Linking Feeding and Reproductive Ecology in Beluga (*Delphinapterus leucas*) and Narwhal (*Monodon monoceros*)

by

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Abstract

Beluga whales (*Delphinapterus leucas*) and narwhals (*Monodon monoceros*) are arctic specialists.   Both species show philopatry to their summer grounds, though the reason for this site tenacity is not well understood. Aside from migration routes, little is known about other aspects of monodontid ecology, such as their mating and feeding ecology. An understanding of the feeding ecology of a species may provide some insights into their mating ecology, and vice versa. The purpose of this thesis is to relative testes mass and dietary biomarkers to gain insights in the mating and feeding ecology of both species, as well as possible links between the two.

Relative testes and brain masses and body masses of odontocetes were collected from the literature and analysed for correlations between sexual size dimorphism (SSD), relative brain mass, and relative testes mass. Results indicate that odontocete species follow a pattern of increasing SSD with decreasing testes mass. An examination of reproductive tracts from belugas and narwhal collected across the Canadian arctic was performed to examine differences in beluga and narwhal mating systems. Belugas were found to have larger relative testes masses, and narwhal testes masses were correlated with tusk length, indicating that sperm competition may play a larger role in the beluga mating system than for narwhal, and narwhal tusks may be honest indicators of male fitness.

Investigations of narwhal and beluga feeding ecology using dietary biomarkers were conducted. In the summer, belugas appear to be congregating and feeding in the estuary plume during the summer, as opposed to along ice floe edges in the spring. Spring diets are representative of diets consumed during the beluga mating season, and no sexual segregation in carbon isotopes or fatty acids was apparent. There was no evidence for sexual segregation in feeding habits outside the mating season, either. Conversely, narwhal showed some evidence of sexual segregation outside the mating season, and the sexes may be feeding in different food webs.

Results suggest that belugas may have a more promiscuous mating system, while narwhals are more polygynous. Implications for conservation for both species are discussed.

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Thesis Format and Claim

This thesis follows a manuscript format. Chapters 2, 3, 4, and 5 are written as manuscripts with Abstract, Introduction, Methods, Results, and Discussion sections. Chapter 1 introduces the theme and background information upon which the manuscript chapters are based, and Chapter 6 synthesizes the results of the chapters.

Chapter 1. Kelley, T.C., Higdon, J.W., and Ferguson, S.H. Large testes and brain masses in odontocetes: The influence of mating system on encephalization. Submitted to the Canadian Journal of Zoology. Trish Kelley developed and wrote the paper, and collected and analysed the data with the guidance of the co-authors.

Chapter 2. Kelley, T.C., Stewart, R.E.A., Yurkowski, D., Ryan, A., Ferguson, S. H. Mating ecology of beluga (*Delphinapterus leucas*) and narwhal (*Monodon monoceros)* as estimated by reproductive tract metrics. A revised version of this manuscript was submitted to Marine Mammal Science. Trish Kelley measured the reproductive tracts with the assistance of D. Yurkowski and A. Ryan, and developed and wrote the paper and analysed the data with the assistance of the co-authors.

Chapter 3. Kelley, T. C., and Ferguson, S.H. Stable Isotopes and Fatty Acids Differentiate Between Summer and Spring Feeding in Belugas (*Delphinapterus leucas*) in Hudson Bay. Submitted to Aquatic Biology. Trish Kelley performed the sample preparations, data analysis, and analysed and wrote the paper with the guidance of the co-author.

Chapter 4. Kelley, T.C. and Ferguson, S.H. Sexual segregation in two closely related species: beluga whales (*Delphinapterus leucas*) and narwhal (*Monodon monoceros*). Submitted to Polar Biology. Trish Kelley performed the sample preparations, data analysis, and analysed and wrote the paper with the guidance of the co-author.

1 General Introduction

1.1 Beluga and narwhal biology

Beluga whales (*Delphinapterus leucas*) are Arctic specialists. They are an important part of the Canadian Arctic and are an important species in the diet of local Inuit communities. They have a circumpolar distribution, and in Canada range from the Beaufort Sea to their southernmost habitat, the Gulf of St. Lawrence (Brodie 1989; Kleinenberg 1969; Smith et al. 1990). Beluga whale evolutionary history and ecology is closely linked to the sea ice and the extreme seasonal variation of the Arctic (Smith et al. 1990). Belugas grow up to 550 cm long and weigh up to 1500 kg, with males growing larger than females and exhibiting a more robust build (Brodie 1989; Smith et al. 1990; Stewart 1994). Belugas calve every 3 years and have a gestation period of 14 – 14.5 months (Brodie 1971; Sergeant 1973). Although they are believed to mate in late winter to early spring, beluga mating behaviour has been seen at other times of the year and is not well understood (Smith et al. 1990). Beluga whales return to traditional summering grounds yearly, and seem to be separated into stable populations in their summering grounds based on matrilineal philopatry (Richard 2005). Belugas are opportunistic feeders, and consume a variety of prey including Arctic cod (*Boreogadus saida*), capelin (*Mallotus villosus*), redfish (*Sebastes marinus*), halibut (*Reinhardtius hippoglossoides*) and shrimp (*Pandalus borealis*) (Kelley et al. 2010; Loseto et al. 2009).

Narwhal are closely related to beluga, and the two species comprise the family Monodontidae (May-Collado et al. 2007). In Canada, narwhal inhabit the Eastern Arctic, with a range extending from Northern Hudson Bay in the south to Ellesmere Island in the North, and the West coast of Greenland to Peel Sound (Hay and Mansfield 1989). Males reach sexual maturity at a length of 420 cm, and females reach sexual maturity at a body length of 360 cm (Hay and Mansfield 1989). Reproduction data on narwhal are deficient, but they are believed to calve every three years, mate in early to late spring and have a gestation period of 13 – 16 months (Best and Fisher 1974; Heide-Jørgensen and Garde 2011). The feature most striking in narwhal is the male tusk, a maxillary tooth that grows through the maxillary bones and skin and can grow up to 3-m long (Best 1981). The tusk erupts at a body length of 260 cm and attains a length of 150 cm at sexual maturity, but continues to grow as narwhals physically mature (Best 1981). The function of the tusk has been the topic of some debate; it has been suggested the tusk serves as a sensory organ (Nweeia et al. 2014), though it is most likely related to a mating system in which males compete for females through physical competition or display (Best 1981; Silverman and Dunbar 1980). The presence of the tusk suggests a more polygynous (one male mates with multiple females) mating system than that for belugas. This potential difference in narwhal life history strategies, coupled with the relatedness of the two species, make narwhal an ideal contrast for understanding beluga feeding and mating ecology.

1.2 Conservation Concerns

For most animal species, conservation programs involve the conservation of genetic diversity (Whitehead et al. 2004). While genetic diversity is important for ensuring a viable population, learned and inherent behaviour necessary for the survival of the species is also integral to conservation of endangered or threatened species (Côté 2003). Most cetacean species have large brains, sophisticated communication abilities and complex social structures that include stable groups within a population for at least some part of the year (Whitehead 2003). Matrilineal philopatry exists in several cetacean species (Connor 2000; Richard 2005; Whitehead and Mann 2000), including beluga and narwhal (Heide-Jørgensen et al. 2012; Richard 2005), where juveniles learn foraging techniques, food patch locations, and migration routes from their mothers and other conspecifics (Whitehead and Mann 2000). It is crucial that an understanding of the behaviour of the Arctic cetacean species be obtained, as climate change will affect the northern latitudes sooner and with greater intensity than more southern latitudes (Ernakovich et al. 2014; Johannessen et al. 2004; Kasischke et al. 2013).

Habitat loss in the form of decreased sea ice caused by anthropogenic climate change may impact narwhal and beluga, species that both utilise sea ice-covered habitat for most of the year (Laidre et al. 2008). Narwhal are particularly dependant on dense pack ice, occupying pack ice habitat throughout the winter (Laidre et al. 2008); this is likely a critical habitat for them as the majority of their annual energy intake occurs in the winter months (Laidre et al. 2003; Laidre et al. 2004; Laidre and Heide-Jørgensen 2005). Beluga may use loose pack ice covered water to escape killer whale (*Orcinus orca*) predation (Frost et al. 1992) and potentially to feed on Arctic cod (Laidre et al. 2008). Reduction of sea ice may also increase anthropogenic effects on narwhal and beluga, as shipping routes through the arctic open up (Wilson et al. 2004), creating a potential for abandonment of critical habitat by marine mammals due to anthropogenic noise (Erbe and Farmer 2000). Decreases in the extent of sea ice cover, or the duration of sea ice cover, may result in ecosystem shifts, such as increased submarine water irradiance and warmer waters altering phytoplankton and primary productivity (Arrigo et al. 2008; Mundy et al. 2009; Pabi et al. 2008; Qu et al.

