of LCMUFAs and requirement of blubber fatty acid fluidity. This study provided baseline documentation of the fatty acid composition of beluga whale blubber, and valuable insight into

how blubber composition may change should thin blubber become more commonplace in this population of whales.

2.2 Introduction

Marine mammal blubber plays a large role in body shape, thermoregulation, streamlining, and buoyancy, and as it is composed of thick layers of lipids, it is also used as a main energy store (Strandberg et al., 2008; Larrat & Lair, 2021; Macmillan et al., 2023). The primary components of lipids are fatty acids, forming about 95% of the structure of a lipid molecule (Moghadasian & Shahidi, 2017). The physical and chemical properties of lipids are determined by the types and proportions of the fatty acids in the lipid, as well as their positioning (Moghadasian & Shahidi, 2017). Saturated fatty acids (SFAs) are the least chemically reactive, are produced endogenously, are typically solid at room temperature and have a higher melting point than unsaturated fatty acids (Moghadasian & Shahidi, 2017; Knothe & Dunn, 2009). Monounsaturated fatty acids (MUFAs) are generally liquid at room temperature and solidify around 4°C (Moghadasian & Shahidi, 2017). While some MUFAs can be synthesized within the body, long-chain MUFAs (LCMUFAs) need to be obtained from diet (Iverson et al., 2004) or derived from their precursors (Grahl-Nielsen et al., 2005). Similarly, many polyunsaturated fatty acids (PUFAs) can be synthesized within the body via precursor fatty acids and desaturase enzymes (Das, 2006), but the majority of PUFAs are obtained directly from diet (Moghadasian & Shahidi, 2017).

Stratification of lipids within blubber is the result of biosynthesis, deposition, transport and mobilization of lipids (Grahl-Nielsen et al., 2005) and has been documented in multiple marine mammal species, including ringed seal (*Pusa hispida*) (Strandberg et al., 2008), minke whale (*Balaenoptera acutorostrata*) (Olsen & Grahl-Nielsen, 2003), bowhead whales (*Balaena mysticetus)* (Budge et al., 2008), Baikal seal (*Pusa sibirica*) (Grahl-Nielsen et al., 2005), and others. The inner layer of blubber is the most metabolically active (Koopman et al., 2002), acting as the primary site of lipid mobilization (Strandberg et al., 2008) and is the most representative

of what has been consumed (Koopman et al., 2002; Budge et al., 2006; Guerrero & Rogers, 2017; Choy et al., 2019). The outer layer acts as an insulator and is important in streamlining and buoyancy (Olsen & Grahl-Nielsen, 2003). The middle layer of blubber has often been omitted in the study of marine mammal blubber stratification. However, it has been documented to contain a continuous gradient in fatty acid composition throughout the layer, with individual fatty acids increasing or decreasing in concentration from inner to outer blubber **(**Strandberg et al., 2008; Olsen & Grahl-Nielsen, 2003).

While diet plays a large role in the fatty acid composition of blubber (Iverson et al., 2004; Grahl-Nielsen et al., 2005), most marine mammal species exhibit a similar pattern of blubber composition (Koopman et al., 1996; Hooker et al., 2001; Olsen & Grahl-Neilsen, 2003; Jay et al., 2021; Jackson et al., 2022), (e.g., a greater abundance of 14, 16, 18 carbon MUFAs in the outer layer, and LCMUFAs and PUFAs in the inner layer), implying that similar physiological mechanisms are occurring across multiple species to support the functional roles of blubber. Water temperature has been shown to play a role in the thickness and stratification of blubber with cold-water species demonstrating thicker blubber with greater rates of stratification (Iverson, 2009; Samuel & Worthy, 2004; Koopman, 2007), potentially due to the need for a proper thermal gradient to keep the insulating properties of blubber functional (Liwanag et al., 2012). To achieve this gradient, the lipids need to remain in a fluid state (Pond, 1998). The body surface of marine mammals is only slightly above the temperature of the surrounding water (the average summer Arctic Ocean surface temperature is 2.82 ± 0.39°C (Carvalho & Wang, 2020)) (Irving & Hart, 1957; Hart et al., 1959; Melero et al., 2015; Favilla et al., 2022), and therefore, the outer layer of blubber needs fatty acids with a lower melting point (i.e.. fatty acids that will not solidify in colder temperatures) to maintain fluidity (Strandberg et al., 2008). The effect of

environmental temperature on fatty acid composition has the potential to explain inter-species similarities in blubber composition.

Beluga whales (*Delphinapterus leucas*) are a toothed whale belonging to the Monodontidae family that are near circumpolar in distribution (Stewart & Stewart, 1989) and are the most common Arctic odontocete (Laidre et al., 2015). They are thought to greatly influence ecosystem structure (Loseto et al., 2009), and act as an indicator species for the Arctic marine ecosystem (Choy et al., 2017). One of the world's largest beluga whale populations is the Eastern Beaufort Sea (EBS) population, last estimated at 38,451 (Marcoux et al., 2025). This population has one of the widest documented home ranges, spanning over 2000 kilometres, where they winter in the Bering Sea and summer in the Canadian Beaufort Sea (Storrie et al., 2023). In Canada, Inuvialuit, Inuit of the Canadian Western Arctic, continue their sustainable traditional harvest of EBS beluga in their summering grounds, and the Inupiat, Inuit Northern in Alaska USA, harvest them during the spring migration (Ostertag et al., 2018; Lowry et al., 1989).

Fluctuations in blubber thickness in adult male EBS belugas were observed throughout 2000-2015 (Harwood et al., 2014, Choy et al., 2017, Macmillan et al., 2023), and a decline in size-at-age of individuals in the EBS whale population was observed from 1989-2008, hypothesized to be due to changing prey availability (Harwood et al., 2014). Inuvialuit harvesters in Tuktoyaktuk have reported that over time beluga blubber thickness has declined (Ostertag et al., 2018), and both the Tuktoyaktuk and Paulatuk Hunter's and Trapper's Committees (HTCs) have recently reported whale health as a primary concern, and a desire to understand the cause of thinning blubber was stated as a priority by the Paulatuk HTC (FJMC priorities, 2022-2023; 2023-2024).

While beluga blubber has been used to examine diet via fatty acid profile analyses to characterize the main prey (Dahl et al., 2000; Thiemann et al., 2008; Smith, 2009; Loseto et al., 2009; Choy et al., 2019; Choy et al., 2020), the compositional changes of fatty acids throughout blubber depth or across differing blubber thicknesses have not been well studied in beluga whales. It is necessary to understand how a shift in blubber thicknesses linked to changes in prey availability and quality in a warming environment will impact beluga whales. Recent observations of thinning beluga blubber call to attention the need to understand the typical blubber fatty acid composition of the full depth of blubber from skin to muscle in beluga whales, and how changes in blubber thickness affect the fatty acid profiles. The ability to study belugas with varying blubber thicknesses provides an opportunity to examine how belugas will be physiologically affected by reduced blubber thickness. Therefore, the objectives of this study are to describe the vertical stratification of fatty acids in EBS beluga blubber, evaluate how fatty acid composition differs throughout blubber stratification and across thickness, and to explore how fatty acid melting point and delta-9 desaturation rates reflect the effect that environmental temperature has on fatty acid composition in beluga whales. Based on what is currently known about marine mammal blubber, I hypothesize that vertical stratification of fatty acids will be present throughout the depth of EBS beluga blubber and predict that it will reflect stratification patterns and delta-9 desaturation rates similar to other Arctic marine mammals, where the innermost blubber layer contains dietary and higher melting point fatty acids, and the outermost layer contains fatty acids with lower melting points and greater rates of delta-9 desaturation.

2.3 Methods

2.3.1 Study Area

Figure 2.1 Map of the study area in NT Canada, highlighting the Tarium Niryutait Marine Protected Area and the Anguniaqvia Niqiqyuam Marine Protected Area in orange. Communities of Tuktoyaktuk* and Paulatuk* are indicated. Source: Canadian Geographic (2017).

The EBS beluga whale population migrates from the Bering Sea to the Beaufort Sea in late May to early June where they occupy areas including the Mackenzie Estuary, the Amundsen Gulf, Viscount Melville Sound, and the Eastern Beaufort Slope in the Canada Arctic Basin, during summer months (Storrie et al., 2022; Richard et al., 2001; Mayette et al., 2023). They spend a few days aggregating in the Mackenzie Estuary, with peak use in early to mid July (Fraker &

Fraker, 1979), before expanding into their wide summer range. By December they will return to the Bering Sea to winter (Storrie et al., 2023).

During their seasonal migration, belugas are sustainably harvested by Inuvialuit, Inuit of Canada's Western Arctic, as they pass through coastal communities and camps in the Inuvialuit Settlement Region (ISR) (Ostertag et al., 2018). The Inuvialuit and their ancestors have hunted beluga for centuries, and the traditional subsistence has, and continues to be of important economic, dietary, and cultural importance (McGhee, 1988; FJMC, 2013). Inuvialuit have developed a deep understanding of EBS beluga behaviour, migration, and health from information passed down through generations (Berkes, 2017; Ovitz et al., 2023). Tuktoyaktuk is one of the most active communities that harvests belugas (Harwood et al., 2002; Harwood et al., 2020), and hunters will often bring the whales to Hendrickson Island to flense the animal, before bringing it back to Tuktoyaktuk for processing (Ostertag et al., 2018). Hunting in this area mainly occurs during the month of July and lasts four to six weeks (Harwood et al., 2002; Harwood et al., 2020).

In Paulatuk, a small community located in Darnley Bay (Arnold et al., 2011), belugas are harvested more opportunistically than in Tuktoyaktuk and were not hunted more consistently and in larger numbers until the 1990s, when beluga migration patterns changed and they began to migrate closer to Paulatuk (Ostertag et al., 2018). For this reason, harvesters in Paulatuk may be less selective in choosing whales to harvest than in other communities (Ostertag et al., 2018). Hunting in Paulatuk takes place when the sea ice clears and beluga enter the area, typically in late July or August, (Harwood et al., 2002); however, with an earlier ice melt, belugas are entering Darnley Bay sooner and thereby supporting earlier harvests (Harwood et al., 2020).

2.3.2 Sample Collection and Study Design

EBS belugas are co-managed by the Federal government (Fisheries and Oceans Canada (DFO)) and Inuvialuit (Fisheries Joint Management Committee (FJMC)) as per the Inuvialuit Final Agreement land claim (IFA, 1984). Inuvialuit have led on conservation management that includes the collection of beluga harvest data in cooperation with the local Hunters and Trappers Committees (HTCs) as described in the Beaufort Sea Beluga Management Plan (FJMC, 2024). Together, DFO and Inuvialuit established two marine protected areas in the ISR under Canada's Oceans Act (1997) (Loseto et al., 2018); the Tarium Niryutait Marine Protected Area (TN MPA, established in 2010) and the Anguniaqvia Niqiqyuam MPA (AN MPA, designated in 2016) (Figure 2.1). The TN MPA is comprised of three separate parcels in the Mackenzie Estuary and was created to conserve the EBS population of beluga whales and their supporting habitat. The AN MPA was established with an objective to conserve multiple marine species including the EBS beluga. The two MPAs are the first steps towards a Marine Protected Area Network that allows for a connection between the two areas as part of a conservation approach to support the migratory EBS belugas with diverse habitat use (Loseto et al., 2018).

Conservations efforts both under the MPAs and the Beaufort Sea Beluga Management Plan (FJMC, 2024) support the beluga harvest monitoring program and the collection of MPA monitoring indicators (FJMC, 2024) that include beluga data such as size, sex, age, blubber thickness as well as the collection of tissues for various monitoring programs (e.g. contaminants (Loseto et al., 2015)). Hunter-based beluga monitoring programs have taken place in the Mackenzie Delta since 1973, where number of whales harvested was recorded, and since 1980, body length, fluke width, sex, and age of whales have also been documented (Harwood et al., 2002). Data collection from beluga hunting in Paulatuk began in 1989 and mirrors data collected

by Tuktoyaktuk (Harwood et al., 2002). Every year, traditional whaling camps obtain data measurements and samples of beluga tissue, that are sent to the Freshwater Institute in Winnipeg for laboratory processing and analyses.

For this study, blubber samples were obtained from beluga whales harvested in the summers of 2022 and 2023 via the beluga monitoring program in partnership with Inuvialuit harvesters during their annual subsistence hunts from two locations: Hendrickson Island, near Tuktoyaktuk NT, and Darnley Bay, where the town of Paulatuk NT is located. As part of the harvest collection, condition of beluga whales was acquired qualitatively from the harvester and monitor reports, and quantitatively from blubber depth and girth measurements. A fist-sized blubber sample was dissected slightly dorsal to the pectoral flipper from each beluga. Each sample contained the full depth of blubber including the skin and blubber/muscle interface.

Of the whales harvested in 2022 and 2023, 20 blubber samples from individual whales were selected for analysis from 2022, 10 from Hendrickson Island, and 10 from the Paulatuk collection (Table 2-1). A total of 24 blubber samples from individual whales were used for analysis from 2023: 14 from Hendrickson and 10 from Paulatuk. Samples were selected for having varying degrees of blubber thickness. Any samples that looked compromised (yellow, wounded, smelled rancid) were omitted. Male belugas are typically sought out by hunters due to their larger size, as well as in efforts to maintain a stable beluga population by avoiding mother-calf groups (Sudlovenick et al., 2024). Therefore, most available beluga tissue samples come from male beluga whales, and of the 44 individual whales used in this study, only four were female, all from 2023. It was recognized that an unequal sex ratio may result in skewed findings. Therefore, both the inclusion and exclusion of females was explored during data exploration where it was determined that sex did not influence our results, and it was therefore decided that

analysis would go forward with the inclusion of the four females. Age data was available after selecting the tissues via dissection of eyeballs and examination of the lens (Pleskach et al., 2016); however, only adults are harvested which helped control for effects of age (Table 2-1). In this study, whales harvested by Tuktoyaktuk hunters and flensed at Hendrickson Island will be referred to as 'Hendrickson whales', and whales harvested by Paulatuk hunters in Darnley Bay will be referred to as 'Paulatuk whales'.

2.3.3 Body Condition Metric

Body length varied between beluga individuals from 330.2 cm to 459.7 cm. Previous research has demonstrated that body length and blubber thickness in beluga whales are positively related (Choy et al., 2017); therefore, to examine differences in fatty acid composition between varying blubber thicknesses while accounting for differences in body length, a standardized blubber thickness measure was recorded for each individual. This measure was determined by dividing total blubber thickness by total body length. This standardized blubber thickness measure was used as a body condition value. Three categories of body condition were created, where those individuals whose value fell into the lower third of the measured standardized blubber thickness range were considered a "thin" individuals, those belugas with values that were in the middle third of the range were considered "medium" individuals, and finally individuals in the upper third of the range of values were considered "thick" individuals (Table 2-1). The terms 'body condition' and 'standardized blubber thickness' may be used interchangeably in this study.

Table 2-1 Biological details of the 44 beluga whales sampled for this study.

2022 Whales Location	Avg age ± SE (years)	Avg length ± SE (cm)	Avg blubber thickness ± SE (mm)	N thin whales	N medium whales	N thick whales	N whales	N females	N males
Hendrickson	21.3 ± 2.1	407.4 ± 7.3	30.6 ± 4.4	5	2	3	10	0	10
Paulatuk	28 ± 4.6	396.6 ± 11.8	19.3 ± 1.8	4	6	0	10	0	10
2023 Whales									
Hendrickson	25.2 ± 2.8	394.5 ± 10.3	33.5 ± 2.8	4	5	5	14	4	10
Paulatuk	30.9 ± 5.4	410.2 ± 5.3	24.3 ± 2.7	9	1	0	10	0	10

2.3.4 Laboratory Analyses

At Fisheries and Oceans Canada, Freshwater Institute in Winnipeg, Manitoba, blubber samples were partially thawed and the outer sides that were exposed to air trimmed to dispose of oxygenated tissue. The full depth of each blubber sample was measured to the nearest millimetre from the skin/blubber interface to the blubber/muscle interface. Blubber samples were then subsampled at intervals of 5 mm from the muscle/blubber interface (inner layer) to the blubber/skin interface (outer layer). As the blubber samples varied in size, and were cut starting with the inner layer, the outer layer ranged in length from 2 to 5mm. Each layer was subsampled to equal 0.5 (± 0.1) grams and the Folch method for lipid extraction was followed (Folch et al., 1957). Each subsample was placed in a 20 ml test tube, and 7 ml chloroform diluted with 0.01% butylated hydroxytoluene (BHT) (v/v/w) followed by 3.5 ml methanol with 0.01% BHT was added to each test tube; test tubes were then placed in the freezer overnight.

The following day, test tubes were vortexed for 30 seconds and the blubber samples within the test tubes were squished using a glass rod to release the lipids from the connective tissue of the blubber. The leftover blubber tissue was removed using a pair of metal tweezers, placed in a separate vial and set aside to dry under the fume hood overnight. An amount of 3.5 ml of 0.7% NaCl was added to the test tubes containing the released lipids, which were then vortexed for 5-10 seconds and left to sit to allow the samples to separate into two layers. The top layers were discarded with a waste pipette prior to a scoop of $NaSO_4$ being added. Caps were secured on the test tubes which were then shaken and left to sit for five minutes to allow the $NaSO_4$ to absorb any excess water from the samples. The solvent was transferred to a clean 15 ml test tube, rinsed twice with 2 ml $CHCl_3$, and transferred to a third, pre-weighed test tube. Test tubes containing the solvent were placed in a hot water bath under an Organomation Associated

Inc. Nitrogen Evaporator (N-evap 111) to be evaporated under nitrogen. Total lipid weight was obtained using a four-decimal Mettler Toledo scale (model AL204) and total lipid percent was calculated by dividing lipid weight by total weight of the blubber sample (~0.5 g).