2006). Increased water temperature may also increase interspecific competition for prey species as other piscivorous cetaceans expand their range northward (Laidre et al. 2008). Pilot whales (*Globicephala macrorhynchus*), harbor porpoises (*Phoceona phoceona*), and killer whales currently move into the Arctic in the summer to feed; warmer water temperatures may encourage these species to move northward earlier in the year and stay longer (Laidre et al. 2008). Killer whales moving into the Arctic may also increase predation pressure on narwhal and beluga (Campbell et al. 1988; Shelden et al. 2003).

Both belugas and narwhals exhibit a philopatric migration to estuaries (belugas), and fjords and bays (narwhal) (Caron and Smith 1990; Hay and Mansfield 1989; Richard 1994, 2005; Richard et al. 1990; Silverman 1979), despite potential increased predation by land-based predators such a polar bears (*Ursus maritimus*) and humans (Hobbs et al. 2005; Watts et al. 1991). A number of explanations for this behaviour have been suggested, such as prey movement (Kleinenberg 1969; but see Hobbs et al. 2005; Sergeant 1973), calving and warm water advantage (Brodie et al. 1981; Sergeant and Brodie 1975) (though see Watts et al. 1991), and predator avoidance (Brodie 1971; Richard 2005). Though a greater proportion of females with calves are typically reported near shore (Caron and Smith 1990; Mansfield et al. 1975), visual identification of individuals comprising a herd may be biased towards more immediately identifiable individuals (Born et al. 1994) and may not reflect true herd composition. Both males and females of both species take part in annual migrations to summering grounds.

Though the role of estuaries in beluga ecology is not well understood, they seem to hold some adaptive significance, as individuals return to the same estuary in successive years

(Caron and Smith 1990), even after repeated disturbances from hunting activity and boat traffic (Smith et al. 1990). Site tenacity may make belugas more vulnerable to the impacts of anthropogenic alterations to beluga habitat. Hydroelectric developments may affect the heat budget of estuaries, both by reducing the overall outflow and creating seasonal fluctuations of freshwater to meet electricity demand (Sergeant and Brodie 1975). Annual return to disturbed areas does not mean whales have developed tolerance to the disturbance, but may underline the importance of the area and the absence of better options (Brodie 1989).

An understanding of beluga and narwhal feeding and mating ecology, including prey species, critical areas for feeding, mating, and calving, is crucial to mitigating the potential impacts of climate change and associated anthropogenic activities in the Arctic.

1.3 Mating Systems and Feeding Ecology

Feeding ecology can be linked to mating systems. In order for mature members of a species to successfully reproduce, they must obtain adequate energy to compete for mates (males) or for gestation and lactation (females). Resource distribution influences the carrying capacity of a given habitat, which in turn influences the reproductive opportunities for species within that habitat (Lindström 2001). In addition to increased mating opportunities, group living confers a number of advantages, such as increased predator detection and defense (Hass and Valenzuela 2002; Molvar and Bowyer 1994; O'Brien 1991), and increased hunting and foraging efficiency (Bekoff et al. 1981; Kleiman and Eisenberg 1973; Molvar and Bowyer 1994). Gregarious species would be expected to increase their group size during mating season in order to increase genetic diversity and the number of potential mates (Bronson 1989). The maximum group size in social species however, is limited by a number of environmental variables; one of the most influential is food availability.

Distribution of resources determines the distribution of females, and the distribution of females dictates the number of females a male can potentially mate with (Emlen and Oring 1977). If resources are clumped, females are predicted to congregate, and males may have access to multiple females (Andersson 1994). If males must compete for females through physical competition, then males with larger bodies will more often be successful and therefore pass on their genes, which can lead to increased sexual size dimorphism within the species (Darwin 1879; Mesnick and Ralls 2002; Weckerly 1998). Differences in body size can influence energetic demands, and thus males and females from dimorphic species may have vastly different energy budgets (Ruckstuhl and Clutton-Brock 2005). An understanding of both the feeding and mating ecology of a species is important to develop a management plan for that species.

1.3.1 Environmental Influences on Mating Systems

Mating systems reflect the efforts of males and females to maximize their lifetime reproductive success. The term 'mating system' describes a general behavioral strategy employed by a species to acquire mates. It includes the number of mates acquired, the method of mate acquisition, the presence or absence of pair bonds, and the extent of parental care provided by each sex (Emlen and Oring 1977). Mating systems are categorized along a continuum of the relative importance of sperm competition, especially when observations of mating activity and social interaction are difficult to observe. ‘Polygyny’, in which one male mates with several females and sperm competition is low, is at one end of the continuum, and ‘polygynandry’ or ‘promiscuity’, in which both males and females mate with a number of mates and sperm competition is high, is at the other. ‘Monogamy’ describes mating system in which each individual mates with one mate, and lies between the ends of the continuum (Thornhill and Alcock 1983). In mammals, monogamy is quite rare (Clutton-Brock 1989). It is unlikely that cetacean species are monogamous, so cetacean mating systems are typically described as either polygynous or polygynandrous (Connor et al. 2000).

Sexual selection is expected to be exerted most significantly on the sex that invests the least in offspring (Trivers 1972). The development of sex-specific traits, especially traits that seem costly to maintain and produce, would likely have come about via linkage with some underlying adaptive trait (Zahavi 1975) and exaggerated through Fisher runaway processes (Fisher 1930). However, the extent to which sexual selection can operate on populations is limited by a number of ecological factors (Emlen and Oring 1977) such as resource availability, the spatial distribution of resources and mates, and the temporal distribution of mates (Bronson 1989).

Sexual selection is believed to be the main cause of sexual size dimorphism (SSD) (Darwin 1879; Ralls 1977; Weckerly 1998). In polygynous species where males compete physically for females or control of resources or territories, increased body size would be advantageous (Darwin 1879). Increased body size in males has been correlated with increased reproductive output; increased body size in females in correlated with increased offspring fitness (Andersson 1994; Shine 1989). One would predict, then, that the evolution of increased body size would follow Fisher runaway processes, and organisms would become increasingly larger (Fisher 1930). Increasing species size would likely increase SSD, as SSD increases with increasing body size in species where males are larger than females, but decreases when females are larger than males (i.e. Rensch,s Rule (Rensch 1959)), and males with the highest levels of physical competition would benefit from the highest degree of sexual dimorphism (Dale et al. 2007). A systematic comparison of mammalian taxa shows that Rensch's Rule does not hold across all species (Weckerly 1998). Instead, a great deal of variation occurs across taxa and mating systems, occurring as the result of a number of environmental factors.

Probably the most influential environmental factor on body size is the availability of resources. Animals increasing in size would require an increase in energy intake, as increased tissue development would require more energy to develop and maintain (Weckerly 1998). Sexual selection-driven SSD then, may be limited by the availability of nutrients. This is the main reason cited for the existence of larger males and smaller females in most mammalian taxa (Ralls 1977). When females are larger than males, the cause is typically associated with the need for increased resource sequestering for successful development of the fetus or for lactation, especially when either of these functions are performed during a time of fasting or low food availability (Ralls 1976). The importance of food availability in the development of body size likely contributes to the inverse relationship between group size and body size (Blanckenhorn 2000). Species with successful group-living strategies would be unlikely to benefit from increased body size.

Risk of predation limits body size growth as well. Increased body size accompanies a decrease in manoeuvrability, so predator avoidance and prey capture mechanisms would increase the risk of early death (Hosken and Blanckenhorn 1999). Increased size in prey species also increases visibility(LaBarbera 1989). In organisms for which manoeuvrability is important in mate acquisition, such as cetaceans, lack of manoeuvrability may decrease the likelihood of successfully mating.

A third influence on body size is life span. Longer-lived organisms have more time to invest in growth. Short-lived species should not be selected for increasing body size, as continued growth throughout life would divert energy away from activities such as reproduction (Brown et al. 1993).

1.3.2 Seasonality of Mating

The availability of food fluctuates not only across space, but through time. No habitat has a continuous excess of food, and fluctuations in food availability across seasons influences female estrus in many species. Though males can theoretically mate at any time of year, many undergo testicular regression in non-breeding seasons (Connor et al. 2000; Harcourt 1995; Millesi et al. 1998; Parsons and Grier 1992), likely to conserve energy when mating attempts would not be successful. Seasonal peaks in birth and ovulation in females have likely arisen from a number of factors, such as seasonal food availability and timing with migration to less predator-infested habitats (Whitehead and Mann 2000). Distribution of receptive females across time can limit male monopolization of multiple females. Strong seasonality limits the abilities of males to access multiple females (Van Hooff and van

Schaik 1992), and if congregation of a species occurs because of seasonal prey distribution, increased male competition for female choice may occur (Whitehead and Mann 2000).

1.3.3 Sexual Segregation

Sexual segregation is linked to both mating systems and to seasonality. Sexual segregation is the separation of males and females within a population, either in habitat use, social interactions, or spatial use (Ruckstuhl and Clutton-Brock 2005). Sexual segregation has been reported in a number of cetaceans, and typically occurs in species with higher SSD (Mysterud 2000). SSD, as previously discussed, is often the result of sexual selection and seen in the greatest extremes in polygynous mating systems (Ralls and Mesnick 2002). An increase in body size is associated with increased energetic demands, which can result in an increased home range size (Jarman 1974). Availability of food and seasonality of mating systems can lead to sexual segregation in habitat use. Highly dimorphic species may exhibit larger degrees in segregation in habitat use, as the larger bodied males have a higher absolute energy requirement outside the breeding season (Breed et al. 2006).

1.4 Estimating Mating System

An understanding of a species mating system can be gained through detailed behavioural observations and genetic markers, however, in cetaceans detailed behavioural analyses are difficult to obtain. Measuring the relative testes mass (the mass of the testes in respect to total body mass) can be used as a proxy for understanding the importance of sperm competition in a species, as larger testes are correlated with higher levels of sperm competition, which are a result of increased promiscuity (Short 1997). A larger testes size relative to body size allows for the production of more sperm per ejaculate (Gomendio and Roldan 1993), and a longer penis allows for sperm deposition closer to the site of fertilization in the female reproductive tract (Dixson 1998). This trend has been found across mammalian taxa (Brownell and Ralls 1986; Connor et al. 2000; Dixson 1998; Kenagy and Trombulak 1986), and can be used as an indicator of mating system in species for which other means of assessing mating systems are unavailable (Gomendio et al. 1998; Perrin and Mesnick 2003). Though Kenagy and Trombulak (1986) included 5 odontocete species in their mammalian study, and Connor et al. (2000) examined the relationship between delphinid genera, this trend has not been directly examined at the species level in cetaceans.

Also unexamined in cetacean is the relationship of brain size to mating system. Though brain size is influenced by a number of factors, such as allometry (Pitnick et al. 2006), habitat complexity (Safi and Dechmann 2005), sensory abilities (Barton et al. 1995), complexity of social interactions (Dunbar 1992, 1998), it may also be influenced by mating system (Pitnick et al. 2006; Shillaci 2006, 2008). Mating can be regarded as a cooperative behaviour between conspecifics with the end result being offspring; however both sexes often have conflicting interests when selecting mates (Arnqvist and Rowe 2005). When one sex seeks to manipulate the other, cognitive capacity, and therefore brain size, is hypothesized to increase (Machiavellian Intelligence Hypothesis) (Rice and Holland 1997). If this hypothesis is true for odontocetes, comparative measurements of brain size may also be used to establish mating system for species for which direct observation of mating behaviour is unavailable.

1.5 Using Dietary Biomarkers to Understand Feeding Ecology

Isotopes are atoms of an element with the same number of protons and electrons as other atoms, but with a different number of neutrons. Stable isotopes refer to those isotopes that are energetically stable due to a similar number of protons and neutrons (Sulzman 2007). Stable isotope analyses of animal tissues are useful in studies of diet and migration, as they reflect local food webs (Hobson 1999). Stable isotopes are incorporated from prey into predator tissue with progressive enrichment, the “trophic enrichment factor”; an enrichment in the heavier isotope in the predator relative to the prey (Michener and Kaufman 2007). The relatively higher trophic enrichment factor of nitrogen allows for the determination of trophic levels an organism occupies in relation to other organisms in the food web, while the low trophic enrichment factors of carbon and sulphur allow for the determination of primary productivity in food webs, and can give insights into marine vs. terrestrial and benthic vs. pelagic feeding (Michener and Kaufman 2007).

Animal lipids are comprised primarily of fatty acids (Budge et al. 2006). They make ideal biomarkers as many fatty acids are transferred from predator to prey with no or with few predictable changes (Kelley et al. 2010; Loseto et al. 2009); this is especially true for fatty acids in marine ecosystems, as many marine predators cannot synthesize fatty acids, so fatty acids present in predators are reflective of their prey (Budge et al. 2008). Fatty acid analysis provides information on the types of prey consumed, as well as the transfer of specific fatty acids through the food web (Budge et al. 2006; Budge et al. 2008; Loseto et al. 2009).

1.6 Thesis Approach and Objectives

The overall objective of this thesis is to link feeding ecology and mating ecology in belugas and narwhals to better understand the social dynamics and habitat use of these two important Arctic species.

The underwater mating systems of odontocetes are difficult to observe, but comparative studies on terrestrial mammals have shown an inverse relationship between relative testes mass (testes mass after accounting for species differences in body size) and SSD. Similar studies have not been conducted using only odontocetes. Cetaceans in general do not conform to the linear allometric relationship used to account for the influence of body size on testes size in terrestrial mammals (MacLeod 2010); it is important to establish a relationship between SSD and testes mass before relative testes masses can be used to infer odontocete mating systems. In Chapter 2, data on testes mass and body mass were summarized from the literature for 38 odontocetes and analyzed together with new data on beluga and narwhal. Data on brain masses were also summarized from the literature, to determine if brain mass was a useful morphological measurement to assist in estimating mating systems, especially if brain masses are available for species for which a paucity of data exists to estimate mating systems. A negative relationship between brain mass and mating system was found in bats (Pitnick et al. 2006), primates (Shillaci 2006) and ungulates (Shultz and Dunbar 2007). The relationship between cetacean testes mass and brain size has not been examined. Results from this chapter lend support to using testes mass and SSD to estimate mating systems for belugas and narwhals; an analysis of this relationship is conducted in Chapter 3.

Chapter 3 uses reproductive tracts collected from both male and female belugas and narwhals from across the Canadian Arctic to infer the importance of sperm competition in both species. To determine if narwhal tusks were honest advertisements of male fitness, they were regressed agaonst testes mass. General linear models were used to determine the differences in relative testes mass between species as well as within species, for stock, and to establish the allometric relationship between testes mass and body mass for both species. Results from this chapter are used to estimate mating systems for belugas and narwhal.

Chapter 4 examines beluga feeding ecology for the Western Hudson Bay beluga population. Belugas move from the Hudson Strait where they overwinter into Hudson Bay following the ice edge of the melting sea ice (Richard 1994, 2005). This chapter uses dietary biomarkers to understand beluga estuary use and the variation in feeding behaviour for a population of whales on their summering grounds, and their late winter and early spring diets when they start their migration. Dietary biomarkers indicated a shift in feeding behaviour between spring and summer; beluga diets from different seasons cannot be pooled to determine feeding ecology year round. If beluga diets are linked to mating ecology, samples used to study diet must be collected at the same time in the beluga reproductive cycle under investigation.

Chapter 5 also uses dietary biomarkers, but compares male and female feeding ecology in belugas and narwhals outside the mating season to determine if sexual segregation occurs in the Western Hudson Bay population of belugas and the Baffin Bay population of narwhals. Sexual segregation has been reported in beluga populations in the Beaufort Sea

(Loseto et al. 2006; Loseto et al. 2009) and the Gulf of St. Lawrence (Lesage et al. 2001), as well as in Canadian narwhal populations (Watt et al. 2013). Results are discussed in terms of the relative importance of sperm competition, as described in Chapter 3, for both species.