Transesterification of lipids occurred to prepare the fatty acid methyl esters (FAME) by adding 1.5 ml DCM with 0.01% BHT to vials (tube A) containing blubber lipids, followed by 3.0 ml of Hilditch reagent. Test tubes (tube A) were flushed with nitrogen, capped, and vortexed, and placed on a heating block at 100°C for one hour. Samples were allowed to cool to room temperature before 3 ml of hexane and 1ml of distilled water were added and subsequently capped and vortexed. Liquids in the sample test tubes were left to separate for approximately five minutes, and the top layer was moved to a new test tube (tube B). A scoop of $NaSO_4$ was added to test tube B, which was then capped and shaken, then the sample was transferred to a final, clean, pre-weighed test tube (tube C). To test tube A, 1 ml of hexane and 1 ml of distilled water was added, the sample was left to sit for approximately five minutes until the liquids separated, and then the top layer was transferred to test tube B to allow the $NaSO_4$ to absorb excess water. The sample was then transferred to test tube C. Another 1 ml of hexane was added to test tube A, and the procedure immediately above was followed once more. Test tube C was placed in the hot water bath and evaporated under nitrogen, until only FAME remained. FAME was weighed and diluted with hexane until a concentration of 100 mg FAME/ ml hexane was achieved before being transferred to a storage vial. Samples were then diluted to ~0.20 mg/ml of FAME to prepare for gas chromatography (GC) analysis which was performed on an Agilent Technologies 7890A GC equipped with a 30 m J&W DB-23 column (0.25 mm I.D; 0.15 μm film thickness). The GC was coupled to a Flame Ionization Detector (FID) that was set to 350°C. Hydrogen was used as the carrier gas which flowed at 1.25 mL/min for 14 minutes, then 2.5 mL/min for 5

minutes. The split/splitless injector was heated to 30°C and run in splitless mode. The oven program was as follows: 60ºC for 0.66 min; 22.82ºC/min to 165ºC with a 1.97 min hold; 4.56ºC/min to 174ºC and 7.61ºC/min to 200ºC with a 6 min hold. Agilent Technologies ChemStation software was used to quantify peaks, and fatty acid standards were obtained from Supelco (37 component FAME mix) and Nucheck (54 component mix GLC-463). Up to 73 fatty acid methyl esters were identified via retention time and known standard mixtures. Fatty acid chromatograms were checked and reintegrated if necessary.

Individual fatty acids were retrieved from the collected samples and described in shorthand nomenclature of A:BnX, where A represents the number of carbon atoms in the chain, B is the number of double bonds, and X is the positioning of the double bond in the carbon chain starting from the terminal methyl group (Gurr & James, 1980).

2.3.5 Data Analysis

Fatty acid results were expressed as a mass percent of total identified fatty acids. Those fatty acids at very low percentage of the total values (i.e. < 0.1%), were removed and remaining fatty acid percentages were recalculated so each sample total would equal 100%. After removal of low percentage fatty acids, 47 fatty acids remained for analysis. Statistical analysis was performed on R (version R-4.5.0) in R Studio (version 2025.05.0-496).

Due to the multivariable nature of the fatty acid data, a principal component analysis (PCA) was used to simultaneously explore relationships within all 47 fatty acids in multi-dimensional planes. Prior to PCA analysis, a value of one was added to each data point, which were then logarithmically transformed to normalize the data (Kenkel, 2006). The first two planes

(PC1, PC2) explaining most of the variability (approximately 63%) were used to summarize the fatty acid variables and examine relationships within the data. All data points were used in the PCA analysis; however, to reduce data complexity and assist with interpretation, the innermost layer was labeled as 'inner', the outermost as 'outer', and the remaining layers in between labeled as 'middle'. It is important to note that the number of layers varied among individuals and ranged from 2 to 11.

To test for differences between fatty acid types among blubber layers, total percentages of MUFAs, SFA, PUFAs, LCMUFAs, and short-chain MUFAs (SCMUFAs) were summed for the innermost, middlemost (as opposed to all middle layers), and outermost blubber layer and then were divided by 100 to convert into proportions. General linear mixed models (GLMMs) (glmmTMB function in the glmmTMB package) with a beta family and logit link function were run to determine if the proportion of each fatty acid type (dependent variable) differed between blubber layers (explanatory variable). Proportions of fatty acid types were highly correlated with one another in each layer; therefore, individual GLMMs were run for each fatty acid type. GLMMs were chosen because they can handle non-normally distributed data and can account for fixed and random effects. The inclusion of random effect variables allows the model to account for variation within individuals and therefore the potential pseudo-replication that may occur. This was necessary as multiple samples were taken from each individual (one for innermost layer, one for middle layer, and one for outermost layer), and therefore individual was included as a random effect variable. The beta distribution was chosen as it works best with proportion data, and the logit link function used as it is the default option for the beta distribution and fit with the final model. To determine legitimacy of the models, residual analysis was performed to check for normality, outliers, over/under dispersion, and homogeneity of variance. Heterogeneity

of variance was found present in all models except the PUFA model, so group-specific dispersion (dispformula argument as part of the glmmTMB function) was added to all but the PUFA model. Pairwise contrasts were then estimated (emmeans package with contrasts method for Tukey adjustment) to determine differences in fatty acid proportions among layers and to control for multiple comparisons within each model.

Proportions of each fatty acid type (SFAs, PUFAs, MUFAs, LCMUFAs, SCMUFAs, omega-3, omega-6, PUFA/MUFA ratio) (dependent variable) found in the entire blubber column were explored among the differing body conditions (explanatory variable) using general linear models (GLMs) (glmmTMB function in the glmmTMB package) with a beta family and logit link function. General linear models were chosen as the proportion data was not normally distributed, and individual models were run for each fatty acid type due to the correlation present between the fatty acid proportions. Residual analysis was performed to determine legitimacy of each model, which confirmed all model assumptions were met. Pairwise comparisons were tested to determine differences in body conditions.

To determine the tendency of each fatty acid to distribute through outer and inner blubber layers, a relative stratification index was calculated for each fatty acid, according to the calculation used by Olsen and Grahl-Nielsen (2003), and adjusted for our 5 mm sampling depth instead of 3 mm:

$$SI = (F_o - F_i)/[(F_o + F_i)/2]$$

Where:

F_o = % of each fatty acid in the outer 5 mm of blubber, and

F_i = % of each fatty acid in the inner 5 mm of blubber.

This calculation yields results wherein positive values indicate more enrichment in the outer layer of blubber, and negative values indicate more enrichment in the inner layer of blubber.

Environmental temperature is thought to impact blubber tissue temperature up to a depth of 21-24 mm from the skin (Hart & Irving, 1959). Therefore, outer blubber will have a lower temperature than blubber closer to the warm body core. To examine the effects of fatty acid melting point on stratification in the outer blubber, a second stratification index was created using the following equation:

$$SI_{outer}=(F_o-F_m)/[(F_o+F_m)/2]$$

Which is an adaptation of the previous stratification index equation, where:

F_o= % of each fatty acid in the outer 5 mm of blubber, and

F_m= % of each fatty acid in the blubber located 20-25 mm from the skin.

At a blubber depth of 21-24 mm from the skin, temperature fluctuations due to the environment should be minimal (Hart & Irving, 1959), and thus fatty acid composition at this depth should not be influenced by temperature. As blubber layers were cut into 5 mm sections, fatty acid values at the blubber depth of 20-25 mm (instead of 21-24 mm) were used as a reference point in the equation. A linear regression analysis was then performed between SI_{outer} values and the melting point of the saturated and monounsaturated fats found in the outer blubber. Of the seven SFAs and 13 MUFAs found in the outer blubber, only five SFAs and eight MUFAs had known melting points recorded in the literature, and therefore seven fatty acid SI values were omitted from the linear regression analysis.

Delta-9 desaturase is an enzyme that inserts a double bond onto a saturated fat, ultimately transforming it into a monounsaturated fat (Strandberg et al., 2008). It is produced as a result of cold exposure and is a major mechanism for cold adaptation in vertebrate lipids (Zerai et al., 2010). The tendency of a saturated fatty acid to undergo desaturase increases with cold exposure, and therefore, should increase with blubber layers situated further away from the body core. To determine the fatty acid Δ9-desaturation rate amongst blubber layers, a Δ9-desaturation index was created, which is the ratio of potentially endogenous Δ9-MUFAs to their matching chain-length SFA complement (e.g., C18:1n7 (MUFA) and C18:0 (SFA counterpart)). The Δ9-desaturation index is calculated according to the equation from Käkelä and Hyvärinen (1996):

$$\sum(\Delta 9 \text{ MUFAs}) : \sum(\text{SFA counterparts})$$

2.4 Results

The most abundant fatty acids found in the entire blubber column were 16:1n7, 18:1n9, 16:0, 20:1n9, 14:0, 22:6n3, 18:1n7, and 22:1n11. These fatty acids made up 59.5-78.9% of the total lipids in the samples. The MUFAs were by far the most abundant fatty acid type in the entire blubber, averaging 67.1% of the total lipid percent, followed by the SFAs at 19.0% and the PUFAs at 13.9%.

2.4.1 Characterizing Blubber Fatty Acid Profiles

A total of 44 individuals were sampled with blubber thicknesses ranging from 10 to 55 mm, yielding between two and 11 blubber layers, 5 mm thick, per individual. A principal component analysis was run to explore how FA profiles were expressed among the blubber layers. The greatest variation within the data was explained by principal component 1 (PC1) (48.8%). Adding coloured-coded ellipses around the data allowed visual analysis to determine that PC1 was related to blubber layer (innermost 5 mm, all middle samples, outermost 5 mm) (Figure 2.2).

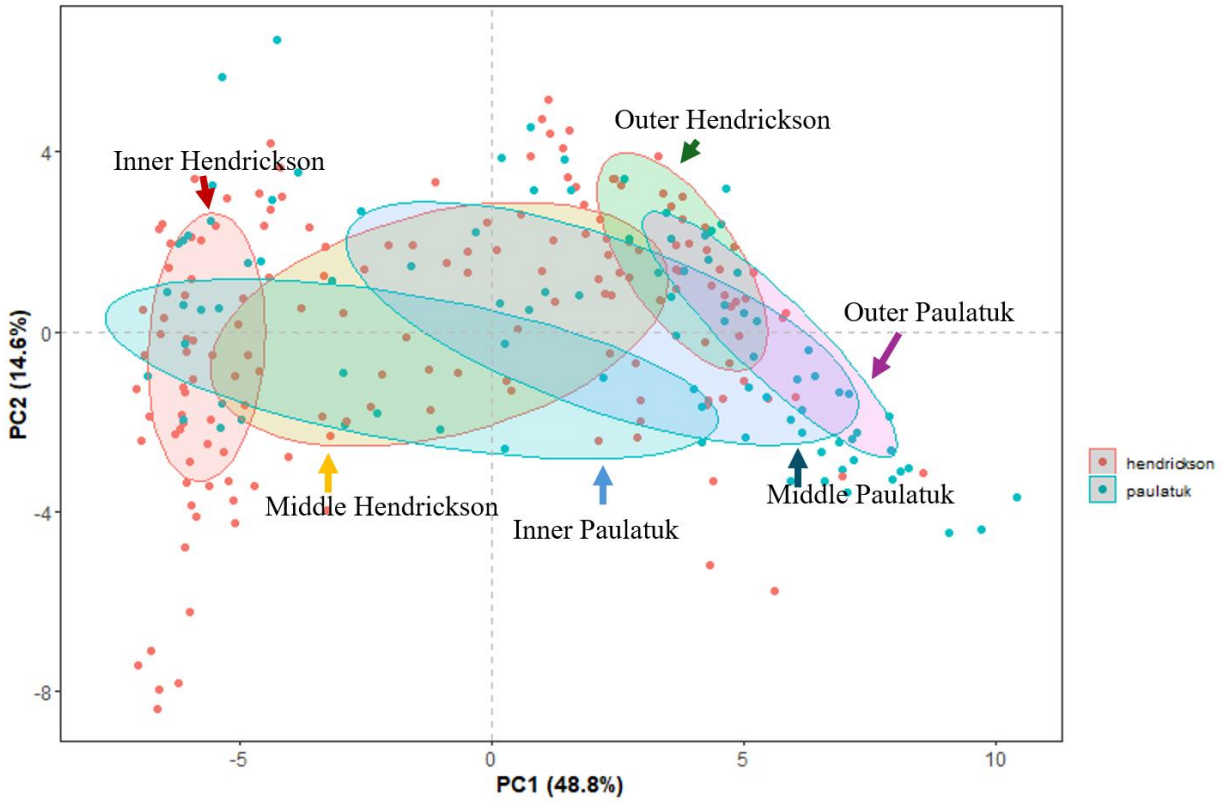

Figure 2.2. Principal component biplot of blubber samples from all individual belugas and every blubber layer from both locations. Samples taken at Hendrickson are represented as a red data point, while samples taken at Paulatuk are presented as a blue point. The ellipses surrounding the points are representative of those most similar from each blubber layer for each location and represent 50% of the data dispersion for each blubber layer.

A separation of ellipses representing the innermost, middle, and outermost blubber layers was observed with samples taken from Hendrickson Island; however, more overlap occurred in the samples taken from Paulatuk as seen in the overlapping ellipses (Figure 2.2). The inner layer ellipse for Paulatuk demonstrated a large overlap with the inner and middle layers from Hendrickson, while the middle Paulatuk layer overlapped with the middle and outer layers from Hendrickson, as well as the inner and outer layers from Paulatuk. The second principal component explained 14.6% of variation within samples and was found to relate to standardized blubber thickness (Figure 2.3). P-values indicated a significant negative relationship was found between PC2 inner ($p = 0.050$) and middle ($p = 0.002$) blubber layers to the standardized blubber thickness/body condition values, yet the R^2 values determined these relationships to be weak (inner: $R^2 = 0.09$; middle: $R^2 = 0.06$). There was no significant relationship between outer layer and standardized blubber thickness/body condition values. The slopes of the relationships between body condition and fatty acids were -30.0 for the inner layer, -22.9 for the middle layer, and 25.2 for the outer layer.

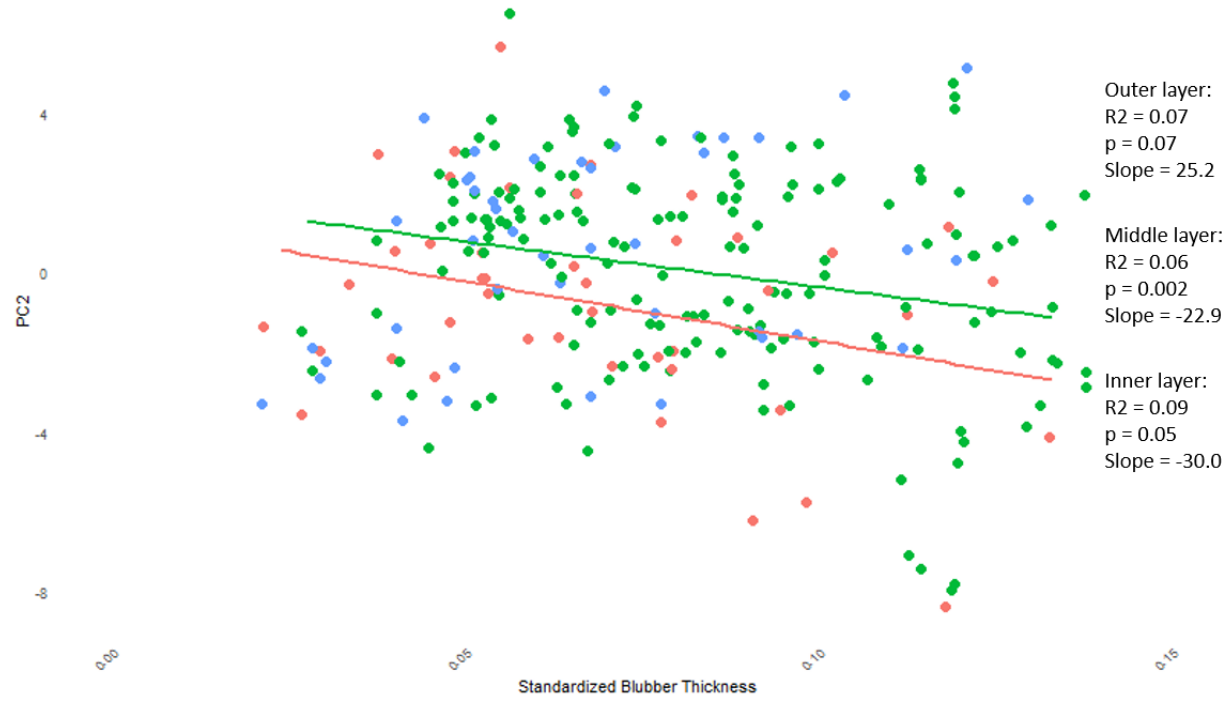

Figure 2.3. Relationship between standardized blubber thickness values of each beluga whale against PC2 (from Figure 2.2 PCA) value. The innermost and middle blubber layers had a statistically significant, yet weak relationship with PC2, while the relationship between the outer blubber layer and PC2 was not significant.

General linear mixed models determined that proportions of MUFAs and PUFAs differed between inner and outer ($p < 0.001$ for both MUFAs and PUFAs), and middle and outer blubber layers ($p < 0.001$ for both MUFAs and PUFAs) (Figure 2.4, Table S-2). The inner layer contained the largest amount of PUFAs, followed by the middle layer, while the outer layer contained the least (Figure 2.4). The inner layer contained a slightly higher amount of saturated fats, but the difference was not statistically significant according to the model. The outer blubber layer contained an overall higher percentage of total MUFAs (Figure 2.4). The proportions of LCMUFAs and SCMUFAs differed significantly between each blubber layer ($p < 0.001$, Table S-2). However, the inner layer of blubber contained a much higher percentage of LCMUFAs,

and the outer layer held the highest percentage of SCMUFAs (Figure 2.4). The middle layer of blubber contained an intermediate amount of MUFAs, LCMUFAs, SCMUFAs, and PUFAs (Figure 2.4).

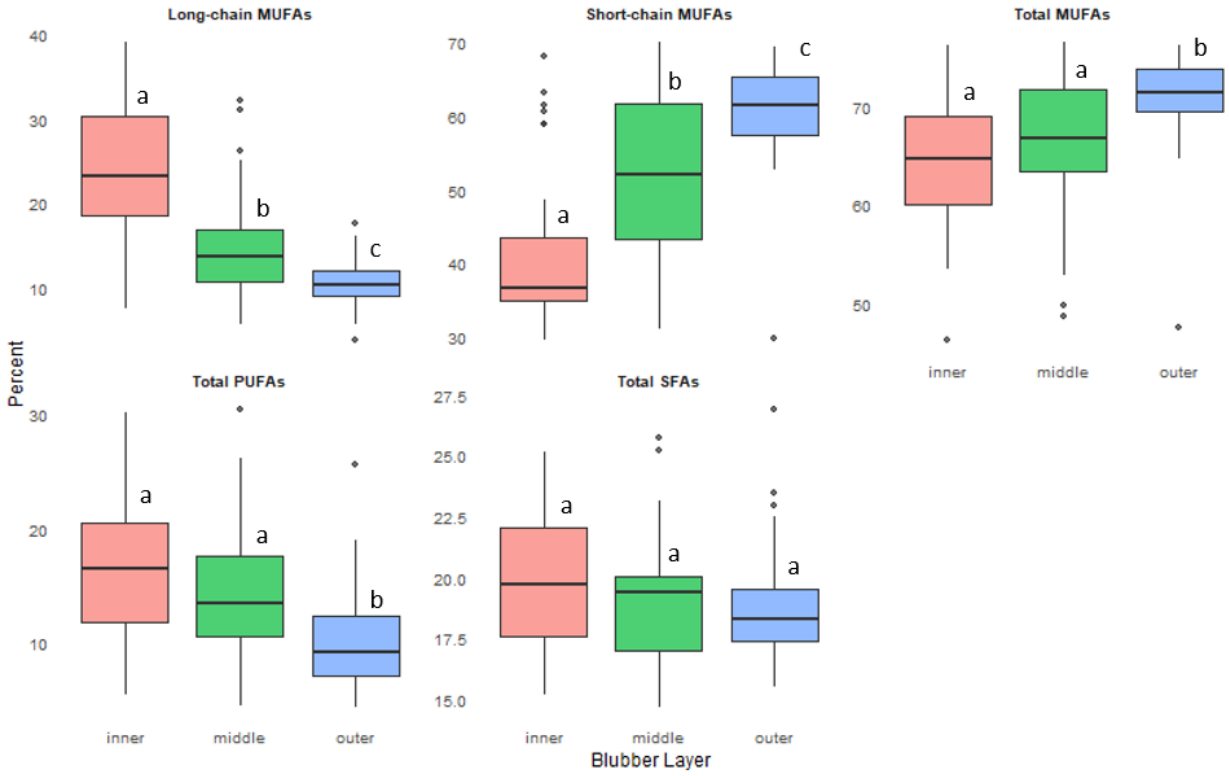

Figure 2.4. Average percentages found of long-chain monounsaturated fats (Long-chain MUFAs), short-chain monounsaturated fats (Short-chain MUFAs), total monounsaturated fats (MUFAs), total polyunsaturated fats (PUFAs), and total saturated fats (SFAs) found in the innermost, middlemost, and outermost blubber layers. Short-chain MUFAs and total MUFAs increased from inner to outer layer, and long-chain MUFAs, total PUFAs decreased from inner to outer layer. Boxes represent the interquartile range (IQR) (middle 50% of data points), the line inside the box represents the median, the whiskers extend to the smallest and largest values within 1.5 x IQR of the box, and the points outside the whiskers are outliers. Boxes in each individual plot that do not share a letter are significantly different ($p < 0.05$) from one another.

General linear models revealed that the proportions of SFAs, MUFAs, PUFAs, PUFA/MUFA ratio, omega-3s, and omega-6s did not significantly differ between any body conditions (Table S-3). There were, however, significant differences in the proportion of LCMUFAs found

between all body conditions ($p < 0.05$, Table S-3), and in the proportions of SCMUFAs between thin and thick individuals ($p < 0.01$, Table S-3) (Figure 2.5). Thick individuals had the largest proportion of LCMUFAs, and smallest proportion of SCMUFAs, while thin individuals had the greatest proportion of SCMUFAs and lowest proportion of LCMUFAs (Figure 2.5). Individuals with medium body condition contained intermediate amounts of LCMUFAs and SCMUFAs.

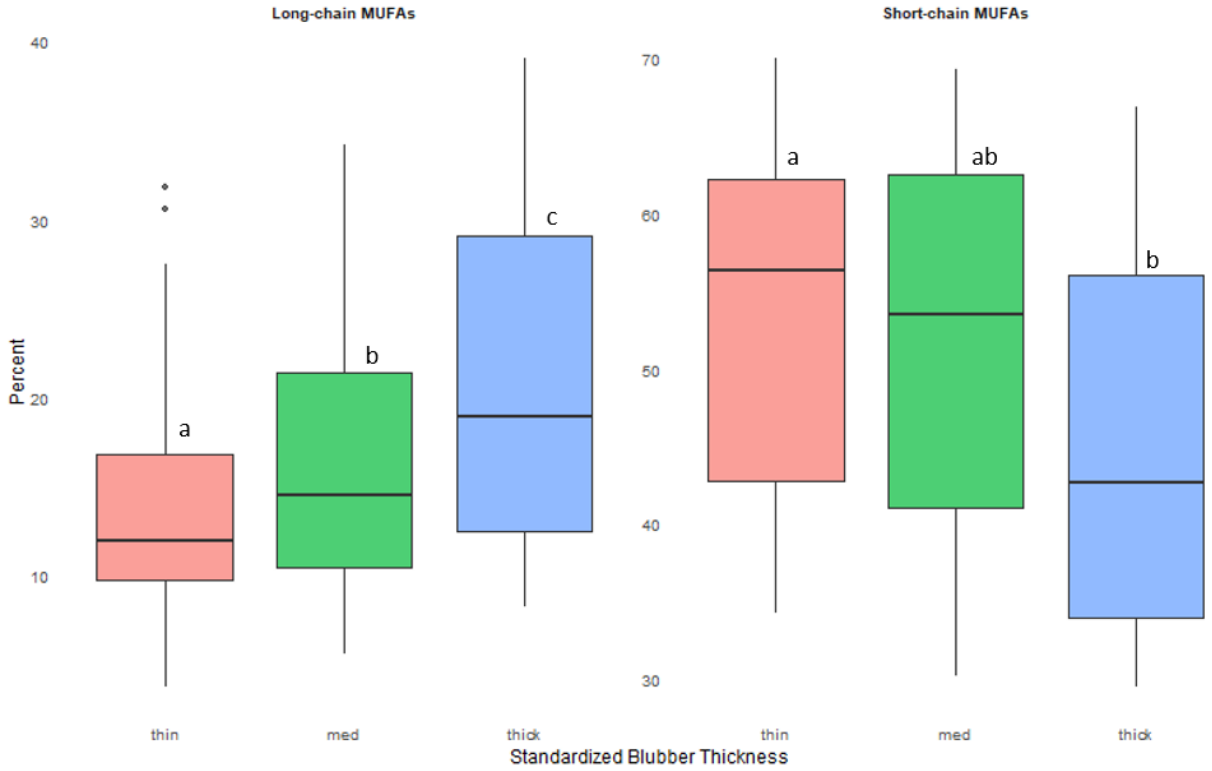

Figure 2.5. Average total percentages of long-chain monounsaturated fatty acids (Long-chain MUFAs) and short-chain monounsaturated fatty acids (Short-chain MUFAs) in the entire depth of blubber found in beluga whales with thin, medium, or thick body condition/ blubber thickness. A greater proportion of long-chain MUFAs were found in whales with thick blubber, and a greater proportion of short-chain MUFAs were found in whales with thin blubber. Boxes represent the interquartile range (IQR) (middle 50% of data points), the line inside the box represents the median, the whiskers extend to the smallest and largest values within 1.5 x IQR of the box, and the points outside the whiskers are outliers. Boxes in each individual plot that do not share a letter are significantly different ($p < 0.05$) from one another.

2.4.2 Stratification Index (SI)

The most stratified fatty acids were C14:1n5, C16:4n3, and C15:0 iso, all of which were found highly enriched in the outer blubber (Figure 2.6). The fatty acid with the greatest enrichment in the innermost layer was C22:1n7. Positive Stratification Index (SI) values indicate fatty acid enrichment in the outermost layer of blubber, while negative SI values indicate inner blubber layer enrichment. A larger absolute SI value is indicative of a higher ratio of abundance in that layer compared to the opposite layer. Non-dietary shorter-chain MUFAs and SFAs accumulated more in the outer layers of blubber, while dietary and long-chain MUFAs and SFAs, C16:0 and C18:0, accumulated in the inner layers. Long-chain PUFAs accumulated in the inner layers, while shorter chain PUFAs, aside from C16:3n4, accumulated more in the outer layers. SI values for C14:1n9 and C18:2n6c were close to zero, indicating an approximate even distribution found in the outer and inner layer.

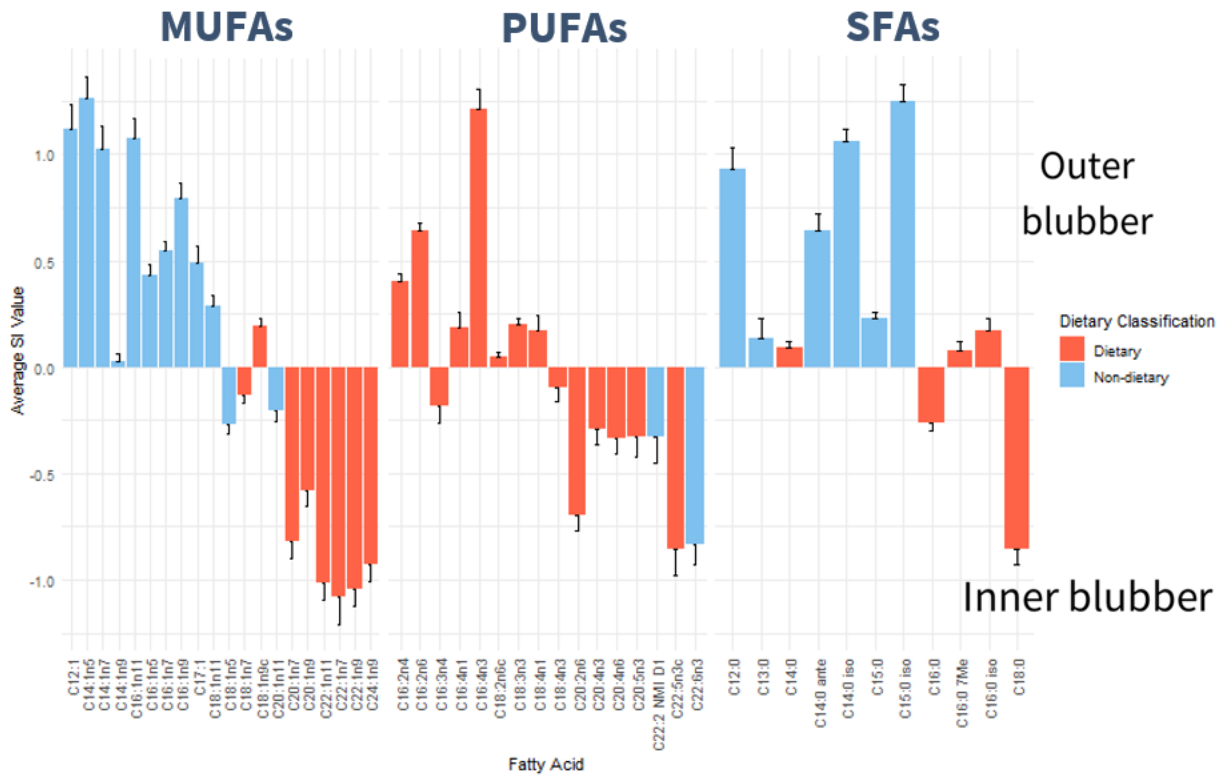

Figure 2.6. Stratification index (SI) for all beluga whales (n= 44) with average SI values for each fatty acid to examine deposition in inner or outer blubber. Positive SI values show enrichment in outer blubber, negative values in inner blubber.

Most fatty acids followed a similar stratification pattern among the three body condition groups (Figure 2.7). However, the thin whales tended to have smaller absolute SI values for most fatty acids, except the long-chain PUFAs, whereas the thicker whales exhibited less stratification. Additionally, the SI value for C18:4n3 and C20:5n3 was positive for thick whales, indicating a greater outer blubber enrichment, while the SI values were negative for thin and medium whales, indicating an inner blubber enrichment.

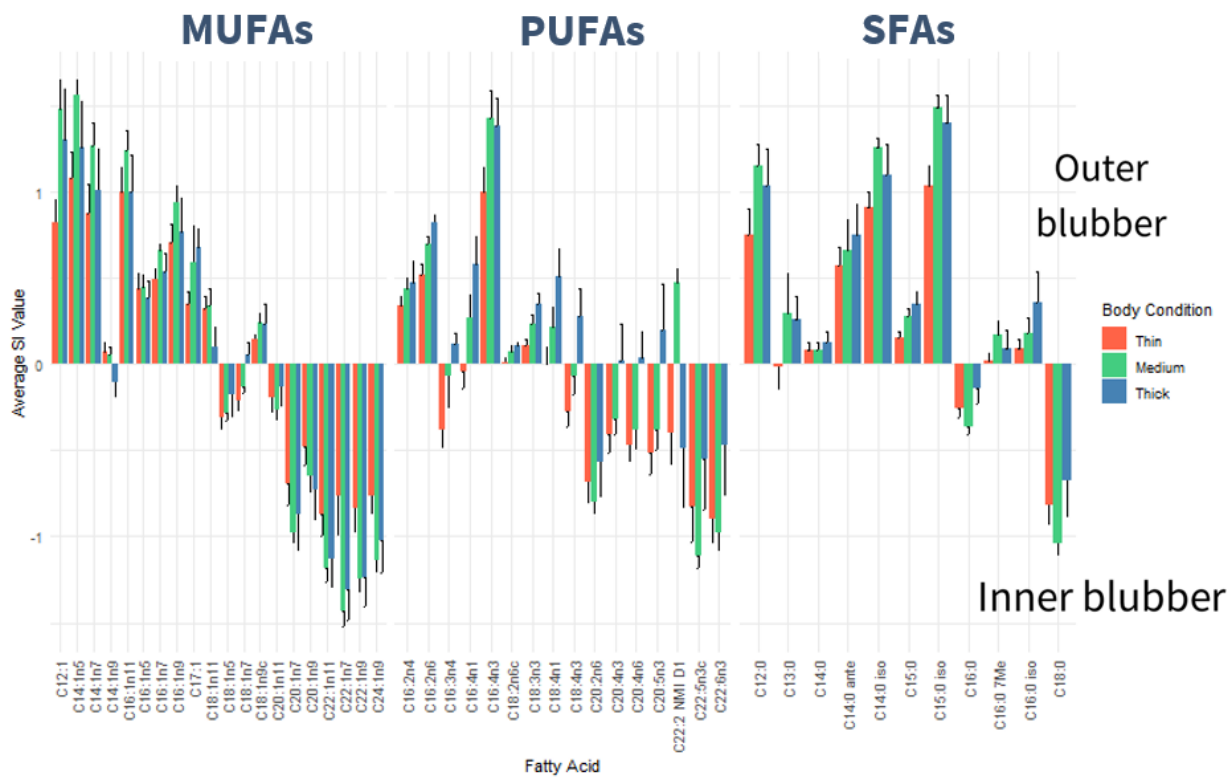

Figure 2.7. Stratification index (SI) examining tendency of fatty acids to distribute in inner and outer layers based on body condition/ blubber thickness. A positive SI value indicates outer blubber layer enrichment, and a negative SI value indicates inner blubber layer enrichment.

2.4.3 Fatty Acid Melting Point and Stratification

For both SFAs ($R^2 = 0.99$, $p < 0.001$) and MUFAs ($R^2 = 0.72$, $p < 0.01$), a strong relationship was shown between fatty acid melting point and average stratification index value for the outermost blubber layer (SI_{outer}) (Figure 2.8). Fatty acids with higher melting points contained lower (more negative) SI values.

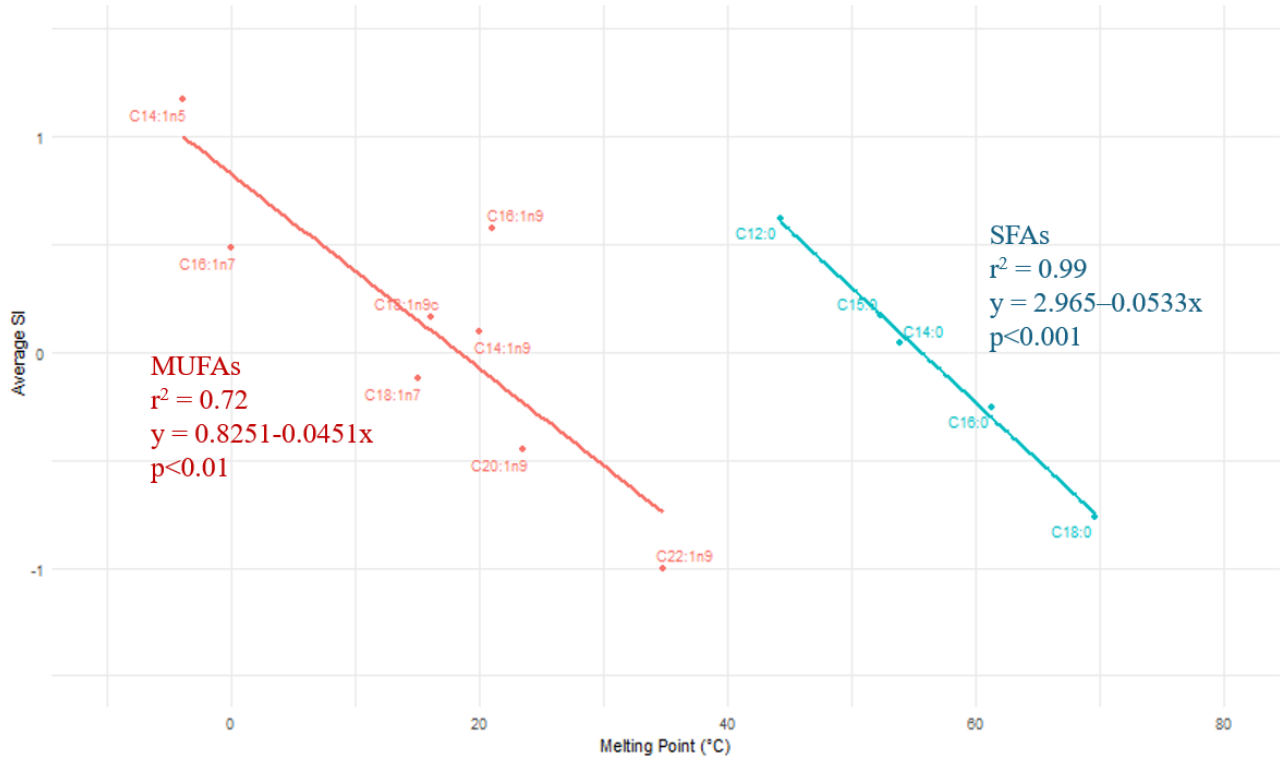

Figure 2.8. Fatty acid melting point (degrees Celsius) on average stratification index (SI_{outer}) values for saturated fatty acids (SFAs; $r^2 = 0.99$, $p < 0.001$) and monounsaturated fatty acids (MUFAs; $r^2 = 0.72$, $p < 0.01$) in the outer blubber layer from all belugas. The melting point of SFAs and MUFAs increases with decreasing SI value.