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2 Large testes and brain masses in odontocetes: The influence of mating system on encephalization

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2.1 Abstract

Little is known about odontocete (Order Cetacea, suborder Odontoceti) mating behaviour, but anatomical adaptations to mating systems in toothed whales mating systems may be similar to those in terrestrial mammals. Species with large testes relative to body size are likely to have polygynandrous (multi-male/multi-female) mating systems, while species with smaller testes and greater sexual size dimorphism (SSD) are predicted to have polygynous mating systems. The sexual conflict/Machiavellian intelligence hypothesis predicts that polygynadrous species have also evolved larger brains both to coerce conspecifics to mate and resist mating attempts by undesirable mates. The costly tissue hypothesis predicts that species investing heavily in testes invest less in brain tissue and vice versa in order to conserve energy.

Residual testes and brain mass (controlling for body size allometry) measurements were used to test the sexual conflict and costly tissue hypotheses in 40 species of odontocetes. Correlations were performed on both raw data and independent contrasts to control for phylogeny. There was a significant positive correlation between residual testes mass and SSD in both data sets, and between residual testes mass and residual brain mass in the non-phylogenetically controlled data set. Results indicate a negative relationship between increased testes masses and SSD in odontocetes.

There was no support for the costly tissue hypothesis, and odontocetes may not be constrained in the development of tissues as observed in terrestrial or aerial mammals possibly due to a reduced cost of locomotion and buoyancy in water. Support for sexual conflict/Machiavellian intellegence hypothesis was found only when phylogenetic effects

were not considered. Group size and complexity of social interactions may be more influential in brain size development than mating system.

2.2 Introduction

Odontocetes, or toothed whales (Odontoceti: Cetacea) live in varied aquatic environments worldwide. There are approximately 67 species of odontocetes (Milinkovitch et al. 1994). Little is known about odontocete mating behaviour, as underwater mating systems are difficult to observe. An understanding of a species' mating systems is crucial to the understanding of the ecology of that species (Côté 2003; Dobson and Zinner 2003). Competition for mates, as well as mate choice, may impact the potential for population growth in species with declining populations (Rowe and Hutchings 2003). Though odontocete mating systems are difficult to observe, they likely utilize the same types of mating systems as terrestrial mammals (Connor et al. 2000). If odontocetes exhibit similar morphological adaptations to sperm competition to terrestrial mammals, morphological measurements may be useful in identifying mating systems.

Sperm competition theory predicts that in most mammals, males should attempt to mate with more than one female in a breeding season and females should attempt to mate with high quality males to increase their fitness (Trivers 1972). Males can compete for fertilization opportunities either precopulation, through methods such as intraspecific fighting and/or behavioural displays, or post-copulation, through methods such as sperm competition and/or mate guarding (Andersson 1994). Contest competition, or aggressive competition between males for access to females (Andersson 1994) has been observed in some odontocete species. When contest competition is important in a species, sexual dimorphism in weaponry or body size is common (Andersson 1994). Aggressive competition tends to select for increased body size in males, resulting in sexual size dimorphism (SSD) (Clutton-Brock et al. 1977; Darwin 1879; Ralls 1977). High degrees of SSD are most commonly associated with mating systems, where a female's ability to express their polygamy potential is limited by limited access to multiple males (Dixson 1998; Rose et al. 1997; Weckerly 1998). In odontocetes, the sperm whale, *Physeter macrocephalus* exhibits a high degree of SSD (Connor et al. 1998); there is also evidence of fighting in mature male *P. macrocephalus*, fights have been directly observed (Caldwell et al. 1966), as have broken teeth (Clarke and Paliza 1988) and head scarring (depigmented scars resulting from aggressive male-male interactions) (Best 1979). Head scarring has also been observed in a number of odontocete species and may serve as a signal of male quality in some cetacean species (MacLeod 1998). Sexual dimorphism in odontocetes may not always manifest as significant dimorphism in size, as manoeuvrability may be an important factor in underwater contests (Connor et al. 2000). Dimorphism in weaponry is also observed in narwhal (*Monodon monoceros*), as males possess tusks (Best 1981; MacLeod 1998; Silverman and Dunbar 1980). Sexual dimorphism may also manifest in propulsion structures such as flippers or caudal peduncle flukes in species that utilize scramble competition (Boness et al. 2002).

Comparative studies of terrestrial mammals have shown a positive correlation between polygynandry (mult-male/multi-female mating systems) and testes size (Connor et al. 2000; Dixson 1998; Kenagy and Trombulak 1986), as larger testes are needed to produce the elevated quantities of sperm required for success in sperm competition. This finding potentially suggests that researchers can substitute testes measurements for visual observations of mating behaviour in species in which observations are difficult to obtain; with the caveat that relative testes mass is a proxy for detailed genetic and observational data (Calhim and Birkhead 2007; Perrin and Mesnick 2003). A positive correlation between polygynandry and testes size in cetaceans has been shown (Connor et al. 2000; Kenagy and Trombulak 1986; MacLeod 2010). Comparisons of relative testes mass among odontocete species may provide some information on the potential importance of sperm competition within this lineage.

A third potential morphological measurement to consider in classifying mating system is brain size. The relationship between brain size and mating system has not been examined in odontocetes. Brain size, like testes size, is explained largely, but not entirely, by allometry (Kenagy and Trombulak 1986; Pitnick et al. 2006). Group size, complexity of social interactions and complexity of mating system (Dunbar 1992, 1998; Shillaci 2006, 2008), advanced sensory abilities (Barton et al. 1995), and habitat complexity (Safi and Dechmann 2005) are all correlated with the evolution of a large brain in a number of mammal groups. Primate brain evolution has received the most attention (Dechmann and Safi 2009), though studies on the factors influencing evolution of brain size for chiroptera (Dechmann and Safi 2009) and carnivores have also been performed (Dunbar 1998). Pitnick et al. (2006) found a negative correlation between female promiscuity and brain size in bats, though foraging ecology, a predictor of both brain size and mating system (Emlen and Oring 1977) may better explain the trend in brain sizes (Dechmann and Safi 2009). No general correlation between testes and brain mass has been found across mammalian taxa (LeMaître et al. 2009), but cetaceans were not included in the study. Because of the myriad factors influencing brain size, broad generalisations regarding mammalian brain size and promiscuity may not be possible to make across taxa.

Sexual conflict theory (Arnqvist and Rowe 2005; Rice and Holland 1997; Trivers 1972) predicts that males and females have conflicting interests when selecting mates (Arnqvist and Rowe 2005). Underlying this conflict is differential investment in offspring and gametes made by males and females. Females theoretically produce fewer, more costly gametes and males produce numerous, inexpensive gametes, and in mammals, females invest heavily in offspring. Odontocetes invest highly in both gestation and lactation, resulting in extended interbirth intervals (3-6 years, *Tursiops* species (Mitcheson 2008)*,* 5.3 years, *Orcinus orca* (Olesiuk et al. 1990)). This difference in reproductive investment can lead to different mating strategies to increase reproductive fitness in males and females (Trivers 1972). When males can secure multiple mates, the potential result is an increase the number of offspring for that male. The same is not true for females acquiring multiple mates (Bateman 1948), but females can benefit from the acquisition of multiple mates when it confuses paternity and protects offspring from infanticide, which is a possibility in bottlenose dolphins (*T. truncatus*) (Connor et al. 2000; Dunn et al. 2002; Patterson et al. 1998). Females also benefit from mating with multiple males by increasing the possibility of fertilization, and to help establish social bonds . Pacific white-sided dolphins (*Lagenorhynchus obliquidens*) and bottlenose dolphins are both polyestrous (Robeck et al. 1994; Robeck et al. 2009) and mate multiple times in a season. Conversely, Hawaiian spinner dolphins (*Stenella longirostris longirostris*) display extensive copulatory behaviour among individuals of both sexes and all ages during the summer months (Perrin and Mesnick 2003). When males attempt to mate with multiple females, females should try to avoid fertilization by lower quality males (Parker 1979). Intersexual conflict arises over mating timing and rate; males can overcome female resistance through physical coercion

(Clutton-Brock and Parker 1995; Rowe et al. 1994), but this is rare in most species. Furthermore, males likely have a reduced ability to physically coerce females into mating in an underwater environment where individuals can move in three dimensions (Connor et al. 2000); though *Tursiops* species form stable alliances of 2-3 males and aggressively herd females, an activity that is associated with reproduction (Connor et al. 1996; Scott et al. 2005).