2.4.4 Delta-9 Desaturase

In all body conditions/standardized blubber thicknesses, the delta-9 desaturase (D9) values were higher towards the outermost layers of blubber and decreased towards the inner blubber (Figure

2.9). The D9 value in both the medium and thick individuals plateaued around 25-30 mm from the skin, where values did not further decrease. D9 values slightly increased at the 35-40 mm and 50-55 mm distances from the skin in thick individuals, and at 40-45 mm from the skin in medium individuals. D9 values in thin individuals continuously decreased towards the innermost layers of blubber then slightly rose at the distance of 25-30 mm from the skin.

Subsamples from 0-5, 5-10, and 10-15 mm from the skin in thin and medium individuals had D9 values significantly different than the reference point (20-25 mm from the skin) (Mann-Whitney U Test, $p < 0.05$). Thick individuals had D9 values significantly different from the reference point at the 0-5 and 5-10 mm sample points ($p < 0.05$).

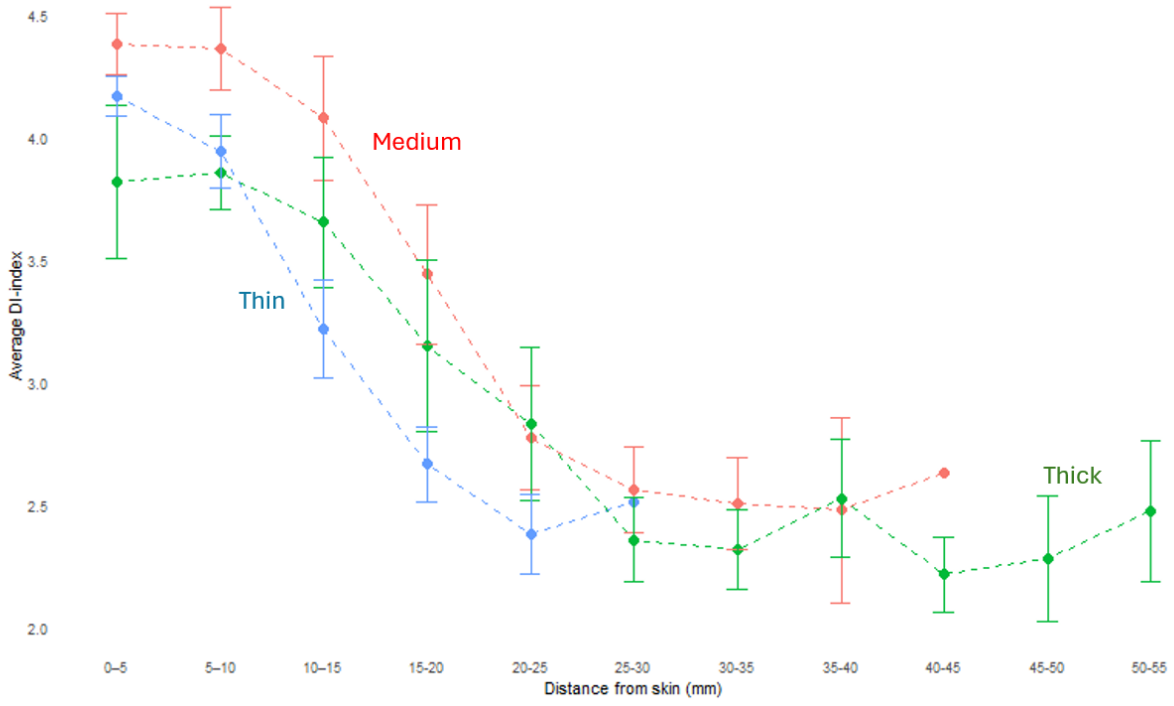

Figure 2.9. Each point represents the average delta 9-desaturase index value against distance sample was taken from the skin for belugas of differing standardized blubber thickness (thin: n=22; medium: n= 13; thick: n= 9). Subsamples from 0-5, 5-10, and 10-15 mm from the skin in thin and medium individuals had D9 values significantly different than the reference point (20-25 mm from the skin) (Mann-Whitney U

Test, $p < 0.05$). Thick individuals had D9 values significantly different from the reference point at the 0-5 and 5-10 mm sample points ($p < 0.05$).

2.5 Discussion

Vertical stratification of fatty acids was found in the blubber of beluga whales, particularly in the proportions of MUFAs and PUFAs that differed between blubber layers. Fatty acids with higher melting points were found more abundantly in the innermost layer, while fatty acids with lower melting points were more abundant in the outermost layer, supporting the concept of the influence of temperature on blubber composition, specifically by demonstrating how each blubber layer contains fatty acids with appropriate melting points to maintain fluidity in accordance with the variability of the depth of permeation of cold temperatures from the surrounding environment. The hypothesized influence of environmental temperature on fatty acid stratification was further emphasized by a decrease in delta-9 desaturase the further the blubber was from the skin. That is, the delta-9 desaturase process supported the stratification of blubber to reflect ideal melting points required to meet the physiological needs of the blubber whereby the fatty acids with higher melting points were located closer to the warm body core where they could remain fluid. Stratification patterns were similar between individuals with differing body condition/standardized blubber thicknesses; however, the proportions of long-chain and short-chain MUFAs varied between individuals among the thin or thick blubber groups, demonstrating the influence of blubber thickness on fatty acid stratification.

Beluga blubber was predominantly composed of MUFAs (67%), followed by SFAs (19%) and then PUFAs (14%), and had the highest fatty acid concentrations of 16:1n7, 18:1n9, 16:0, 20:1n9, 14:0, 22:6n3, 18:1n7, and 22:1n11. These findings are consistent with other studies

of marine mammal blubber, including beluga blubber. MUFAs were found to be the predominant fatty acid type in sperm whales (*Physeter macrocephalus*), Pacific walrus (*Odobenus rosmarus divergens),* southern elephant seals (*Mirounga leonina*), finless porpoise (*Neophocaena phocaenoides*), and beluga (Jackson et al., 2022; Jay et al., 2021; Best et al., 2003; Tang et al., 2021; Dahl et al., 2000). Dahl et al. (2000) similarly found SFAs 14:0 and 16:0, the MUFAs 16:1n7, 18:1n9 and 20:1n9, and PUFAs 22:6n3 as the major fatty acids extracted from the full column of beluga blubber. However, they also found 20:5n3 as one of the most abundant fatty acids, while the whales in our study contained higher concentrations of 18:1n7 and 22:1n11. Beluga blubber sampled from across the Canadian Arctic as well as near Svalbard has been shown to contain high levels of 16:1n7, 18:1n9, 20:1n9, 14:0, and 16:0 (Thiemann et al., 2008), fatty acids that correspond to the top five most abundant in our dataset. Pacific Walrus blubber and ringed seal blubber (*Pusa hispida*) (Jay et al., 2021; Strandberg et al., 2008) have also been demonstrated to contain high levels of the MUFAs 16:1n7, 18:1n9, and 18:1n7, as well as high abundance of the omega-3 fatty acids 20:5n3, 22:5n3, 22:6n3, and SFA 16:0.

2.5.1 Blubber stratification

The PCA revealed the inner, middle, and outer layers of blubber to be chemically distinct from one another, as observed along PC1, with some differentiation between locations as well. In this analysis, all data retrieved from the layers in between the innermost and outermost layers were labelled as ‘middle’, which may explain some of the overlap observed between ellipses. The gradient in fatty acid composition found throughout the middle layers aligns with observations in ringed seals and leopard seals (*Hydrurga leptonyx*), where the middle layer of blubber acted as a transition layer between the inner and outer layer (Strandberg et al., 2008; Guerrero et al., 2016). Differences in composition between locations might represent variability in prey type and

availability, as regional differences in fatty acid signatures have been previously reported in beluga whales across the Canadian Arctic (Thiemann et al., 2008). Additionally, differences in blubber thickness/ body condition may explain some of the differences between the two locations with respect to the degree of blubber layer overlap along PC1, as whales from Paulatuk had an overall lower body condition/ thinner blubber than whales from Hendrickson. The thinner blubber prevalent in Paulatuk whales coincided with fewer middle blubber layers, and because of this, the middle layers are more likely to overlap with the innermost or outermost layer as the compositional change occurs more quickly from inner to outer layer.

A divergence in fatty acid composition between the innermost and outermost layers was expected, given the findings from other marine mammals, such as bottlenose dolphins (*Tursiops truncatus*), minke whales (*Balaenoptera acutorostrata*), sperm whales (Samuel & Worthy, 2004; Olsen & Grahl-Nielsen, 2003; Jackson et al., 2022), as well as beluga (Bernier-Graveline et al., 2021; Dahl et al., 2000, Thiemann et al., 2008) which have all demonstrated compositional changes throughout blubber depth. The innermost and middle layers were found to be significantly related to standardized blubber thickness, but the outer layer was not. The greater variability in the inner and middle layers with changes in blubber thickness, and the relative consistency in the composition of the outermost layer provides further evidence towards the accepted theory that the innermost layer is the most metabolically active, while the outermost layer largely plays a structural and more stable role where fatty acid mobilization is less frequent.

The proportions of fatty acid types found in each layer align with findings in past studies of marine mammal blubber. The innermost layer contained the greatest concentration of PUFAs, most of which are dietary in origin. No statistically significant difference was found in the proportion of SFAs between any layers. Stratification of SFAs throughout blubber depth has

been found in other cetaceans, such as minke and bowhead whales (Budge et al., 2008, Olsen & Grahl-Nielsen, 2003). The outer layer of blubber was dominated by the MUFAs, similar to other findings in the literature (Jackson et al., 2022). However, when MUFAs were examined by chain length, the inner layer contained the highest percentage of LCMUFAs, while more SCMUFAs resided in the outer layer. The MUFAs comprised 67% of the blubber, 50% of which were short-chain, and 17% long-chain. The LCMUFAs are mainly obtained by diet, while SCMUFAs are endogenously created, providing explanation of why the LCMUFAs are found in the innermost, metabolically active layer while the endogenously created, more stable SCMUFAs are found in the outer layer. The greater proportion of SCMUFAs (50%) of the total MUFAs explains why most MUFAs are primarily found in the outer blubber layer. While the proportion of SFAs did not significantly differ between layers, the middle layer of blubber contained an intermediate percentage of LCMUFAs, SCMUFAs, and PUFAs in between the amounts found in the innermost and outermost layer, further demonstrating how the middle blubber may act as a transitionary layer in beluga whales, similar to ringed seals and leopard seals (Strandberg et al., 2008; Guerrero et al., 2016). This is, however, contradictory to findings in minke whales, where some LCMUFAs (20:1n9 and 22:1n11) were found in highest abundance in the middle blubber layer (Olsen & Grahl-Nielsen, 2003).

2.5.2 Body condition and FA composition

There were no differences in the ratio of PUFA:MUFA in whales in body condition category of thick relative to thin, contrary to findings by Bernier-Graveline et al. (2021) where a significantly larger PUFA:MUFA ratio was found in St Lawrence Estuary belugas in good body condition compared to moderate and poor body condition whales. Bernier-Graveline et al. (2021) also found a greater abundance of the omega-6 fatty acid, arachidonic acid, and omega-3 fatty acids,

EPA, and DHA in whales in good body condition, whereas no differences in overall proportion of omega-3 or omega-6s were found between whales with differing body condition in this study. This result was surprising as omega-3 and omega-6 fatty acids are mainly acquired from diet and thus would be expected to be found in higher amount in whales with larger lipid stores i.e. greater body condition. All whales examined in this study did, however, contain a larger proportion of omega-3 fatty acids (80.2% of total PUFAs) compared to omega-6s (9.4% of total PUFAs), similar to findings in bowhead whales where omega-3s made up 79% of total PUFAs and omega-6s were 17% of total PUFAs (Budge et al., 2008).

The only differences in total proportion of fatty acids between body condition groups were in the LCMUFAs and SCMUFAs. The LCMUFAs were significantly different among the body condition groups, as the thick whales had the most LCMUFAs, and the thin whales had the least. The proportion of SCMUFAs significantly differed between the thin and thick whales, as the thin whales contained more SCMUFAs than the thick whales. SCMUFAs can be endogenously created, whereas LCMUFAs are typically acquired by diet. The C20 and C22 MUFAs in particular are well known markers for a diet consisting of Arctic cod *(Boreogadus saida)*, a preferred prey item of EBS beluga, as identified using fatty acid analyses from harvested belugas sampled at Hendrickson Island and near Paulatuk (Loseto et al., 2009; Choy et al., 2020) and was supported in stomach content evaluations (Quakenbush et al., 2015). The finding of greater proportions of SCMUFAs in the thin whales thus may provide evidence that the thin whales were not feeding or at least not feeding as much on LCMUFA-rich Arctic cod. If the animal was not receiving enough LCMUFAs from diet, an increase in the endogenous synthesis of SCMUFAs, as well as the influence of environmental temperature, discussed later on, could explain the higher proportion of SCMUFAs found in thin whales.

2.5.3 Stratification Index

Fatty acids C14:1n5, C16:4n3, and C15:0 iso, found in the outer layer, were the most stratified fatty acids in the blubber. Similarly, Waugh et al. (2014) found C14:1n5 to be one of the most stratified fatty acids in humpback whale (*Megaptera novaeangliae*) blubber. However, they also found that the LCMUFAs, PUFAs, and SFAs exhibited lower degrees of stratification, whereas the beluga blubber used in this study contained rather highly stratified fatty acids in all fatty acid groupings, particularly when SI values in differing body condition were examined.

Overall, the whales with a medium body condition value contained more highly stratified fatty acids, and whales with thin body condition had less stratified fatty acids, demonstrating a more even distribution across blubber layers. The lower stratification seen in the thin whales may be explained by the same concept that explained the greater overlap among the ellipses for inner, middle, and outer layers in the PCA for Paulatuk individuals. That is, Paulatuk whales were overall thinner than Hendrickson whales, and thin whales will contain fewer middle layers than thick whales. If the middle layers are acting as a transition layer from inner to outer blubber, then individuals with fewer middle layers will experience a quicker transition of fatty acid composition from inner to outer blubber, resulting in a greater overlap of fatty acids. A larger rate of change in fatty acid composition throughout the blubber depth in thin individuals compared to medium or thick has also been demonstrated in ringed seals (Strandberg et al., 2008).

Thick whales in our study demonstrated a lower rate of PUFA stratification, and in some cases contained PUFAs with an SI value that indicated outer blubber enrichment when thin and medium whales contained inner blubber enrichment for the same fatty acids. One example of this can be seen in 20:5n3 or EPA, which is commonly referred to as an essential fatty acid as it is

obtained from diet, aside from the small amounts that can be synthesized from precursor fatty acids (Das, 2006). As an important dietary fatty acid, it is known to be predominantly stored in the innermost, most metabolically active blubber layer, and has also been shown to be one of the most readily mobilized fatty acids during energy depletion (Iverson et al., 1995). As a dietary, easily mobilized fatty acid, it would be expected that it would be primarily found in the innermost layer, as was the case in humpback whales (Waugh et al., 2014), and, in this study, the case in which all body condition types were combined (Figure 2.6). While proportion of total PUFAs found in the blubber did not differ between body condition types, those with thick blubber will have an overall larger store of lipids, which might be indicative as to why they are able to store them in a less active blubber layer. Additionally, if whales with good body condition are in a state of net positive calorie intake as opposed to negative intake and are not relying on blubber stores for nutrition, which may be the case in good body condition whales, this may help to explain why thicker whales are storing more of this fatty acid in the outer blubber layers. In a study of blubber stratification in ringed seals of differing blubber thicknesses (Strandberg et al., 2008), 20:5n3 was found to accumulate more in the outermost layer of blubber when all blubber thicknesses were combined; however, the reasoning for why this occurred was not provided.

2.5.4 Delta-9 desaturation

The cold Arctic marine waters likely influenced the fatty acid composition of the blubber, whereby fatty acids with higher melting points were found more abundantly in the inner layers of blubber near the warm body core, to maintain fluidity. As melting point of the fatty acids decreased towards the outer blubber, the SI value increased. This supports the concept that the

blubber lipids need to remain in a semi-fluid state for peak insulative properties and is further demonstrated by the results of the delta-9 desaturation analysis.

Regardless of the body condition group, delta-9 values were the highest in the outermost layers of blubber and decreased towards the muscle, and in the whales with thick and medium blubber, leveled out between 20 and 30 mm from the skin. These findings align with delta-9 measurements calculated from the blubber of ringed seals (Strandberg et al., 2008), where delta-9 values plateaued around 21-24 mm from the skin, the blubber depth in which the temperature from the environment should no longer permeate the tissue (Hart & Irving, 1959). Despite variations in body condition characterized here by standardized blubber thicknesses, the delta-9 values plateaued around the same point in all individuals, supporting the hypothesis that fatty acid composition of the blubber is at least in part influenced by the temperature of the surrounding environment and the need to maintain core body temperature. A decreasing delta-9 value from outer to inner blubber was also found in sea lions and phocids (Liwanag et al., 2012), but interestingly, delta-9 values did not differ significantly between blubber layers in fur seals (Liwanag et al., 2012), an Arctic species who contains blubber but relies primarily on fur for insulation. Perhaps in fur seals the insulative fur enables the blubber to remain at a temperature warm enough to maintain fluidity without the need for endogenous desaturation. However, this same study revealed an overall lower proportion of unsaturated fatty acids in the fur seal blubber compared to sea lion and phocid blubber, suggesting fur seal blubber may be less adapted for cold temperatures in general, and the activation of delta-9 desaturation by cold temperatures may be a process only found in cold-water species that rely exclusively on blubber for insulation.

In individuals with thick blubber (better body condition), the rate of delta-9 desaturation ceased before reaching the innermost blubber layer. However, if the full depth of an individual's

blubber is less than 20 mm, as was the case for some individuals in this dataset (two individuals had a total blubber depth of 10 mm), the external temperature would presumably permeate through the entire depth of blubber, meaning endogenous desaturation of lipids would be required all the way throughout to the innermost blubber layer as a means to avoid lipid solidification that may occur in fatty acids with higher melting points. The innermost layer of blubber in all individuals was found to contain higher amounts of fatty acids with high melting points like the LCMUFAs and PUFAs. Thick whales had higher proportions of LCMUFAs while thin whales had a greater proportion of SCMUFAs. Given that the rate of delta-9 desaturation is relatively constant despite differences in blubber thickness, the difference in proportion of LCMUFAs (with a high melting point), and SCMUFAs (with a low melting point) between thick and thin individuals is a plausible explanation of how a whale is able to maintain lipid fluidity in the blubber even when a reduced thickness allows the external environment to affect the full depth, and may explain why the thicker whales contained a higher proportion of LCMUFAs and the thin whales a higher proportion of SCMUFAs. The higher melting point of LCMUFAs may have a limited impact in thicker whales as they are stored in the deeper layers of blubber where the external temperature does not impact the tissue. However, if the whales' blubber is too thin, the entire depth of blubber can be affected by the external temperature, essentially cooling the tissue, and therefore fatty acids with lower melting points are needed for the lipids to remain in a semi-fluid state, making it disadvantageous for whales with thin blubber to store large amounts of LCMUFAs.

The seemingly strong influence of the thermoregulatory role of blubber on fatty acid composition shown here has been demonstrated in other studies, as well. For instance, beluga whale calves with differing body sizes have been found to contain large differences between

dietary fatty acid signature and blubber fatty acid signature, with smaller calves having the largest difference from full size whales (Birkeland et al., 2005). While examining fatty acid composition in the inner layer of varying age (and thus varying size) beluga calves, Birkeland et al. (2005) found that the youngest, and therefore the smallest calves had the lowest proportion of LCMUFAs in the inner blubber layer, which increased abruptly in the larger calves. The fatty acid composition of the blubber gradually changed to be more like their mothers with age. Temperature effects on the fatty acid composition in the blubber of phocids have also been speculated on, as harbour (*Phoca vitulina*) and grey seals (*Halichoerus grypus*) inhabiting more temperate habitats have been shown to contain less SCMUFAs than ringed seals inhabiting Arctic habitats (Fredheim et al., 1995; Severinsen et al., 2000). Fatty acid composition of the blubber in marine mammals is often described as being largely influenced by the fatty acid composition of prey; however, results from this study and others showcasing the large variability of fatty acids in relation to melting point suggest that the effects of environmental temperature may play a larger role in the composition of the blubber than previously thought.

2.6 Conclusion

The fatty acid stratification patterns exhibited in beluga whales in this study were similar to those that have been previously reported for other Arctic marine mammals. Fatty acid melting point had a large influence on fatty acid composition of the blubber, despite assumed differences in body condition reflective of different blubber thicknesses. While it is suspected that marine mammals in poor body condition will have more difficulty diving and hunting due to a lower oxygen storage capacity (Choy et al., 2019), the results of this study raise the additional question of whether individuals with thinner blubber may further incur higher energetic costs associated with the endogenous desaturation of fatty acids that are necessary throughout the entire blubber depth, compared to those with thicker blubber. The plateau of delta-9 desaturation rates around 25-30 mm from the skin could be used in future monitoring to indicate a minimum blubber thickness required to avoid excess energy expenditure to maintain core body temperature. This may be a potential issue that warrants further investigation.

2.7 Acknowledgements

Thank you to the Tuktoyaktuk and Paulatuk Hunters and Trappers Committees, the Fisheries Joint Management Committee, and the subsistence beluga whale hunters for their contribution of knowledge, samples from their harvest, and support to undertake this research. We thank the beluga monitors and sampling crew at Hendrickson Island and Paulatuk for retrieval of samples and biological data. Thank you to Bruno Rosenberg, Megan Wardekker, Dana Neumann, and the support staff in the Biotracers laboratory at the Freshwater Institute in Winnipeg, Manitoba for your laboratory and technical work. This research was funding by Fisheries and Oceans Canada, the Fisheries Joint Management Committee, and the University of Manitoba.

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Chapter 3. Total lipid percent in the blubber of Eastern Beaufort Sea beluga whales (*Delphinapterus leucas*) and its use as a body condition indicator.

3.1 Abstract

Measurement of energy stores in marine mammals is commonly used as an indicator of body condition, the overall health of an animal. Multiple methods of measuring body condition are currently used, including total lipid percent found in the adipose tissues, and total blubber depth; however, these metrics are often used as a standalone measure without incorporating potential effects of other variables that may affect an individual's energy stores. Total lipid percent was measured in the innermost, middlemost, and outermost layers of blubber in beluga whales (*Delphinapterus leucas*) from the Eastern Beaufort Sea population to determine whether stratification of percent lipids is present. This analysis showed that the middle layer of blubber contained the highest percentage of lipid. The effect of commonly measured covariates, including age, harvest location, blubber thickness, and body length on total lipid percent found in each layer of the blubber was analyzed and, no significant relationship found. The muscle lipid percent was found to be significantly related to the lipid percent in the outer layer of blubber, as well as the percentage of protein in the muscle. Results from this study indicate the middle layer of blubber is a significant storage site of lipids for belugas regardless of size, age, or location, and should be included in future studies of blubber analysis. Results from this study also suggest that the outer layer of blubber is not an accurate representation of total lipid percent in the blubber yet can be indicative of lipid stores in the muscle tissue. Findings from this study enhance our understanding of energy stores in beluga whales, which increase our ability to accurately measure body condition and overall individual health, allowing for better tools to monitor beluga population health.

3.2 Introduction

Blubber is an adapted layer of fat found just below the skin in marine mammals, whose purposes include buoyancy, streamlining, thermoregulation, and energy storage (Strandberg et al., 2008; Larrat & Lair, 2021; Macmillan et al., 2023). It is primarily composed of adipocytes, which are lipid-filled cells surrounded by a matrix of collagen and elastin fibers (Iverson, 2009). Under normal conditions in mammals, the number of adipocytes becomes fixed early in life (Faust et al., 1978), which enlarge or shrink in size with an increase or decrease in lipid accumulation (Struntz et al., 2004). Lipids are the preferred choice for energy reserves, as they are energy-dense, containing 9kcal/gram, compared to the 4kcal/gram that protein and carbohydrates contain (Moghadasian & Shahidi, 2017; Kwon et al., 2020).

Blubber is the primary storage site for lipids in marine mammals (Iverson, 2009). However, the total amount of lipids found in blubber has been shown to vary widely across species, with Arctic marine mammals containing blubber with a higher lipid percent than marine mammals found in more temperate or tropical zones (e.g., 81.3-94.9% in the inner blubber of narwhal (*Monodon monoceros*) compared to 36.3-81.4% in common dolphins (*Delphinus delphis*); Koopman, 2007). Additionally, the total lipid percent found throughout the depth of blubber in individuals varies in some species (fin whales (*Balaenoptera physalus*), Aguilar & Borrell, 1990; sperm whales (*Physeter macrocephalus*), Jackson et al., 2022), while remaining relatively constant in others (Pacific walrus (*Odobenus rosmarus divergens*), Jay et al., 2021). Although blubber contains the largest lipid store, other body areas act as energy storage sites that the animal may draw upon when needed, such as the liver, pancreas, and skeletal muscle (Olsen et al., 2021). Differences in muscle lipid percent have been reported to vary with diet, age, and season, suggesting skeletal muscle lipids may play an important role as an energy reserve

(Adamczak et al., 2023). Previous research has shown that muscle lipid percent varies in proportion to blubber lipid percent in Weddell seals (*Leptonychotes weddellii*) (Trumble et al., 2010) and fin whales (Lockyer, 1986), indicating the potential for muscle lipid percent to reflect overall lipid storage levels. However, muscle lipid percent has also been shown to vary widely between species (Adamczak et al., 2023), as has the relationship between muscle lipid percent and body condition. An inverse relationship between muscle lipid percent and body condition has been documented in mice and European eels (*Anguilla anguilla*), where starvation led to an increase in skeletal muscle lipid percent, yet contrary findings have been documented in other animals (McCue, 2010).

Body condition is a measure of the overall health of an animal, and an animal with a better body condition will have more energy available for migration, hunting, will possess a stronger immune system and higher reproductive success, and will be able to handle calorie deficits much more easily than an animal in poor body condition (Atkinson & Ramsay, 1995; Merila & Svensson, 1997; Moller et al., 1998; Cotton et al., 2006). The amount of lipid reserves an animal contains is often used as an indicator of body condition (Larrat & Lair, 2021; Macmillian et al., 2023). There is no standardized approach for measuring body condition; however, measurements of total lipid percent, blubber thickness, or body girth are commonly used in marine mammals (Beck et al., 1993; Evans et al., 2003; Macmillan et al., 2019; Cunen et al., 2021; Macmillan et al., 2023; Bernaldo de Quiros et al., 2024). To use any of these metrics as a body condition indicator, the relationship to other biological variables must be understood and considered. For example, blubber thickness and total lipid percent have been shown to vary independently from one another in ziphiids and balaenopterids (Kershaw et al., 2019). Additionally, if total blubber thickness were to be measured to determine body condition, the

length of the whale, age, and sex should be accounted for because baseline lipid content may vary between sexes and ages, with differences in foraging habits and habitat selection. Scaled mass index is more commonly being used as a body condition indicator, which is a measure that can be obtained non-invasively, such as from aerial photogrammetry (Sherrill et al., 2024) that accounts for the expected relationship between body length and weight (Peig & Green, 2009). This method has been shown to be in good agreement with visual evaluation of beluga carcasses (Larrat & Lair, 2021); however, being a non-invasive evaluation tool, this method does not include any biochemical analyses of the body tissues. The body site from which a species prioritizes lipid mobilization is another necessary factor when judging body condition from metrics such as total blubber thickness or lipid percent, as the catabolism of non-blubber lipid tissues may not impact these factors but rather another metric such as girth, thereby leading to an increased blubber thickness to girth ratio (Larrat & Lair, 2021), giving the perception of a superior body condition. These relationships may be especially important to consider in the study of Arctic species, which may prioritize thick blubber due to the many functions that go beyond lipid storage.

The Eastern Beaufort Sea (EBS) beluga whale (*Delphinapterus leucas*) population is one of the world's largest, containing an estimated 38,451 individuals (Marcoux et al., 2025) who migrate between the Inuvialuit Settlement Region (ISR) in the Northwest Territories in the summer, and the Bering Sea in Alaska in the winter (Storrie et al., 2023). Throughout 2000-2007 and 2011-2014, a larger variability in blubber thickness has been documented in EBS adult male belugas (Harwood et al., 2014, Choy et al., 2017, Macmillan et al., 2023), and a decline in size-at-age of individuals was reported from 1989-2008 (Harwood et al., 2014). This is hypothesized to be due to a change in prey availability due to climate change (Harwood et al., 2014). Inuvialuit

harvesters from Tuktoyaktuk have also reported a decline in blubber thickness over time (Ostertag et al., 2018), and both the Tuktoyaktuk and Paulatuk Hunter's and Trapper's Committees (HTCs) have expressed concern over EBS beluga whale health, and understanding the cause of thinning blubber was identified as a priority by the Paulatuk HTC (FJMC priorities, 2022-2023; 2023-2024).

The importance of understanding how commonly used biological metrics relate to each other to better estimate body condition is stressed by the recent observations of thinning beluga blubber. Thus, the objectives of this study are to explore how total lipid percent differs throughout the full depth of beluga blubber, as well as to examine the relationship between blubber lipid percent and covariates including age, harvest location, blubber thickness, and body length to evaluate whether these factors need to be considered when interpreting lipid percent results. I hypothesize that lipid percent will vary throughout the depth of blubber and predict that the middle layer will contain the highest total percent lipid. I also hypothesize that standardized blubber thickness (body condition) will impact the total lipid percent found in the blubber, and predict that thinner whales, and those from Paulatuk will have a lower lipid percent found in the blubber, based on what has been observed in other marine mammals with thinning blubber. The final objective is to examine the relationship between these covariates and total lipid percent found in the skeletal muscle to determine whether more information can be gained on beluga body condition by examining muscle tissue lipids, as well as to see if there is any evidence of the utilization of muscle lipids in beluga whales that coincide with blubber lipid use. This analysis will also help to verify whether total lipid percent is an accurate indicator for body condition, and whether shallow-depth biopsy darts can be used as an alternative to more invasive procedures such as obtaining total blubber depth or body girth.

3.3 Methods

3.3.1 Study Area

Figure 3.1 Map of the study area in NT Canada, highlighting the Tarium Niryutait Marine Protected Area and the Anguniaqvia Niqiqyuam Marine Protected Area in orange. Communities of Tuktoyaktuk* and Paulatuk* are also labelled. Source: Canadian Geographic (2017).

Two marine protected areas (MPAs) in the ISR in the Western Canadian Arctic were established by Fisheries and Oceans Canada (DFO) under the Canada's Oceans Act (1997) in partnership with Inuvialuit, Inuit of the Western Canadian Arctic (Loseto et al., 2018). The Tarium Niryutait MPA (TN MPA) was established in 2010 with a goal of conserving the EBS beluga whale population and their surrounding habitat and is comprised of three separate areas of the

Mackenzie Estuary. The Anguniaqvia Niqiqyuam MPA (AN MPA) was designated in 2016 and was created with an intention to conserve numerous marine species, including the EBS beluga. With both MPAs being co-developed with Inuvialuit, a key goal of the MPAs is to ensure the long-term health of the population to support the traditional sustainable harvest of belugas by Inuvialuit. EBS belugas are highly migratory (Loseto et al., 2018), and the addition of the two MPAs create a connection between the two regions.

EBS belugas migrate yearly from the Bering Sea to the Canadian Arctic, arriving in the Mackenzie Estuary, Viscount Melville Sound, the Amundsen Gulf, and the Eastern Beaufort Slope in late May to early June (Storrie et al., 2022; Richard et al, 2001; Mayette et al., 2023). They will aggregate at the mouth of the Mackenzie River for a few days, with peak activity occurring in early to mid July (Fraker & Fraker, 1979), prior to moving into their summer range. By December they will migrate to the Bering Sea until the following spring (Storrie et al., 2023).

The Inuvialuit have hunted beluga for centuries (McGhee, 1988). Today, belugas are sustainably harvested during their annual migration as they pass through coastal communities and camps in the ISR and are considered a culturally significant and important food source (FJMC, 2013). The most active community that harvests beluga is in Tuktoyaktuk, where whales will often be brought to Hendrickson Island for flensing before heading back to Tuktoyaktuk for processing of the meat and muktuk (Ostertag et al., 2018). Hunting here usually occurs during the month of July and lasts four to six weeks (Harwood et al., 2002).

Paulatuk is a small community located in Darnley Bay (Arnold et al., 2011). Belugas were not consistently hunted here and in greater numbers until the 1990s, when migration patterns shifted and belugas began migrating closer to Paulatuk (Ostertag et al., 2018). Belugas around Paulatuk are harvested more opportunistically than by Tuktoyaktuk, and thus, hunters

may be less selective when pursuing whales than in other communities (Ostertag et al., 2018). Hunting in Paulatuk occurs in late July or August, when belugas have left the Mackenzie estuary and have started moving to offshore areas to feed (Harwood et al., 2002). The harvest of beluga whales by the Inuvialuit occurs sustainably as traditional knowledge and practices that have been passed down for generations are used (Ostertag et al., 2018; FJMC, 2013). This has instilled a deep-rooted understanding and knowledge of EBS beluga migration, health, and behaviour that is invaluable to western science research.

3.3.2 Sample Collection and Study Design

A long-term health management and collaborative research program for EBS belugas is currently maintained via joint efforts by the federal government and the Fisheries Joint Management Committee (FJMC), who work in tandem with the Tuktoyaktuk and Paulatuk HTCs to manage beluga conservation efforts in the ISR. During the annual hunt, research teams will work alongside Inuvialuit hunters at traditional whaling camps to obtain data measurements and tissue samples of belugas, which are then sent to the Freshwater Institute in Winnipeg for biological data collection and further analysis. Hunter-based beluga monitoring in the Mackenzie Delta began in 1973, where number of harvested whales was recorded, and in 1980, documentation of body length, fluke width, sex, and age of the whales was included (Harwood et al., 2002). In 1989, data collection from beluga hunts began in Paulatuk (Harwood et al., 2002).

In the summers of 2022 and 2023, beluga whales were harvested during the annual subsistence hunts at Hendrickson Island and near Paulatuk NT. A fist-sized blubber sample containing the full depth of blubber, including the skin and blubber/muscle interface was

removed slightly dorsal to the pectoral flipper from each beluga. A similar sized sample of muscle tissue was excised from the latissimus dorsi muscle from each beluga. Condition of the whale's overall health was acquired qualitatively from the harvester and monitor reports and quantitatively from blubber depth and girth, and body length measurements. Sex of the whales was determined at the time of harvest, and age of whales was acquired from the dissection of eyeballs and examination of the lens (Pleskach et al., 2016).

From the whales harvested in 2022, 20 blubber samples from individual whales were used for analysis: 10 from Hendrickson Island, and 10 from Paulatuk. From the whales harvested in 2023, 24 blubber samples from individual whales were used for analysis: 14 from Hendrickson and ten from Paulatuk. Samples were selected for having varying degrees of blubber thickness. Any yellow, wounded, rancid smelling, or other samples that looked compromised were not selected. Muscle samples were taken from the matching individuals that blubber samples were obtained, so a blubber sample and corresponding muscle sample could be examined from each individual. Hunters typically target male belugas due to their larger size and to support sustainable population management (Sudlovenick et al., 2025). Therefore, most samples available for this study came from male beluga whales. Only four of the 44 individual whales sampled for this study were female. The objective of this study was to examine how different variables may affect lipid percent in the blubber. However, our dataset contained a highly unbalanced sex ratio (40 males, four females), therefore, concerns of unreliable results from sex effect due to low female sample size prompted the decision to exclude the four females from the data analysis (Table 3-1). Juvenile whales are not harvested and therefore only adults were used in this study, controlling for the effects of maturity. Blubber samples were frozen at -20C° before being shipped to the Freshwater Institute in Winnipeg, Manitoba. Going forward,

whales harvested by Tuktoyaktuk hunters and flensed at Hendrickson Island will be referred to as 'Hendrickson whales', and whales from Paulatuk hunters in Darnley Bay will be referred to 'Paulatuk whales'.