Physical traits (Johnson et al. 1993; Sinervo and Basalo 1996) and behavioural modifications, such as songs and displays (Mann 2001) are both methods of attracting mates. To increase fitness, males are predicted to evolve strategies to monopolize females, and females should evolve strategies to avoid monopolization by undesirable males. Both males and females are predicted to develop increased cognitive capacities to effectively manipulate, and avoid manipulation by, members of the opposite sex (Rice and Holland 1997). In polygynandrous or polyandrous mating systems, females mate with more than one male, and most often choose their mates (Birkhead 2000; Orians 1969). Selection pressure favouring the ability of males to fertilize multiple females increases in polygynadrous and polyandrous mating systems. Female resistance to seduction would likely simultaneously increase (Rice and Holland 1997). This can create an evolutionary arms race in which both sexes are simultaneously under selection to subvert the mating investment made by the other sex (Pitnick et al. 2006; Rice and Holland 1997). Thus, one prediction of the '*Machiavellian intelligence hypothesis*' is for increased cognitive capacities, and therefore brain size in species with polygynadorous mating systems, as compared to those with monogamous or polygynous mating systems (Pitnick et al. 2006; Rice and Holland 1997). Increased brain size, specifically neocortex size, would confer reproductive advantages for individuals who could successfully manipulate others (Byrne 1996; Byrne and Whiten 1997; Whiten and Byrne 1999).

Both brain and testicular tissue, however, are energetically costly to produce and maintain. In mammals, basal metabolic rate (BMR) increases to account for the extra energetic burden created by increased brain mass (Martin 1998). Species can evolve larger brains without increasing their BMR by decreasing their investment in other energetically expensive tissues. Aiello and Wheeler (1995) noted a decrease in intestinal mass in anthropoid primate species with large brains, which they called the 'Costly Tissue Hypothesis'. Though the original hypothesis tested the energetic trade-off between encephalization and gut length, trade-offs have been observed between encephalization and a decrease in other costly tissues, such as muscle mass (Isler and van Schaik 2006) and testes mass (Pitnick et al. 2006; Shillaci 2006). Here, I test these two alternative predictions of odontocete mating systems: (1) *the Machiavellian Intelligence/sexual conflict hypothesis* predicts larger brain sizes in polygynandrous species (Rice and Holland 1997; Shillaci 2006). The sexual conflict hypothesis states that both sexes should attempt to alter the behaviour of the opposite sex to avoid detrimental outcomes imposed by the opposite sex (e.g., reduced reproductive potential, injury, infanticide) while simultaneously obtaining beneficial outcomes (e.g., protection, optimal mating rates, good genes) (Rice and Holland 1997). Species with a greater degree of polygynandry are under greater pressure to avoid being manipulated, as well as to manipulate their conspecifics. This requires greater cognitive capacity in species with higher levels of polygynandry. (2) The alternative is the *costly tissue hypothesis*. Brain, testicular, and intestinal tissues are all energetically costly to develop and maintain. This hypothesis predicts that species that invest heavily in one metabolically expensive tissue, such as testes, will invest less in other such costly tissues; therefore, species that have developed large testes and therefore, in response to sperm competition should also have relatively decreased brain mass (Aiello and Wheeler 1995).

2.3 Methods

2.3.1 Data Collection

Data on average testes mass and average male and female body mass for 38 odontocete species were collected from the literature (Appendix 1); data for *Delphinapterus leucas* and *Monodon monoceros* were collected (Chapter 3). Testes masses the weight of the epididymides. Epididymide weight accounted for an average of 17.5% of the total weight of the testes across all species for which information on testes mass with and without epididymides was present. Epididymide weight was estimated at 17.5% of the total weight of the testes; this amount was added to testes masses for which the literature specified the weight of the testes without the epididymides. Testes mass in the literature was reported as either an average for the species, or a single value. The brain mass measurement used was the average for the species, as sex-specific data were not always available. Brain mass measurements are either fresh brain mass measurements or measures of cranial volume. Brain mass data were available for only 34 of the 40 species examined (missing for *Lissodelphis borealis*, *Lagenorhynchus hosei*, *Peponcephala electra*, *Lagenorhynchus obscurus, Sousa guanensis* and *Cephalorhynchus commersonii*). The humpback dolphin (*Sousa plumbea)* was also missing brain mass data, and mass was estimated as equal to the rough-toothed dolphin (*Steno bredanensis*), as the two species have similar skull dimensions and skull specimens are often confused for each other (Jefferson and

Kaczmarski 2001). When available, male and female body mass and brain mass were collected from the same sources that provided the testes mass data. Body mass data was not available for 5 of the 40 species, and was calculated following Trites and Pauly (1998). All measurements are from sexually mature animals, as defined in the literature. Details on the number of individuals sampled, and how body mass, brain mass and testes mass was calculated are listed in Table 7.1 (Appendix 1).

2.3.2 Preliminary Analysis

A preliminary statistical analysis was performed to determine the relationship between SSD, testes mass and brain mass. All data were expressed in grams and standardized using a $\log_{10}$ transformation before analysis. Additive models (AM), using male body mass as the independent variable and brain mass and testes mass as the dependent variables, were created to determine relative brain and testes size by controlling for allometry (MacLeod 2010). Residuals from the AMs represent relative testes and brain masses. SSD was calculated by dividing the body mass of the heavier sex by the body mass of the lighter sex, and then subtracting 1 from the resulting ratio (Shine 1994). Species with females heavier than males are denoted by negative (-) values while species where males were heavier were assigned positive (+) values (Shine 1994). Correlations between SSD, relative brain mass and relative testes mass were determined using Pearson correlation coefficients. AMs were created using the MCGV package in R (Wood 2006; Wood 2004); all other analyses were completed using Sigmaplot (Systat Software 2008) and SYSTAT (Systat Software 2004).

2.3.3 Phylogenetic Analysis

To properly control for the effects of common ancestry, independent contrasts (Felsenstein 1985) were calculated on $\log_{10}$-transformed data for each species with a complete data set (n=20-1). Analyses were conducted using MESQUITE v. 2.0 (Maddison and Maddison 2007) on the cetacean phylogeny of Nikaido et al. (2001). No branch length data were available, so phylogenetic analyses were based on an arbitrary branch length model. Diagnostics for branch length fit were determined by the PDAP module for MESQUITE (Garland Jr et al. 1992; Midford et al. 2003). The analysis was performed using Nee's branch length transformations (Garland Jr et al. 2002). The strength of the phylogenetic signal was determined by using the PHYSIG code (Bloomberg et al. 2003).

To test for significance of bivariate relationships after controlling for body size and phylogeny, Felsenstein's independent contrasts (FIC) calculated from the PDAP module of MESQUITE were used. A least squares linear regression with the model constrained to pass through the origin using the FIC values was performed. The residuals were used to calculate Pearson correlation coefficients (Systat Software 2004) to test relationships among the variables.

2.4 Results

2.4.1 Preliminary Analysis

There was a negative relationship between $\log_{10}$ testes mass and SSD ($r=-0.42$, $p<0.01$). Conversely, a significant positive relationship was found between $\log_{10}$ brain mass and

$\log_{10}$ testes mass (r=0.42, *p*=0.01), suggesting that species with higher levels of sperm competition also exhibit increased brain mass. A significant negative relationship was found between SSD and brain mass (r=-0.35, *p*=0.05) (Table 2.1).

2.4.2 Phylogenetic Analysis

The phylogenetic signal as determined by PHYSIG showed a significant fit of the tree to the data for testes mass and female body mass (Table 2.2).

Tests for the relationship between $\log_{10}$ testes mass and SSD were repeated using the FIC values generated to control for phylogenetic relatedness. The relationship between $\log_{10}$ testes mass and SSD remained significant (r=-0.38, *p*=0.02), but the relationship between $\log_{10}$ brain mass and $\log_{10}$ testes mass was no longer significant (r =0.30, *p*=0.07). The relationship between brain mass and SSD remained following phylogenetic control (r=-0.41, *p*=0.01) (Table 2.3).