Table 3-1 Biological details of the 40 beluga whales sampled for this study.

2022 Whales Location	Avg age ± SE (years)	Avg length ± SE (cm)	Avg blubber thickness ± SE (mm)	N thin whales	N medium whales	N thick whales	N whales	N females	N males
Hendrickson	21.3 ± 2.1	407.4 ± 7.3	30.6 ± 4.4	5	2	3	10	0	10
Paulatuk	28 ± 4.6	396.6 ± 11.8	19.3 ± 1.8	4	6	0	10	0	10
2023 Whales									
Hendrickson	25.2 ± 2.8	394.5 ± 10.3	33.5 ± 2.8	3	3	4	10	0	10
Paulatuk	30.9 ± 5.4	410.2 ± 5.3	24.3 ± 2.7	9	1	0	10	0	10

3.3.3 Body Condition Metric

A standardized blubber thickness measure was created so blubber thicknesses between individuals could be compared while accounting for differences in body length, as body length ranged from 330.2 cm to 459.7 cm (Kulchycki, Chapter 2). This was calculated by dividing total blubber thickness by total body length and was measured for each individual. This standardized blubber thickness measure was also used as a measure of body condition, where individuals whose blubber thickness value fell into the lower third of the range was considered a thin individual, those whose value fell into the middle third of the range was considered medium, and those whose value fell into the upper third of the range was considered a thick individual. Going forward, the terms standardized blubber thickness and body condition may be used interchangeably in this paper.

3.3.4 Laboratory Analysis

Blubber samples were partially thawed, and the outer sides were trimmed to dispose of oxygenated tissue. The full depth of each blubber sample from the skin/blubber interface to the blubber/muscle interface was measured to the nearest millimetre, then subsampled at intervals of 5 mm. The blubber closest to the muscle was called the inner layer, and the blubber closest to the skin was called the outer layer, and the middlemost 5 mm sample was called the middle layer. The blubber samples varied in size, and were cut starting with the inner layer, therefore, the outer layer ranged in length from 2 to 5 mm. The Folch method was followed to perform lipid extraction (Folch et al., 1957). A subsample of each layer was taken to equal 0.5 (± 0.1) grams, which was then placed in a 20 ml test tube, 7 ml chloroform diluted with 0.01% butylated hydroxytoluene (BHT) (v/v/w) and then 3.5 ml methanol with 0.01% BHT was added, before being placed in the freezer overnight.

Test tubes were vortexed for 30 seconds and the blubber samples were squished using a glass rod inside of the test tubes to release the lipids from the blubber. A metal pair of tweezers was used to remove the remaining blubber tissue, which was then placed in a separate vial and set aside to dry under the fume hood overnight. A total of 3.5 ml of 0.7% NaCl was added to the test tubes containing the lipids, before being vortexed for 5 to 10 seconds and set aside to allow for separation into two layers. The top layer of each sample was discarded using a waste pipette and then a scoop of $NaSO_4$ was added. Caps were assembled on the test tubes so they could be shaken and left to rest for five minutes to allow the $NaSO_4$ to absorb any excess water from the samples. The remaining solvent was transferred to a new 15 ml test tube, rinsed twice with 2 ml $CHCl_3$, and moved to a final, pre-weighed test tube. The final test tubes containing the solvent

were placed in a hot water bath and evaporated under nitrogen using an Organomation Associated Inc. Nitrogen Evaporator (N-evap 111). A four-decimal point Mettler Toledo scale (model AL204) was used to obtain total lipid weight, then the total lipid percent was calculated by dividing lipid weight by weight of the blubber sample (~0.5 g).

Muscle content has significantly less lipid content than blubber, so a modified Folch method was used (Folch et al., 1957). Muscle samples were weighed, freeze dried, and weighed again to determine total water percent in each sample. Approximately 0.2 (± 0.03) grams of the freeze-dried muscle tissue from each sample was placed in a 25 mL test tube (tube A) before adding 3 mL methanol with 0.01% BHT, shaking, and allowing to sit for a few minutes. Then 6 mL $CHCl_3$ was added to the test tubes which were then capped, shaken, and allowed to sit in the freezer overnight.

The test tubes were briefly vortexed, and the solvent was transferred to a second test tube (tube B) before 3 mL 2:1 chloroform/methanol was added to tube A, which was then vortexed briefly. The rinse solvent was transferred to tube B, and tube B was discarded. A total of 7 mL of 0.88% NaCl was added to tube B, which was then capped, shaken, and allowed to sit for approximately five minutes so the liquids in the sample could separate. The top layer was discarded, and a scoop of $NaSO_4$ was added to the test tubes and caps were assembled so the tubes could be shaken and left to sit for five minutes so any excess water could be absorbed. The solvent was then transferred to a pre-weighed 15 mL test tube (tube C). The $NaSO_4$ in tube B was rinsed twice with 2 mL $CHCl_3$ and the solvent was transferred to tube C before tube C was placed in a hot water bath and evaporated under nitrogen. Total lipid weight was obtained and total lipid percent calculated by dividing lipid weight by the weight of the muscle sample (~0.2

g). Total protein percent in the muscle tissue was measured by subtracting total lipid percent and water percent from total weight of the muscle sample.

3.3.5 Data Analysis

Prior to analysis, total lipid percent was divided by 100 to convert into proportion data. All statistical analysis was performed on R (version R-4.5.0) in R Studio (version 2025.05.0-496). To determine if differences in total lipid percent throughout the depth of blubber occurred, a general linear mixed model (GLMM) (glmmTMB function in the glmmTMB package) was used with a beta distribution and logit function to examine the effect that blubber layer (explanatory variable) has on the lipid content of the sample (response variable). A GLMM was chosen as the model for non-normally distributed data, and incorporates both fixed and random effects. As multiple samples within each blubber sample (i.e., inner, middle, and outer blubber layers) were collected from each individual, the need to account for variation within individuals, and thus the possibility of pseudo-replication was necessary. Individual was therefore included as a random effect variable in the model. A beta distribution was used because it works best with proportion data, and the logit link function was chosen as it is the default for the beta family. Residual analysis was used during data exploration to check for normality, outliers, homogeneity of variance, and over/under-dispersion, and to therefore determine the legitimacy of the model, during which, heterogeneity of variance was deemed present. To account for this, the final model included group-specific dispersion (dispformula argument as part of the glmmTMB function), which models the variance by group (blubber layer) instead of as a whole. One outlier was removed from the data set due to an ultralow lipid percent value, likely due to human error during the extraction process.

To test for the effects of age, standardized blubber thickness, harvest location, and body length (explanatory variables) on the total lipid percent of each blubber layer (inner, middle, outer) (response variable), separate general linear models (GLMs) (glm function from base R) with a gamma family and a log link function were run for each blubber layer. The lipid percent data were not normally distributed, which is why general linear models were chosen over linear models. Gamma distributions are used when the response variable contains positive, continuous proportion data, and the use of a log link function ensures the return of positively fitted values. To check for collinearity between explanatory variables and to therefore avoid multicollinearity in the final models, variance inflation factors (VIFs) for each covariate were calculated (vif function in the car package). VIFs calculate how much the variance of an estimated regression coefficient increases if the explanatory variables were to be correlated. All VIF scores were < 2, meaning no correlation was present and all covariates could therefore be included in the model (Zuur et al., 2010). Residual analysis was used to determine legitimacy of the model as above, with addition of confirming linearity between predictor and response variables. Harvest year was not included in the final models, as the addition of it resulted in too many explanatory variables (n/10 = 40/ 10 = 4 explanatory variables at maximum), putting the model at risk of overfitting. However, when year was included during exploratory analysis, it was still deemed non-significant in all three models (Table S-5).

Prior to examination of covariate effects on muscle lipid percent, a correlation matrix was constructed to determine if a significant relationship exists between lipid percent in the inner, middle, or outer blubber layer and the total lipid percent found in the muscle tissue. Results indicated a significant negative relationship was present ($p < 0.022$) between the outer blubber lipid percent and muscle lipid percent, and therefore, outer blubber lipid percent was included as

an explanatory variable in the final model. A GLM with a gaussian family and identity link function was used to investigate the effects of body length, harvest location, standardized blubber thickness, and outer blubber lipid percent (explanatory variables) on the total lipid percent found in the muscle tissue (response variable). A gaussian distribution was used as the muscle lipid percent data were approximately normally distributed, and the identity link function is the default function used for gaussian distributions. Similarly, VIF scores and residual analysis were examined to check model assumptions for the final model. Ages ranged from 8 to 63 years and was not included in the model to account for a maximum allowance of four explanatory variables.

To determine if a relationship was present between muscle protein percent (response variable) and standardized blubber thickness (explanatory variable), a GLM with a Gamma family and log link function was used. An additional GLM with Gamma family and log link function was run to explore whether a relationship was present between muscle lipid percent (response variable) and muscle protein percent (explanatory variable). Assumptions were checked via residual analysis, where it was determined that the linearity assumption was not met. Therefore, a polynomial term was added to both models to allow for a better model fit.

3.4 Results

Blubber from whales harvested at Paulatuk was thinner than blubber from whales harvested at Hendrickson Island (Mann-Whitney U test, $p < 0.001$). Blubber from whales at Paulatuk ranged in thickness from 10-35 mm, with an average blubber thickness of 21.8 ± 1.7 mm. Blubber thickness from whales at Hendrickson Island ranged from 15-55 mm, averaging 33.1 ± 2.8 mm.

3.4.1 Total Lipid Percent through Blubber Depth

Blubber lipid percent ranged from 60.9 to 95.2% for the innermost layer, with an average of 83.0 ± 7.4%; 64.9 to 93.3% for the middle layer with an average of 85.7 ± 5.1%; and 67.6 to 93.2% for the outermost layer, with an average of 82.1 ± 6.4% (Table 3-2). Blubber layer had a significant effect on the lipid percent found in the blubber ($p = 0.02$, Table S-4). The middle blubber layer had a significantly higher total lipid percent than the outer blubber layer ($p= 0.02$) but not the inner blubber layer (Figure 3.2). The inner layer had a slightly higher lipid percent than the outer layer, although this difference was not significant (Figure 3.2). The middle layer also had significantly less variability in total lipid percent ($p = 0.04$).

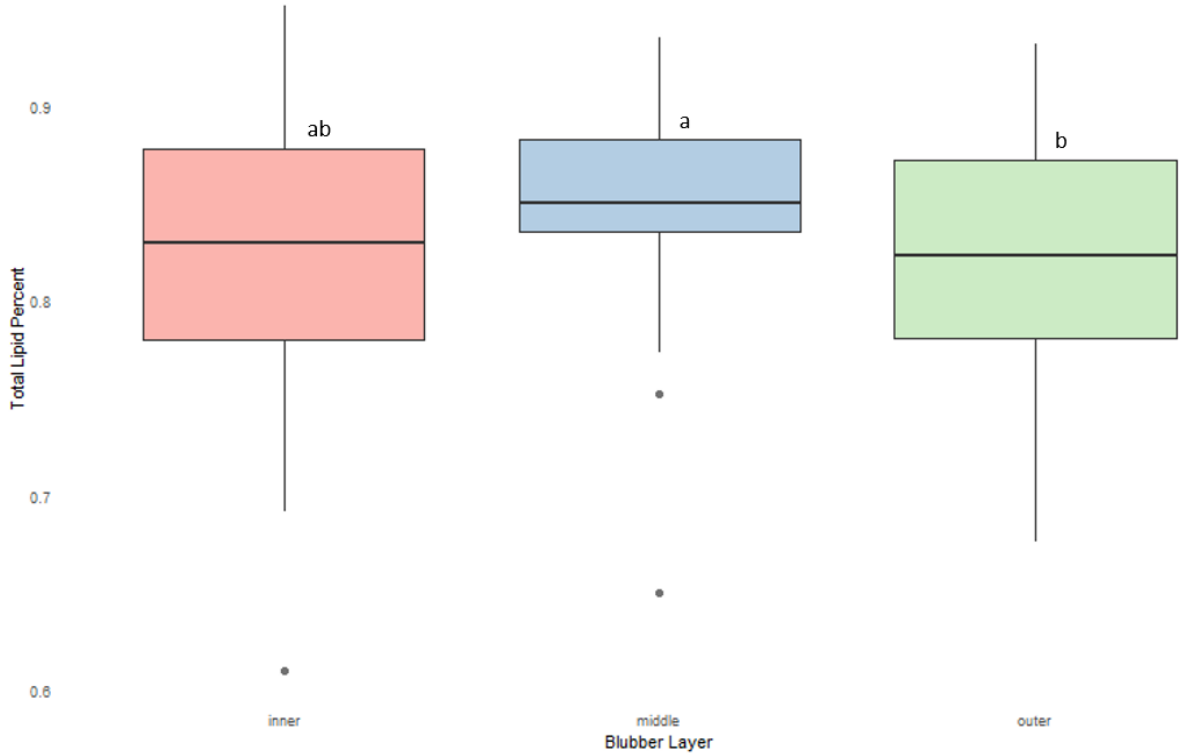

Figure 3.2. Total lipid percent found in the innermost, middle, and outermost blubber layers. The middle layer contained a significantly greater total lipid percent than the outer layer ($p = 0.02$), but not the inner layer. Boxes represent the interquartile range (IQR) (middle 50% of data points), the line inside the box represents the median, the whiskers extend to the smallest and largest values within 1.5 x IQR of the box, and the points outside the whiskers are outliers. Boxes in each individual plot that do not share a letter are significantly different ($p < 0.05$) from one another.

3.4.2 Blubber lipid percent and relationship with other covariates

General linear models were run for all three blubber layers individually, which revealed that none of the measured covariates (body length, harvest location, age, body condition/standardized blubber thickness) had any effect on lipid percent in any of the blubber layers ($p > 0.05$, Table S-5) (Figure 3.3).

Figure 3.3. Total lipid percent of the a) innermost, b) middle, and c) outermost blubber layers plot against age, standardized blubber thickness, and body length (cm), none of which were significantly related.

3.4.3 Muscle lipid percent and relationship with other covariates

Muscle lipid percent ranged from 0.9 to 4.7%. Outer blubber lipid percent had a significant negative relationship with the percentage of lipid found in the muscle tissue (GLM, $p = 0.02$; Table S-5; Figure 3.4).

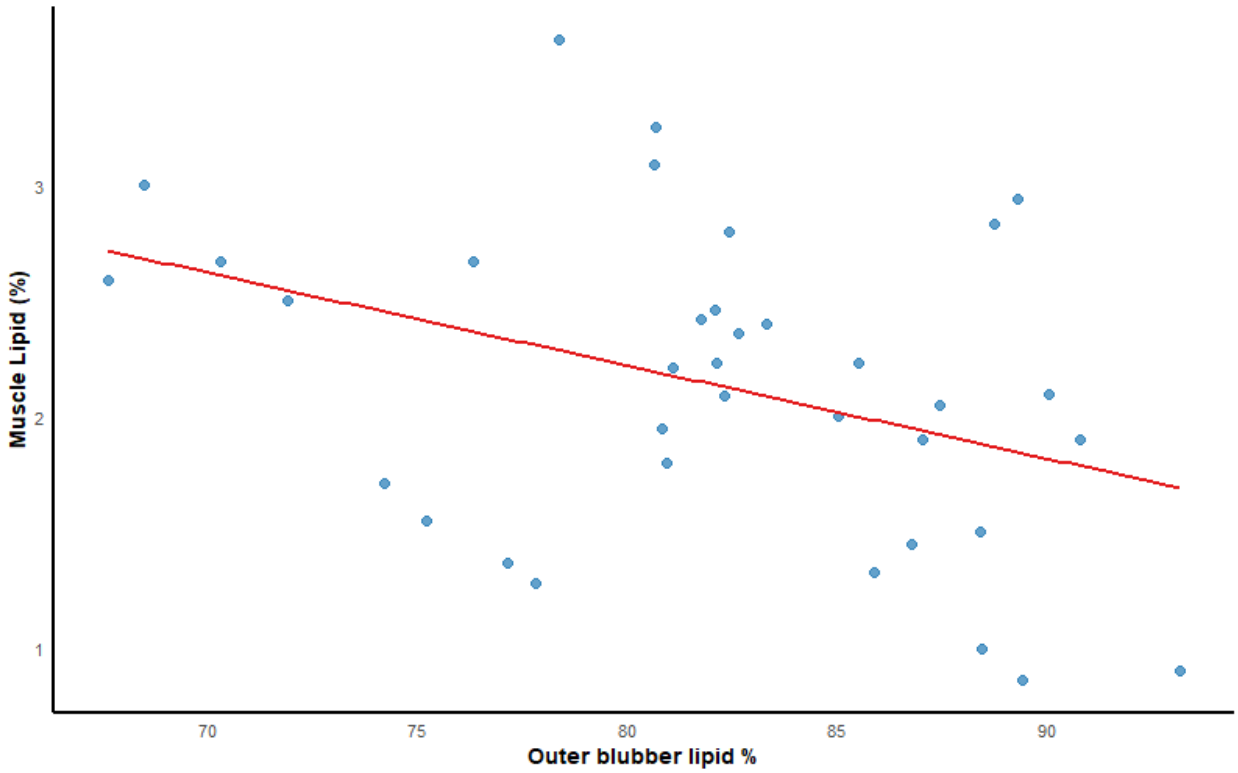

Figure 3.4. The only significant explanatory variable, outer blubber lipid percent, plot against total lipid percent found in the latissimus dorsi muscle. A significant ($p = 0.02$) trend was found where muscle lipid percent decreased with an increase in outer blubber lipid percent.

There was no significant relationship between muscle protein content and body condition/standardized blubber thickness. A significant (GLM, $p < 0.001$; Table S-5) positive relationship did, however, exist between muscle lipid percent and muscle protein percent (Figure 3.5).

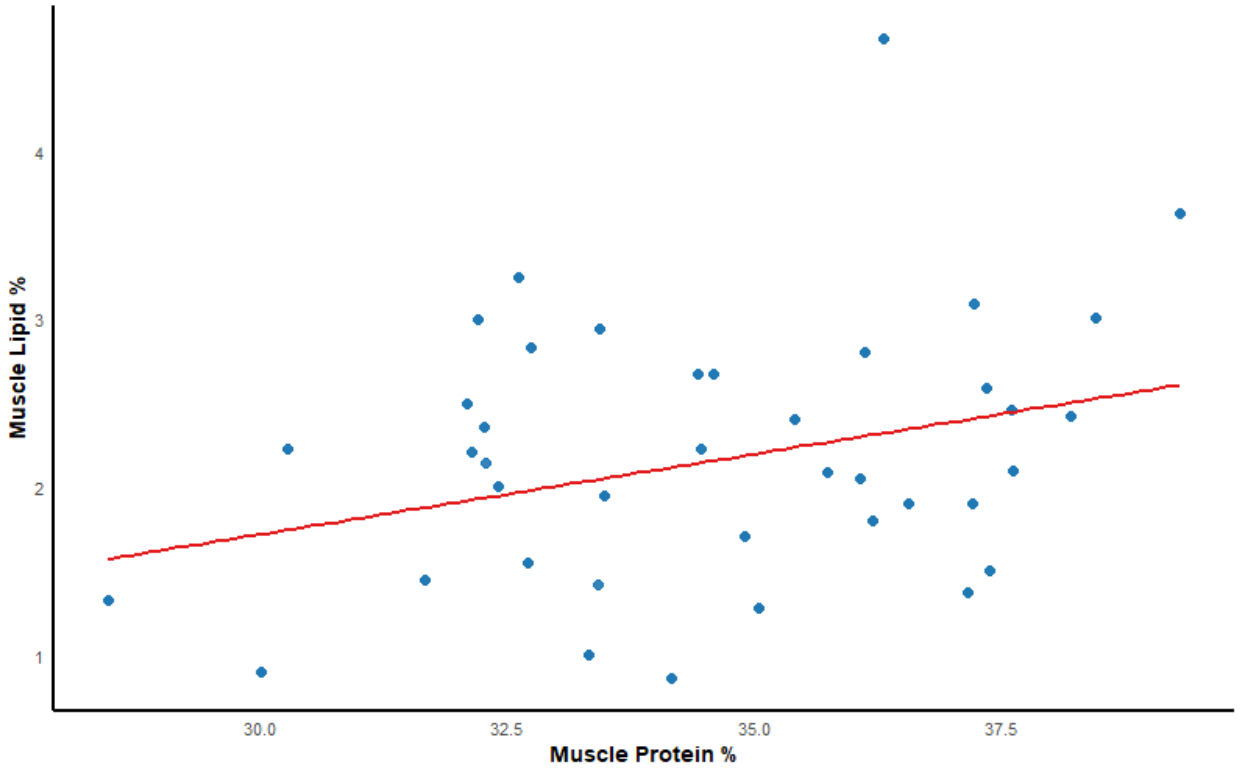

Figure 3.5. A significant ($p < 0.001$) and positive relationship was present between the lipid percent and protein percent found in the latissimus dorsi muscle.

3.5 Discussion

3.5.1 Total Lipid Percent

The total lipid percent found in the beluga blubber (60.9 to 95.2%) aligns with the lipid ranges found in previous studies of beluga blubber, such as in the study of Hudson Bay belugas in Belanger et al. (2025) who found a lipid percent range of 69-99%, and in Krahn et al. (2004), who found a range of 68-85% in Cook Inlet and Bristol Bay belugas. Blubber lipid percent ranges have been documented in many other marine mammals. Blubber lipids ranged from 50-97% among pinnipeds (fur seals, sea lions, phocids), with the phocids containing the highest lipid content. In fin whales, a large range of 34.4-88.2% has been reported (Aguilar & Borrell, 1990) and a similarly large range of 18.3-69.8% in humpback whales (*Megaptera novaeangliae*) (Waugh et al., 2014).

Total lipid percent in blubber has also been shown to vary widely between odontocete species. For example, lipid percent in striped dolphins (*Stenella coeruleoalba*) has been documented as ranging from 25-65%, while a range of 63-79% has been reported in Blainville's beaked whales (*Mesoplodon densirostris*) (Bernaldo de Quiros et al., 2024). In a study of 30 different odontocete species (Koopman, 2007), which included various dolphin species, monodontids, porpoises, beaked whales and sperm whales, lipid percent ranged from <25% to >83%. This same study demonstrated that all examined cold-water species contained much higher lipid percent in their blubber (minimum 60%, often above 80%) compared to species that inhabit temperate or tropical waters. This is likely related to the insulating effects of lipid, where an increase in lipid content coincides with reduced blubber conductivity, and therefore retention of body heat (Worthy & Edwards, 1990).

Percent lipid was not explained by age, harvest location, blubber thickness, or body length; thus, the range in lipid percent may be due to life history strategies or other variations not tested in this study. Capital breeding species, which go through cycles of feasting and fasting, store more lipid during the feeding season to then use for reproduction and restorative processes and will therefore exhibit greater variation in blubber lipid percent throughout their life compared to income breeders, which are individuals who maintain a relatively constant feeding schedule (Irvine et al., 2017). Therefore, when comparing individuals of the same species, it is important to consider the migration or reproductive period in which samples were collected. Toothed whales are considered income breeders (Derous et al., 2020), with relatively constant energy stores year-round. However, Belanger et al. (2025) found significant differences in lipid content across seasons in beluga whales. Variation in prey abundance or other population-specific factors can affect lipid content in blubber. For example, Jay et al. (2021) found interannual differences in the lipid content of blubber of Pacific walrus, which use a mixed strategy of income and capital breeding (Noren et al., 2014). The differences are hypothesized to be due to dietary differences between sampling years.

In this study, all individuals were sampled using the same method, blubber samples were taken from the same body location and were from freshly subsistence-harvested beluga. During the subsampling process in the laboratory, blubber samples were cut from a semi-solid state in efforts to lessen likelihood of lipid loss. Therefore, differences in percent lipid in the blubber samples used in this study likely stems from habitat variations or feeding preferences.

3.5.2 Lipid Percent through Blubber Depth

While slight, lipid percent was significantly higher in the middle layer (avg. 85.7%) compared to the outer blubber layer (avg. 82.1%), but not the inner blubber layer (avg. 83.0%). The outer layer contained a lower lipid percent than the inner layer; however, was not statistically significant. Similar findings were reported in Krahn et al., (2004) who found that lipid percent was relatively uniform throughout beluga blubber, with the middle layer containing a slightly greater lipid percent than the inner or outer layers. The middle layer was found to contain a greater lipid percent in the blubber of sperm whales (Jackson et al., 2022), as well as in short-finned pilot whales (*Globicephala macrorhynchus*) (Bagge et al., 2012). Additionally, while total lipid percent was not reported, the middle blubber layer has been shown to expand or shrink in ringed seals (*Pusa hispida*), depending on total blubber thickness of the individual (Strandberg et al., 2008).

Other marine mammals have demonstrated a consistent lipid percent throughout the depth of blubber. No difference in lipid percent has been shown through the depth of blubber in killer whales (*Orcinus orca*) (Krahn et al., 2004), nor between the outer and inner layer in lactating Pacific walrus females (Jay et al., 2021). Various species of beaked whales (Cuvier's beaked whales (*Ziphius cavirostris*), Sowerby's beaked whales (*Mesoplodon bidens*), northern bottlenose whales (*Hyperoodon ampullatus*)) have demonstrated no difference in total lipid percent between inner, middle, or outer layers of blubber, which included cases where individuals died from either chronic or acute conditions (Kershaw et al., 2019). Mixed results have been reported for humpback whales, where a consistent lipid percent has been found throughout the blubber depth, with an exception to lactating females, who demonstrated a greater lipid percent found in the outer blubber layer compared to the inner layer (Waugh et al., 2014),

while in a study by Kershaw et al. (2019), all individuals held a greater lipid percent in the outer layer than the inner layer.

Lipid percent throughout blubber depth has shown to vary widely in some marine mammal species, such as minke whales (*Balaenoptera acutorostrata*) (Kershaw et al., 2019) and female fin whales (Aguilar & Borrell, 1990), with total lipid percent increasing from innermost to outermost blubber, aside from in pregnant fin whales, where lipid percent decreased from innermost to outermost blubber, although this finding was not significant (Aguilar & Borrell, 1990). Male fin whales, however, did not demonstrate lipid percent variation throughout the blubber layers (Aguilar & Borrell, 1990). Risso's dolphins (*Grampus griseus*), short-finned pilot whales, and pygmy sperm whales (*Kogia breviceps*) have all shown stratification in lipid percent, with the inner blubber layer containing a greater lipid percent than the outer layer (Koopman, 2007). Previous studies have demonstrated how lipid percent throughout the blubber tends to vary widely between species and sex. While only one species was used in our study, the beluga whale, all our individuals were male. Had it been possible to include more females, larger variations in blubber lipid percent throughout the depth of blubber may have occurred, particularly if varying reproductive statuses were involved.

3.5.3 Relationship between blubber lipid percent and other covariates

I hypothesized that lipid percent would relate to body condition, noting that better body conditions would support more lipid stores. Total lipid percent in the blubber was not significantly related to body length, harvest location, age, or standardized blubber thickness (body condition) for any of the blubber layers (inner, middle, outer). Varying results have been

documented in the literature regarding the relationship of blubber lipid percent and other biological factors. Across the 30 different odontocete species examined by Koopman (2007), only one significant relationship between body length and blubber lipid percent was found, which was in the outermost layer of blubber in harbour porpoises (*Phocoena phocoena*), where lipid content declined with an increase in body length. Age class, body condition (length/girth), and blubber thickness also exhibited no relationship to blubber lipid percent in various stranded ziphiids and balaenopterids (Kershaw et al., 2019), nor was a relationship found between blubber thickness and lipid percent in stranded sperm whales (Evans et al., 2003). A significant correlation between blubber lipid percent and body condition was found between fed and fasted southern humpback whales (Bengtson Nash et al., 2013); however, only the outer blubber layer was analyzed.

Struntz et al. (2004) found that when all life history categories of bottlenose dolphins (*Tursiops truncatus*) were combined (fetal, juvenile, subadult, adult), lipid content and blubber thickness displayed no significant relationship. However, when life history categories were treated separately, fetal and adult dolphins demonstrated a significant relationship between blubber lipid percent and blubber thickness, with lipid percent increasing with thickness. Although age was included as an explanatory variable in our study, all individuals were considered adults, and so we were not able to evaluate different ontogenetic stages.

The same authors found a relationship between blubber lipid percent and body condition in bottlenose dolphins, where dolphins in emaciated body condition had blubber lipid percent similar to fetal dolphin blubber, up to 48% less than robust adult blubber (Struntz et al., 2004). Kershaw et al. (2019) found a relationship between cause of death in balaenopterids and lipid percent in the blubber, where individuals who died of a chronic condition had a lower blubber

lipid percent than those who died of acute trauma, indicating a gradual decline in health in those with a chronic condition, reflected by the animals depletion of their lipid stores. In contrast, Bernaldo de Quiros et al. (2014) found no relationship between blubber lipid percent and body condition in striped dolphins or Blainville's beaked whales. These authors did, however, find that total amount of lipids (adipose tissue) in the body were related to body condition. This, along with the relationship found by Struntz et al. (2004) between blubber lipid percent and body condition in bottlenose dolphins in emaciated condition, may provide evidence that there is a certain lipid storage threshold that may need to be passed for lipid percent in the adipose tissue to begin significantly decreasing.

The variability in how body condition is measured makes comparison between studies difficult. For example, in the aforementioned studies, Kershaw et al. (2019) used body length divided by girth to define body condition, while Struntz et al. (2004) determined body condition based on convexity or concavity of the animals epaxial surface. The body condition measurements used by Bernaldo de Quiros et al., (2014) were calculated by dividing total body mass by total body length squared, also known as Quetelet's index, while our study used a standardized blubber thickness measurement, acquired by dividing blubber thickness over total body length. As no standardized method of measuring body condition exists, and the defining labels are often determined by the available individuals, an animal in 'poor' body condition in one study may be labeled as 'moderate' or even 'good' in another. While our study considered the individuals with the thinnest standardized blubber measurement as having a 'thin' body condition, the individuals were likely not in supremely poor health, as Inuvialuit hunters will purposely try to avoid sick or old whales (Ostertag et al., 2018).

Our inability to test for sex effects resulted from a low sample size of females, yet if sex could have been included as a covariate, it is possible it may have been significantly related to total lipid percent, particularly had varying reproductive statuses been involved. This has been demonstrated in sperm whales, where females contained overall higher blubber lipid percentages than males (Jackson et al., 2022), in bottlenose dolphins, where pregnant females contained a blubber lipid percent approximately 27% higher than other adults (Struntz et al., 2004), and in fin whales, where a strong relationship was present between reproductive status and average blubber lipid percent, and between reproductive status and lipid percentage stratification in females, but not in males (Aguilar & Borrell, 1990). Female mammals require higher amounts of lipids, particularly during pregnancy and to prepare for lactation (Heldstab et al., 2017).

3.5.4 Relationship between muscle lipid percent and other covariates

The total lipid percent found in the muscle tissue ranged from 0.9-4.7%, which is slightly higher than what has been recorded for most vertebrates (1.0 to 2.0%) (Adamczak et al., 2023). Our results are also greater than the total lipid percent found in the longissimus dorsi muscle in striped dolphins (1.0 to 1.3%) and Blainville's beaked whales (0.60 to 1.1%) (Bernaldo de Quiros et al., 2024).

The muscle lipid percent was significantly related to the lipid percent found in the outer layer of blubber, where a greater lipid percentage in the muscle tissue coincided with a decreased lipid percent found in the outer blubber layer. The relationship between muscle lipid percent and marine mammal body condition has not been well studied, however, contrasting results have been reported in Weddell seals and fin whales, where lipid percent in the muscle varied in

proportion to the lipid percent of the blubber, suggesting muscle lipids were being metabolized with blubber lipids (Trumble et al., 2010; Lockyer, 1986). Additionally, the composition of muscle tissue has been shown to change with body condition in striped dolphins and Blainville's beaked whales, as triglycerides, a main storage lipid type, decreased with a worsening body condition, suggesting lipid catabolism from the muscle tissue during times of caloric deficits (Bernaldo de Quiros et al., 2024). Sex likely plays a role in muscle lipid percent, as differences between male and female harp seals (*Phoca groenlandica*) have been recorded. Pregnant Northwest Atlantic harp seals were documented containing a larger muscle lipid percent than males, and one-month post-partum females had the largest muscle lipid percent, and the lowest blubber lipid percent compared to males and other reproductive status females (Beck et al., 1993). It is difficult to determine why a significant negative relationship was present between muscle lipid percent and outer blubber lipid percent in this study. The outermost blubber layer is the least metabolically active blubber layer (Olsen & Grahl-Nielsen, 2002; Strandberg et al., 2008), and the composition has been shown to remain relatively stable in varying body condition beluga whales (Kulchycki, Chapter 2). Therefore, more research examining muscle lipids in beluga whales needs to be done to further explore this relationship.

In addition to lipid mobilization from muscle tissue, Bernaldo de Quiros et al., (2024) also reported muscle protein catabolism in striped dolphins and Blainville's beaked whales during energy deficit. It is often thought that starving mammals prioritize lipid catabolism before muscle protein breakdown for energy (Strandberg et al., 2008). While both species demonstrated lipid catabolism from both their blubber and muscle tissue, the beaked whales appeared to preferentially catabolize muscle proteins over muscle lipids (Bernaldo de Quiros et al., 2024). Additionally, muscular atrophy of the epaxial muscles in beluga whales during caloric deficit has

been documented, suggesting evidence of muscle catabolism during starvation (Larrat & Lair, 2021). Protein content in the muscle of our beluga samples did not show any significant relationship with standardized blubber thickness (body condition), but did exhibit a positive, significant relationship with lipid content in the muscle. While neither lipid content nor protein content in the muscle tissue were significantly related to body condition in our study, the fact that muscle lipid content increased with muscle protein content may provide evidence that lipid and protein in male beluga muscle is mobilized simultaneously, and thus beluga muscle lipid and protein stores may be reflective of one another.

3.6 Conclusion

This research determined that total lipid percent and blubber thickness were not related and therefore one cannot be inferred by the other; however, a positive, significant relationship between muscle lipid percent and protein percent suggests there is potential that muscle tissue can be incorporated into the analysis of body condition, and that future research may be indicated. The middle layer was determined to be an important storage area in the blubber, in which whales with thin blubber will be lacking. Although it was not possible to include sex, age class, or reproductive status as variables in this study, insight from the literature highlights the importance in the inclusion of females, particularly of differing reproductive stages into future research. The opportunistic nature of marine mammal sampling will likely make this a difficult endeavor; however, the exclusion or underrepresentation of females limits our ability to fully understand the relationships between lipids and body condition.

3.7 Acknowledgements

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Chapter 4. General Discussion

4.1 General Overview

This study documented the stratification and composition patterns of lipids and fatty acids in beluga whale blubber and examined differences in total lipid percent and fatty acid composition among beluga whales (*Delphinapterus leucas*) with varying body condition from the Eastern Beaufort Sea (EBS) population. Influence of biological covariates on the percent lipid found in the blubber and muscle tissue, and the effects of environmental temperature on fatty acid composition of the blubber were also explored. This study was initiated in response to concerns about beluga health by Inuvialuit harvesters, stemming from more frequent observations of belugas containing thinner blubber.

Findings from Chapter 2 revealed that the inner, middle, and outer blubber layers contained different proportions of fatty acid types that were influenced by blubber thickness, highlighting the interplay between body condition and blubber composition. The innermost layer contained a greater proportion of PUFAs and long-chain MUFAs, and the outer layer contained more short-chain MUFAs. The middle layer acted as a transition layer in the proportions of fatty acid types between the inner and outer layers. The outer layer of blubber contained a greater abundance of fatty acids with lower melting points, and higher rates of delta-9 desaturation, demonstrating the impact of environmental temperature on fatty acid composition of the blubber. The whales with thinner blubber contained a greater proportion of short-chain MUFAs and lower relative amounts of long-chain MUFAs than whales with thicker blubber, which may provide evidence of lower feeding rates. A greater abundance of short-chain MUFAs in the whales with thinner blubber may also indicate a need for fatty acids with lower melting points due to the

environmental temperature effects, providing insight into how blubber composition may change should thin blubber become more prevalent in this population of whales.