2.5 Discussion

Understanding mating systems is important for management and conservation of cetacean species. While direct observations of mating behaviour may be difficult, mating systems may be estimated from morphological measurements. There was a significant negative correlation between SSD and relative testes mass in the 40 species in this study, indicating that odontocetes and terrestrial mammals follow similar patterns of male investment in testes mass or increased body mass relative to females (Kenagy and Trombulak 1986; Mesnick and Ralls 2002; Mitani et al. 1996; Weckerly 1998; Zenuto 1999).

Sexual size dimorphism is commonly explained as arising from sexual selection, whereby males have evolved larger body sizes to increase their success in intrasexual conflict, thereby increasing their mating success (Agrawal 2001; Alexander et al. 1979; Heske and Ostfeld 1990; Jarman 1983; Leutenegger and Kelly 1977; Payne and Dorsey 1983; Scudo 1967). However SSD may arise from other factors that favour large, competitive males such as thermoregulation, population density and fecundity advantages in females (Isaac 2005). It is unlikely that SSD is driven by a single selective factor (Weckerly 1998); however polygynous mating systems are typically characterized by more intense, physical intrasexual competition than may be present in monogamous or promiscuous mating systems, and typically show greater degrees of SSD, and testes that are proportionately smaller than those of monogamously and promiscuously mating species (Heske and Ostfeld 1990). This trend has been shown in many vertebrate species (Alexander et al. 1979; Andersson 1994; Dixson 1998; Kenagy and Trombulak 1986; Kleiman 1977; Ralls 1977; Shine 1979, 1989; Wiley 1974; Zenuto 1999) including the odotocente species in this study.

There were significant correlations between brain mass and testes mass (though the relationship was not significant after controlling for phylogeny), and a negative correlation between SSD and brain mass; these correlations support the Machiavellian intelligence/sexual conflict hypothesis. Increased relative testes mass is correlated with the need to produce large quantities of sperm necessary for sperm competition (Gomendio et al. 1998; Møller and Hobson 2004). Testes and brain tissues are energetically expensive, so a reduction in the mass of one or the other would be expected to evolve in species that did not need or were unable to maintain large amounts of costly tissue (Pitnick et al. 2006). Thermogenesis was suggested as a possible explanation for the evolution of large brains in cetaceans (Manger 2006), but there is overwhelming evidence suggesting that cetacean brain size is linked with cognitive capacities (Connor 2007; Connor et al. 1992; Herman 1980; Marino 1998; Marino et al. 2007; Ridgeway 1986). There was no significant correlation between residual testes mass and residual brain mass after controlling for phylogeny, which weakens support for the Machavellian intelligence/sexual conflict hypothesis. This may be a result of the lack of fit of brain mass data to the phylogenetic tree, or the result of other factors having a greater influence on the evolution of brain mass than mating system.

There are myriad factors influencing brain size in mammals, such as sensory ability, group size, and spatial ecology (Barton et al. 1995; Dunbar 1998; Safi and Dechmann 2005). Primate brain size evolution has been linked to group size (Dunbar 1992, 1998), and though primates and cetaceans do not share similar evolutionary histories, odontocetes rank among the most highly encephalized species (Marino 1996). Group size varies significantly between odontocete species, and the Social Brain Hypothesis (Dunbar 1992, 1998) postulates that increased brain size (specifically neocortex size) is the result of increased group size. Primate research has shown that it is not group size alone that determines neocortex size, but rather clique size within groups and complexity of social interactions (Dunbar 1992; Dunbar and Shultz 2007; Marino 1996, 1998). Variations in social systems may play a role in brain evolution, as might the complexity of interactions within groups. However these variables may be difficult to measure and quantify. A complete analysis of odontocete group size, foraging behaviour, group structure, and brain size may illuminate the extent to which group dynamics influences brain size.

The data did not support Aiello and Wheeler's (1995) costly tissue hypothesis, despite support for this hypothesis in other mammalian groups. Pitnick et al. (2006) found significant support for the hypothesis when testing for correlations of brain mass, testes mass, and mating system in bats. Bats, like cetaceans, are highly social animals that communicate acoustically (Matsumura 1979) and live in diverse habitats, and both orders have evolved relatively large brains (Barton et al. 1995; Dechmann and Safi 2009; Marino 1998; Marino et al. 2004; Safi and Dechmann 2005). But cetaceans and bats occupy very different habitats. Because cetaceans live in water, the evolutionary constraints on their weight and body size may be more relaxed than that of bats. Flight is energetically costly in bats (Speakman and Racey 1991) and significant increases in tissue weight would make it more costly; so the maximum size their testes and brains can obtain is limited. Increased testes size increases fitness when sperm competition occurs (Andersson 1994; Kenagy and Trombulak 1986), but only so far as the mass of the testes does not impede flight ability.

The environment in which bats and whales compete for mates and copulate is quite different. Although bats compete for mates in flight, they mate in their roosts (Storz et al. 2001), thus confining copulation to a 2-dimensional plane. Cetacean mating takes place in the water, and whales can move in 3-dimensions through the medium either to solicit copulation or to escape unwanted mating attempts. For these species, manoeuvrability may be more important than size (Connor et al. 2000).

The complex interaction between brain development and mating systems is not well understood. Sperm competition was associated with decreased brain mass in bats (Pitnick et al. 2006), but was not significantly linked to brain size in primates, though increased SSD indicating inter-male physical competitions, was linked with decreased brain mass (Schillaci 2006). An investigation across mammalian taxa showed no evolutionary trade-off between brain mass and testes mass (LeMaître et al. 2009) but this study did not include cetaceans, and variations in feeding ecology between disparate taxa may obscure variation in brain size due to mating system (Dechmann and Safi 2009). The prediction made by Rice and Holland (1997), that polygynadrous species should develop large brains, was not supported by this investigation of odontocetes. There are potential problems with using brain size as a proxy for intellectual ability (Healy and Rowe 2007), but until extensive experimental research has mapped out the functions of all areas of the brains of all vertebrates, it may be the best current metric for interspecies comparisons.

To summarize, the data did not support the Machiavellian Intelligence/Sexual Conflict Hypothesis as testes mass and brain mass, and SSD and brain mass were positively correlated both before and after controlling for phylogeny. Odontocete species conformed to the same trend regarding SSD and testes mass as terrestrial species. Though this study is far from exhaustive in its inclusion of odontocete species, the results suggest that relative testes mass may be a potential metric available for assessing and interpreting odontocete mating systems where direct observation has not been possible.

	$\log_{10}$ Residual Brain Mass (g)	SSD	$\log_{10}$ Residual Testes Mass (g)
$\log_{10}$ Residual Brain Mass (g)		-0.42*** 40	0.42*** 34
SSD			-0.35** 34

Table 2.1. Pearson Correlation coefficients for non-phylogenetically controlled residuals. Cells contain the correlation coefficient and number of samples. Significant correlations are indicated as: $^{**}p \leq 0.05$, $^{***}p \leq 0.001$.

	$\log_{10}$ Residual Brain Mass (g)	SSD	$\log_{10}$ Residual Testes Mass (g)
$\log_{10}$ Residual Brain Mass (g)		-0.41** 33	0.30 33
SSD			-0.38** 39

Table 2.2 Pearson correlation coefficients for phylogenetically controlled residuals. Cells contain the correlation coefficient and number of samples. Significant correlations are indicated as $^{**}p \leq 0.05$.

	Diagnostic Correlation	Expected MSE_0/MSE	Observed MSE_0/MSE	MSE_{tree}	MSE_{star}	K	P
$\log_{10}$ Male Mass (g)	-0.54	1.61	0.97	0.48	0.45	0.60	0.07
$\log_{10}$ Female Mass (g)	-0.33	1.61	1.06	0.31	0.30	0.64	0.03**
$\log_{10}$ Testes Mass (g)	-0.26	1.61	1.03	0.30	0.30	0.64	0.01**
$\log_{10}$ Brain Mass (g)	-1.63	1.61	0.69	1.40	1.35	0.44	0.62
SSD	-1.46	1.53	0.05	0.04	0.04	0.34	0.92

Table 2.3 Diagnostics for branch length fit and phylogenetic signal strength. K indicates the amount of phylogenetic signal in relation to expectation under Brownian motion trait evolution. MSE statistics represent mean square error values used to calculate K; MSE_{tree} is the mean square error of the tree, MSE_{star} is the mean square error of a star phylogeny. Phylogenetic signal was significant for female mass, and testes mass. ** indicates significance at $\alpha=0.05$.