Findings from the third chapter revealed that highest lipid content, which occurred in the middle blubber layer, was not influenced by body length, harvest location, body condition (standardized blubber thickness), or age of adults. This suggests the middle blubber layer plays a significant role in the storage of lipids in all belugas across size, adult age, and location. Outer blubber lipid does not best represent the total lipid found in the blubber, but the significant relationship found with muscle lipid percent suggests the outer layer of blubber may be a useful indicator of lipid stores found in the muscle tissue. The positive relationship between muscle protein content and total muscle lipid percent suggested that protein content and lipid content in the muscle tissue may be mobilized simultaneously during energy depletion, as opposed to one energy store being prioritized over the other. Results from this study indicate that the full depth of blubber needs to be included in blubber lipid analyses due to the differences present throughout the blubber layers. The findings from this study also suggest that blubber lipid percent or blubber thickness alone may not be sufficient to accurately reflect body condition in beluga whales, and additional factors should be considered in the assessment of body condition.

4.2 Study significance and limitations

4.2.1 Significance:

This study responded to observations and concerns raised by the Inuvialuit about the belugas they were harvesting and observing. As the first study of its kind, it provided baseline information on the stratification patterns of lipids in EBS beluga blubber, allowing for future

changes to be detected and recorded, providing insight should thinner beluga blubber become more prevalent. This study has supplemented our understanding of lipid composition and mobilization within beluga tissues and has highlighted the role that fatty acid melting point and delta-9 desaturation rates play in blubber composition. The Arctic is warming four times faster than the global average (Rantanen et al., 2022), therefore with an increase in ocean temperatures, it can be expected that biochemical changes in the blubber of marine mammals will occur. Additionally, results from this study stress that there is currently no single metric that provides a perfect measure of body condition in marine mammals, and that a species-specific approach that utilizes multiple variables is likely needed to accurately estimate beluga body condition. While whales with thicker blubber had an overall larger store of lipids in the body, there was no difference in total lipid percent in the blubber or muscle tissue between whales with differing blubber thicknesses, nor were there any differences in the vertical stratification patterns of fatty acids in the blubber. The major contrast between whales with thin or thick blubber presented in the form of fatty acid composition, as thin whales had a higher proportion of SCMUFAs and thicker whales a higher proportion of LCMUFAs. This may indicate a lack of feeding in thin whales, particularly of Arctic cod (*Boreogadus saida*). This also provides insight into how the environmental temperature modifies blubber composition if a whale does not have thick enough blubber. This may lead to a negative feedback loop as the whale will burn more calories maintaining fluid blubber lipids to sustain a proper core body temperature to compensate for a lack of insulative lipid stores, further depleting energy stores. Insights from this study enhance our understanding of the physiological processes that occur in beluga whale blubber, which contribute to informing management and conservation efforts.

4.2.2 Limitations:

Several limitations in the study may have impacted the results. A common challenge when working with fresh blubber tissue is the unavoidable loss of some lipid during blubber extraction from the carcass. Furthermore, precision is reduced when multiple people excise tissue simultaneously in the field. Additionally, as tissue samples are collected opportunistically from subsistence harvested whales, hunter bias was present. Beluga harvesters in this region typically opt for the larger males with white skin and will avoid females with calves, whales undergoing molting, or those that look old or sick (Ostertag et al., 2018). This led to a collection of whales where all individuals were adults, and almost all (Chapter 2) or all (Chapter 3) were male, limiting the representativeness of the results of this study as they may not accurately portray what would be found in female or juvenile belugas.

Another limitation was that the sample size restricted us to use a maximum of four variables in our GLMs, requiring the exclusion of some variables of interest in Chapter 3. With a larger sample size, harvest year and age could have been included in all models, which may have altered results. Harvest year could have influenced results due to differences in prey dynamics or environmental conditions between years. All individuals were adults; however, belugas are a long-lived species (the oldest beluga in our dataset was aged at 56 years old) making age a potential source of variation in tissue lipid content.

4.3 Future research

Future research should continue to support the conservation of the EBS beluga population and their Arctic ecosystem. Not only are beluga whales an integral member of Arctic marine

ecosystems, supporting ecosystem health through top-down predatory pathways (Prasad, 2024), but their health is also an important indicator of the health of the Arctic environment. In addition, belugas are important culturally and nutritionally to the Inuvialuit of the Western Arctic (Friesen and Arnold, 1995; Ostertag et al., 2018; FJMC, 2013), and thus Inuvialuit concerns and Traditional Knowledge should be incorporated into future studies. Ongoing monitoring of blubber thickness, along with broader assessments of beluga health is necessary for monitoring population status and ensuring the safety of consuming beluga as a traditional food source.

Thin whales in this study exhibited lower proportions of long-chain monounsaturated fatty acids, fatty acids associated with a diet of Arctic cod (Loseto et al., 2009; Choy et al., 2020). A natural follow up to this study would be to examine fatty acids in relation to beluga diet, to determine if prey abundance or quality is contributing to the occurrence of thinner blubber in some individuals. Northward movements of fish species distributions have already been documented (Falardeau et al., 2014), which may lead to an altered beluga prey community. Therefore, dietary analysis could determine whether thin beluga individuals contain less long-chain MUFAs due to an overall lack of food, or if they are relying on alternative prey, which would suggest that reduced blubber thickness may become increasingly common in beluga whales as Arctic ecosystems continue to change.

Future research could include the examination of adipocytes in the blubber of Eastern Beaufort Sea belugas. Adipocyte size analysis would offer insight into beluga blubber at a finer scale than lipid percent and could indicate whether whales with thinner blubber are exhibiting altered or disadvantageous cellular structures. A relationship between Hudson Bay beluga adipocyte size and blubber lipid percent has already been explored, where adipocyte size was shown to fluctuate annually but remain stable across seasons, even when lipid content varied

across seasons and years (Belanger et al., 2025). However, these findings come from a different beluga stock with distinct habitat use and migration routes. Analysing adipocytes that come from the same blubber samples used in this study would allow for a direct comparison with lipid percent, enhancing our understanding of lipid storage and mobilization in beluga blubber.

This study demonstrated how delta-9 desaturase activity occurs throughout the full depth of blubber in thin whales. An apt complement to this analysis would be to explore the thermal conductivity of the blubber in these individuals, to examine the extent to which decreased blubber thickness compromises the thermoregulatory function of blubber. A decreased thermoregulatory capacity could lead to an increase in energy expenditure, reducing fitness, and making belugas more vulnerable to changing Arctic conditions, ultimately compromising survival.

4.4 References

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Supplementary Materials

Community Engagement

This research was initiated in response to Inuvialuit harvesters regarding concerns of beluga health, and therefore, the inclusion of Inuvialuit knowledge and ensuring results were disseminated back to communities was of utmost importance. The field work for this project took place in the Inuvialuit Settlement region, where I had the opportunity to observe and learn from the community about the traditional harvest of beluga whales. The most up-to-date results on the total lipid percent and fatty acid composition of beluga blubber were shared via oral presentations at the 2024 FJMC meeting, the Arctic Net 2024 conference, and a 2025 Paulatuk Hunters and Trappers Committee meeting. As concerns regarding 'skinny whales' was voiced as a priority concern, results presented focused on the findings seen in the whales with thin blubber. Results will continue to be shared into the future at upcoming HTC and FJMC meetings.

Supplementary Materials Associated with Chapter 2

Table S-1. Fatty acids retained for analysis after removal of low percentage (<0.1%) fatty acids.

Fatty acid	Classification	Diet
C12:0	SFA	Non-dietary
C12:1	MUFA	Non-dietary
C13:0	SFA	Non-dietary
C14:0	SFA	Dietary
C14:0 ante	SFA	Non-dietary
C14:0 iso	SFA	Non-dietary

Fatty acid	Classification	Diet
C14:1n5	MUFA	Non-dietary
C14:1n7	MUFA	Non-dietary
C14:1n9	MUFA	Non-dietary
C15:0	SFA	Non-dietary
C15:0 iso	SFA	Non-dietary
C16:0	SFA	Dietary
C16:0 7ME	SFA	Dietary
C16:0 iso	SFA	Dietary
C16:1n11	MUFA	Non-dietary
C16:1n5	MUFA	Non-dietary
C16:1n7	MUFA	Non-dietary
C16:1n9	MUFA	Non-dietary
C16:2n4	PUFA	Dietary
C16:2n6	PUFA	Dietary
C16:3n4	PUFA	Dietary
C16:4n1	PUFA	Dietary
C16:4n3	PUFA	Dietary
C17:1	MUFA	Non-dietary
C18:0	SFA	Dietary
C18:1n11	MUFA	Dietary
C18:1n5	MUFA	Non-dietary
C18:1n7	MUFA	Dietary
C18:1n9c	MUFA	Dietary
C18:2n6c	PUFA	Dietary
C18:3n3	PUFA	Dietary
C18:4n1	PUFA	Dietary

Fatty acid	Classification	Diet
C18:4n3	PUFA	Dietary
C20:1n11	MUFA	Dietary
C20:1n7	MUFA	Dietary
C20:1n9	MUFA	Dietary
C20:2n6	PUFA	Dietary
C20:4n3	PUFA	Dietary
C20:4n6	PUFA	Dietary
C20:5n3	PUFA	Dietary
C22:1n11	MUFA	Dietary
C22:1n7	MUFA	Dietary
C22:1n9	MUFA	Dietary
C22:2 NMI D1	PUFA	Non-dietary
C22:5n3c	PUFA	Dietary
C22:6n3	PUFA	Non-dietary
C24:1n9	PUFA	Dietary

Table S-2. General linear mixed models (GLMMs) used to determine proportional differences in fatty acid composition between blubber layers. Wald z-tests are reported. Degrees of freedom are not estimated under maximum likelihood.

Model Used	Effect	Estimate (β)	SE	z value	p value	Post-hoc comparisons
MUFA_adj ~ layer_most + (1 | individual)	Intercept (inner)	0.569	0.044	13.00	< 0.001	inner - middle p = 0.082; inner - outer p < 0.001; middle - outer p < 0.001
	Middle	0.109	0.051	2.14	0.033	
	Outer	0.309	0.044	7.05	< 0.001	
PUFA_adj ~ layer_most + (1 | individual)	Intercept (inner)	-1.619	0.060	-26.85	< 0.001	inner - middle p = 0.061; inner - outer p < 0.001; middle - outer p < 0.001
	Middle	-0.142	0.062	-2.27	0.023	
	Outer	-0.487	0.066	-7.40	< 0.001	
SFA_adj ~ layer_most + (1 | individual)	Intercept (inner)	-1.367	0.027	-50.81	< 0.001	inner - middle p = 0.260; inner - outer p = 0.061; middle - outer p = 0.616
	Middle	-0.037	0.024	-1.57	0.117	
	Outer	-0.055	0.024	-2.26	0.024	
LCMUFA_adj ~ layer_most + (1 | individual)	Intercept (inner)	-1.170	0.075	-15.51	< 0.001	inner - middle p < 0.001; inner - outer p < 0.001; middle - outer p < 0.001
	Middle	-0.563	0.085	-6.61	< 0.001	

	Outer	-0.907	0.073	-12.46	< 0.001	
SCMUFA_adj ~ layer_most + (1 | individual)	Intercept (inner)	-0.364	0.066	-5.55	< 0.001	inner - middle p < 0.001; inner - outer p < 0.001; middle - outer p < 0.001
	Middle	0.452	0.072	6.30	< 0.001	
	Outer	0.791	0.058	13.58	< 0.001	

Table S-3. General linear models (GLMs) used to determine differences in proportions of fatty acid types between differing standardized blubber thicknesses. Wald z-tests are reported. Degrees of freedom and not estimated under maximum likelihood.

Model Used	Effect	Estimate (β)	SE	z value	p value	Post-hoc comparisons
MUFA_adj ~ ratio_label	Intercept (medium)	0.659	0.056	11.70	< 0.001	thin - medium p = 0.999; medium - thick p = 0.824; thin - thick p = 0.784
	Thick	-0.056	0.088	-0.63	0.526	
	Thin	0.000	0.071	0.00	1.000	
PUFA_adj ~ ratio_label	Intercept (medium)	-1.838	0.097	-19.00	< 0.001	thin - medium p = 0.681; medium - thick p = 0.911; thin - thick p = 0.464
	Thick	0.088	0.148	0.60	0.551	
	Thin	-0.112	0.123	-0.91	0.362	

SFA_adj ~ ratio_label	Intercept (medium)	-1.511	0.035	-42.80	< 0.001	thin - medium p = 0.212.; medium - thick p = 0.884; thin - thick p = 0.583
	Thick	0.028	0.055	0.51	0.609	
	Thin	0.083	0.044	1.89	0.059	
LCMUFA_adj ~ ratio_label	Intercept (medium)	-1.669	0.073	-22.76	< 0.001	thin - medium p = 0.0388; medium - thick p = 0.0453; thin - thick p < 0.001
	Thick	0.306	0.108	2.82	0.005	
	Thin	-0.252	0.095	-2.64	0.008	
SCMUFA_adj ~ ratio_label	Intercept (medium)	0.004	0.068	0.06	0.953	thin - medium p = 0.376; medium - thick p = 0.0701; thin - thick p = 0.0018
	Thick	-0.234	0.107	-2.19	0.029	
	Thin	0.122	0.086	1.42	0.155	
Omega_3_avg ~ ratio_label	Intercept (medium)	-2.072	0.112	-18.47	< 0.001	thin - medium p = 0.468; medium - thick p = 0.923; thin - thick p = 0.317
	Thick	0.106	0.170	0.62	0.535	
	Thin	-0.186	0.144	-1.29	0.196	
Omega_6_avg_adj ~ ratio_label	Intercept (medium)	-4.349	0.039	-111.49	< 0.001	thin - medium p = 0.954; medium - thick p = 0.999; thin - thick p = 0.960

	Thick	-0.001	0.061	-0.02	0.987	
	Thin	0.011	0.049	0.22	0.829	
PUFA_MUFA_ratio_avg ~ ratio_label	Intercept (medium)	-6.093	0.107	-56.79	< 0.001	thin - medium p = 0.824; medium - thick p = 0.900; thin - thick p = 0.578
	Thick	0.074	0.168	0.44	0.664	
	Thin	-0.081	0.135	-0.60	0.555	

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Table S-4. General linear mixed model used to explore differences in total lipid percent throughout the depth of blubber.

Model Used	Effect	Estimate (β)	SE	z value	p value	Post-hoc comparisons
Conditional: lipid_prop ~ layer_most + (1 | individual)	Intercept (inner)	1.603	0.079	20.28	< 0.001	inner - middle p = 0.171; inner - outer p = 0.833; middle - outer p = 0.018
	Middle	0.145	0.081	1.80	0.073	
	Outer	-0.050	0.087	-0.58	0.565	

Dispersion: lipid_prop ~ layer_most + (1 | individual)	Intercept (inner)	3.684	0.266	13.85	< 0.001
	Middle	0.925	0.450	2.06	0.040
	Outer	0.378	0.380	0.99	0.320

Table S- 5. General linear models exploring the effects of various covariates on total lipid percent in the blubber or muscle tissue, or protein content in the muscle tissue.

Model Used	Effect	Estimate (β)	SE	z value	p value
total_lipid_percent_inner ~ + age + blubber_depth_body_length_ratio + location + body_length	Intercept	4.549	0.226	20.10	< 0.001
	Age	-0.001	0.001	-0.41	0.686
	Standardized blubber thickness	0.093	0.631	0.15	0.883
	Location: Paulatuk	0.044	0.034	1.30	0.202
	Body Length	-0.000	0.001	-0.60	0.552
total_lipid_percent_inner ~ + age + blubber_depth_body_length_ratio + location + body_length + year	Intercept	-34.970	-0.560	-19.00	0.579
	Age	-0.001	0.001	-0.50	0.620

	Standardized blubber thickness	-0.026	0.662	-0.04	0.969
	Location: Paulatuk	0.040	0.035	1.17	0.251
	Body Length	-0.000	0.001	-0.62	0.542
	Year	0.020	0.031	0.63	0.531
total_lipid_percent_middle ~ + age + blubber_depth_body_length_ratio + location + body_length	Intercept	4.481	0.154	29.03	< 0.001
	Age	-0.001	0.001	-1.20	0.238
	Standardized blubber thickness	-0.536	0.426	-1.26	0.217
	Location: Paulatuk	0.000	0.023	0.02	0.985
	Body Length	0.000	0.000	0.25	0.805
total_lipid_percent_middle ~ + age + blubber_depth_body_length_ratio + location + body_length + year	Intercept	12.401	42.530	0.29	0.772
	Age	-0.001	0.001	-1.12	0.272
	Standardized blubber thickness	-0.520	0.443	-1.17	0.250
	Location: Paulatuk	0.001	0.023	0.04	0.972

	Body Length	0.000	0.000	0.25	0.807
	Year	-0.004	0.021	-0.19	0.853
total_lipid_percent_outer ~ + age + blubber_depth_body_length_ratio + location + body_length	Intercept	4.343	0.209	20.82	< 0.001
	Age	-0.000	0.001	-0.01	0.990
	Standardized blubber thickness	-0.516	0.577	-0.90	0.377
	Location: Paulatuk	-0.009	0.031	-0.30	0.766
	Body Length	0.000	0.001	0.49	0.629
total_lipid_percent_outer ~ + age + blubber_depth_body_length_ratio + location + body_length + year	Intercept	4.074	57.300	0.07	0.944
	Age	-0.000	0.001	-0.01	0.989
	Standardized blubber thickness	-0.517	0.610	-0.85	0.403
	Location: Paulatuk	-0.009	0.031	-0.29	0.771
	Body Length	0.000	0.001	0.48	0.635
	Year	0.000	0.028	0.01	0.996

muscle_lipid ~ blubber_outer + body_length + blubber_depth_body_length_ratio + location	Intercept	7.616	1.935	3.94	0.000
	Outer blubber lipid %	-0.039	0.016	-2.37	0.024
	Standardized blubber thickness	-0.515	4.448	-0.12	0.909
	Location: Paulatuk	0.260	0.236	1.10	2.800
	Body Length	-0.006	0.004	-1.61	0.117
muscle_protein ~ poly(blubber_depth_body_length_ratio, 2)	Intercept	3.545	0.005	756.86	< 0.001
	Standardized blubber thickness (Linear)	-0.125	0.070	-1.78	0.077
	Standardized blubber thickness (Quadratic)	-0.501	0.070	-7.12	<0.001
muscle_protein ~ poly(muscle_lipid, 2)	Intercept	3.545	0.005	740.10	< 0.001
	Muscle lipid % (Linear)	0.390	0.072	5.40	< 0.001
	Muscle Lipid % (Quadratic)	-0.145	0.072	-2.00	0.044