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3 Mating ecology of beluga (*Delphinapterus leucas*) and narwhal (*Monodon monoceros)* as estimated by reproductive tract metrics

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Keywords: odontocete, Monodon monoceros, Delphinapterus leucas, testes mass, tusk length, mating ecology, mating season

3.1 Abstract.

Narwhal and beluga whales are important species to Arctic ecosystems and little is understood about their mating ecology. This understanding is crucial as these species face are vulnerable to climate change impacts. Reproductive tracts from beluga and narwhal between 1997 and 2008 from 5 beluga stocks and 2 narwhal stocks across the Canadian Arctic were collected. Reproductive tract length for males and females, relative testes mass for males, and tusk length for male narwhal were measured. Variation relative to species, body size, stock, maturity, and season was assessed. A significant variation in testes mass across month of harvest and stock for beluga, and no significant difference between stock or date of harvest for narwhal were found. Beluga had significantly larger testes relative to body size than narwhal, suggesting they were more promiscuous than narwhal. A significant relationship was found between narwhal tusk length and testes mass, indicating the tusk may be important in female mate choice. No significant differences were found between narwhal and beluga reproductive tract length for males or females. The mating systems suggested for narwhal and belugas from this data mean both species may respond differently to climate change.

Keywords: Delphinapterus leucas, Monodon monoceros, testes, mating ecology, mating season, tusk

3.2 Introduction

Beluga whales (*Delphinapterus leucas*), and narwhal (*Monodon monoceros*) are closely related and similarly adapted Arctic specialists with evolutionary histories and ecologies closely linked to the sea ice and the extreme seasonal variation characteristic of the Arctic. Both are an integral part of the eastern Canadian Arctic marine ecosystem and both are important species in the diet of local Inuit communities (Hoover et al. 2013). However, little is known about their reproductive ecology. Understanding the reproductive ecology of a species is important to the conservation of that species (Côté 2003; Lee et al. 2011; Rowe and Hutchings 2003; Steyaert et al. 2012). This is especially true for narwhal and beluga, species that face threats to conservation resulting from anthropogenic climate change . Habitat loss in the form of decreased sea ice caused by climate change may impact narwhal and beluga, species that both utilise sea ice-covered habitat for a large portion of the year (Laidre et al. 2008).

Both species are also hunted in subsistence hunts by Inuit. Sustainable exploitation of a species relies on a surplus of animals that can be removed from a population without negatively impacting the viability of the population (Côté 2003). Beluga and narwhal hunts are typically biased towards males, either because they are the largest whales (beluga), or because they are of greater financial importance by possessing a tusk (narwhal) (Hoover et al. 2013; Petersen et al. 2012). Females with calves of both species are protected from hunting pressures (Minister of Justice 2011). Such biased removal can result in skewed sex ratios as well as the removal of sexually-selected traits, which may limit conception rates

(Poole 1989). Both habitat loss and exploitation may lead to Allee effects wherein individual fitness suffers due to low conspecific density (Stephens et al. 1999). In addition, genetic drift and loss of adaptive gene combinations may be lost through hybridization (Kelly et al. 2010; O'Corry-Crowe 2008). Understanding beluga and narwhal mating systems may allow researchers to predict, and managers to mitigate, the impacts of climate change. But direct observation of their mating systems is made problematic by their habitat and distribution.

Beluga and narwhal spend large parts of the year under sea ice, making direct observation of courtship and mating in both species difficult. Morphological measurements of primary and secondary sexual characteristics, as well as sexual size dimorphism can be indicative of the mating system utilized. Males of any species can compete externally or internally for mates. In mating systems, where males compete physically for sole or primary access to multiple females, larger males are selected for as they are the most likely to outcompete rival males and gain access to females (Heske and Ostfeld 1990; Mitani et al. 1996; Ralls and Mesnick 2002; Shine 1989; Weckerly 1998; Zenuto 1999). Investment in secondary sexual characteristics such as horns, tusks, and ornamentation are also typical of species in which males compete externally for females (Andersson 1994; Arnqvist and Rowe 2005; Preston et al. 2003). Large weaponry may be selected for as they presumably intimidate rival males or are preferred by females (Clutton-Brock 1982; Geist 1966). Tusks or horns may also serve as honest indicators of male fitness (Johnstone 2008). Males with these forms of ornamentation also tend to have smaller testes than males which compete via sperm competition (Harcourt et al. 1995; Heske and Ostfeld 1990; MacLeod 2010; Pitcher et al. 2005; Rose et al. 1997; Yurkowski et al. 2011; Zenuto 1999). Males competing for fertilization through sperm competition need larger testes to produce greater quantities of sperm to increase their chances of fertilizing females (Dixson 1998; Ginsberg and Huck 1989; Gomendio et al. 1998). If males of a species have evolved proficiencies at sperm competition, females of the species are likely to develop countermeasures to prevent fertilization by lower quality males (Ginsberg and Huck 1989; Parker 1979; Rice and Holland 1997; Whitehead and Mann 2000). When males compete post-copulation for fertilization, female reproductive tracts are the stage of the contest. Typically vaginas are longer and more convoluted than those of females of less promiscuous mating systems (Gomendio et al. 1998). Females of species with high degrees of sperm competition develop adaptations to limit fertilizations by undesirable mates (Barrett and Warner 1997; Hosken and Blanckenhorn 1999; Ishida et al. 2001; Klusmann 2006) such as increased reproductive tract length and reproductive tract complexity to create an 'obstacle course' to prevent the 'worst' quality sperm from successfully fertilizing eggs (Eberhard 1998). All reproductive morphology and behavioural characteristics have associated energetic costs that result in trade-offs as species adaptations evolve towards the optimum. Measuring beluga and narwhal testes, penis length, and female reproductive tract length may help elucidate the mating systems of both species, and allow a comparison between beluga stocks as well as between beluga and narwhal.

There are at least five beluga stocks in the Canadian Arctic – the Beaufort Sea, Cumberland Sound, Western Hudson Bay, Eastern Hudson Bay, and High Arctic stocks (Richard 1994; Richard et al. 1990; Richard and Pike 1993; Turgeon et al. 2012; Turgeon et al. 2009). There is significant size variation among these stocks (Doidge 1990; Heide-Jørgenson and Teilmann 1994; Stewart 1994). Though males are larger than females in all stocks, the degree to which males are larger than females varies across stocks (Doidge 1990; Heide-Jørgenson and Teilmann 1994) with whales from the Eastern Hudson Bay stock having the smallest difference between males and females (Doidge 1990). Beluga can compete for females internally or externally. Belugas could compete via sperm competition, as pods are often mixed-gender (Brodie 1989; Sergeant 1973) and because females are induced ovulators (Steinman et al. 2011); though sexual segregation has been observed in Beaufort Sea beluga (Loseto et al. 2006; Loseto et al. 2009; Richard et al. 2001). Induced ovulation may be a mechanism that allows females to assess males post-copulation (Larivière and Ferguson 2003), as greater stimulation is required to induce ovulation and abortion of embryos in previously mated females (Adams 1981). This reduces risk for females in mating with multiple males in high density populations, but could also allow for successful mating when population density is low (Larivière and Ferguson 2003).

There are two recognized distinct stocks of narwhal in Canada; the Northern Hudson Bay stock that overwinters in Hudson Strait and summers in Northern Hudson Bay (Richard 1991); and the Baffin Bay stock that overwinters in Davis Strait and summers along the coasts of northern Canada and western Greenland (Richard et al. 2010). Narwhal are believed to have a mating system in which males compete for access to multiple females through external male-male competition. This assumption is based on several aspects of narwhal ecology; the absence of communal, male-female feeding congregations (Best 1981; Best and Fisher 1974), the presence of the tusk only rarely in females (6% of tusked animals (Petersen et al. 2012)), and the observation of tusk involvement in aggressive encounters between narwhal (Silverman and Dunbar 1980). The belief that the narwhal tusk functions in relation to mating is not the only theory explaining its existence. It has been proposed that the tusk is used in acoustic communication (Best 1981; Ford and Fisher 1978), or to detect temperature or salinity differences in the environment (Nweeia et al. 2014), though neither of these functions would be useful to only the male. The narwhal tusk grows allometrically, which may mean that the tusk is more important to adult males than juveniles (Best 1981); suggesting a potential role in mate acquisition as the adaptive function. The investment in the tusk as a male secondary sex trait suggests narwhal should be more likely to engage in pre-copulatory competition for mates than beluga.

The mating season of narwhal and beluga has been estimated. Both species are believed to have a protracted breeding season of a few months. The mating season for beluga is believed to be between April and July (Heide-Jørgenson and Teilmann 1994; O'Corry-Crowe et al. 2003). Similarly, the narwhal mating season has been estimated between February and April (Best and Fisher 1974), although a breeding season of May – June is also possible (Heide-Jørgensen and Garde 2011). Estimates of breeding season are based on the size of fetuses in harvested whales, assuming a gestation period of approximately 14 months (Best and Fisher 1974; Heide-Jørgenson and Teilmann 1994; O'Corry-Crowe et al. 2003). Testicular regression in cetaceans has been recorded for a number of species (Connor et al. 2000; Ferrero and Walker 1993; Hohn et al. 1985; MacLeod 2010; Mogoe et al. 2000; Neimanis et al. 2000; van Waerebeek and Read 1994) but has not been reported in beluga or narwhal. Testes in whales harvested during the spring are expected to be larger than testes in whales harvested at other times of the year.

The aims of this chapter were to 1) measure variation in body size and reproductive morphology among beluga and narwhal stocks; 2) determine if any variation in testes mass and penis length (and potentially mating system) exists between different beluga and narwhal relative to the allometric growth during immature and mature life stages; 3) determine whether beluga and narwhal testes regress outside of the breeding season, and if the observed variation in testes mass can approximate the timing of the breeding season; 4) establish the relationship between tusk length and testes mass for narwhal (if any); and 5) if there are any observable female morphological adaptations between the two species in responseto sperm competition in vagina, uterine, and uterine horn length.

3.3 Methods

3.3.1 Data Collection

Beluga samples used in this study were collected from Inuit subsistence hunts from across the Canadian Arctic through Department of Fisheries and Oceans sample collection programs.. Hunters and Trappers Associations/Organizations/Committees in each community were sent sampling kits with detailed instructions on which samples to collect and how to collect them. Biological samples were shipped frozen to the Freshwater Institute in Winnipeg, MB, Canada, along with a data sheet containing information on length, sex and hunt date and location. Length was measured in a straight line from snout to fluke notch. Samples were collected between 1991 and 2008, and include samples from several beluga populations (Beaufort Sea, Hudson Bay (Eastern and Western), High Arctic, and Cumberland Sound). Most samples were collected in the summer (June- August), though the collections spanned from April to December. Male and female reproductive status was assessed via body length. Beluga populations are known to vary in size, and beluga in different stocks reach sexual maturity at different lengths (Heide-Jørgenson and Teilmann 1994). Belugas were sampled from across the Canadian Arctic, with the majority collected in the eastern Arctic. The whales sampled were assigned to one of 5 stocks (Beaufort Sea *n* =6, Western Hudson Bay *n*=81, High Arctic n=9, Eastern Hudson Bay *n*=65, Cumberland Sound *n*=30) based on the nearest community. Some whales may not be true members of an assigned stock, as stock ranges overlap. Whales harvested in Iqaluit were assigned to the Western Hudson Bay stock, as whales harvested in Iqaluit are most frequently identified as WHB using genetic markers (Turgeon et al. 2012).

Narwhal samples were collected from the Northern Hudson Bay and Baffin Bay stocks following the same procedure as beluga samples from 1990 to 2008, between June and October.

Male and female reproductive tracts from belugas and narwhals were thawed and measured to the nearest cenitmeter in 2010. Length and width of testes were recorded in centimeters, and the weight of the testes, with and without the epididymides, was recorded in grams using an electronic balance (Denver Instruments, Bohemia, New York). Irregularities such as cysts or areas of calcification were noted but not deducted from the testes mass. No histological examination was conducted as freezing tissues destroys cellular structures. Penis length was measured as apical length - the length of the *pars intrapraeputalis* - starting at the apex of the penis as ending at a slight constriction before the basal segment of the penis; described in Bland and Kitchener (2001) and Slijper (1966). Apical penis length was the most reliable measure of penis length, as it was difficult to determine if the sample collected represented the entire length of the penis or if part of the base had been severed during removal. Uterine horn length was measured from the end of the horn to where the horn meets the body of the uterus, along the convex curve of the uterine horn. Average uterine horn length is the average length calculated from measurements of both uterine horns in one whale. The body of the uterus was measured in a straight line from the central junction of the two horns to the proximate side of the cervix. Overall uterine length was the average of uterine horn measurements added to the uterine body length. Vaginal length was measured in a straight line from the distal side of the cervix to the vagina's external opening. There were 38 full mature female reproductive tract beluga samples (Cumberland Sound =5, Eastern Hudson Bay = 15, High Arctic = 2, Western Hudson Bay =16) that allowed the measurement of the entire length of the vagina, from the cervix to the external opening. Average uterine horn length measurements were available for 105 mature beluga females (Cumberland Sound = 18, Eastern Hudson Bay = 30, High Arctic = 5, Western Hudson Bay = 52). Average uterine horn length measurements were available for 16 immature female belugas (Cumberland Sound = 2, Eastern Hudson Bay = 5, High Arctic = 2, Western Hudson Bay =7).

There were 21 narwhal female reproductive tracts (Baffin Bay = 19, Northern Hudson Bay = 2), of which only two were complete tracts, one of which belonged to a fetus. The small narwhal sample size precluded a meaningful interspecific and intraspecific female genital tract comparison to a comparison of the average length of the uterine horns. Average uterine horn lengths were available for 13 mature narwhal females (Baffin Bay = 12, Northern Hudson Bay =1).

Narwhal tusks were measured in the field by hunters as required by DFO Canada narwhal hunting license. The ‘exposed’ tusk measurement was used for analysis. referring to the visible portion of the tusk protruding from the skin on the forehead of the narwhal.

3.3.2 Reproductive Status

Average lengths at maturity used for whales from Western, Eastern and Northern Hudson Bay stocks are listed as ‘Hudson Bay’, for whales from the Beaufort Sea average lengths for whales from ‘Alaska’ were used, and Cumberland Sound average lengths are listed as ‘Cumberland Sound’. There were no average length estimates for whales from the High Arctic stock; whales from this stock were assumed mature at the same lengths as those from Cumberland Sound (Heide-Jørgenson and Teilmann 1994).

Narwhal males were considered mature if they were at least 388 cm long; the minimum length for sexual maturity was estimated as 85% of the average for physical maturity (Garde et al. 2007). Females were considered mature if they had attained a length of 337cm (Garde et al. 2007).

Regressions of testes mass on body mass were performed within each stock for both narwhals and belugas to ensure that individual whales were designated correctly as ‘mature’ and ‘immature’.

3.3.3 Mass

Whale mass was estimated from length using regression equations. Beluga whales from Hudson Bay are shorter than belugas from other beluga populations, as well as leaner

(Sergeant and Brodie 1969). Beluga male weights were estimated from length measurements using the parameters listed in (Stewart 1994) for belugas from Western Hudson Bay, Cumberland Sound, and the High Arctic.

For males from Western Hudson Bay:

$$\text{Mass(kg)} = -2.98\ \text{Length(cm)}^{2.25}$$

For males from Cumberland Sound:

$$\text{Mass (kg)} = -3.38\ \text{Length(cm)}^{2.44}$$

And for males from the High Arctic and Beaufort Sea stock:

$$\text{Mass (kg)} = -3.10\ \text{Length (cm)}^{2.33}$$

The equation for belugas from the Eastern Hudson Bay population was taken from Doidge (1990):

$$\text{Mass (kg)} = -3.84\ \text{Length (cm)}^{2.58}$$

Narwhal regression equations were calculated from data presented by Hay and Mansfield (1989) on 20 males from northern Baffin Island, using a regression analysis in Sigmaplot (Systat Software 2008):

$$\text{Mass (kg)} = -3.70\ \text{Length(cm)}^{2.56}$$

3.3.4 Statistical Analysis

3.3.4.1 Males

‘Testes mass’ refers to the combined mass of both left and right testes from a single beluga or narwhal, excluding the epididymides. The percent the epididymides contributed to the overall testes weight was 23.0% for narwhal, (*n* = 45; standard deviation (SD)=18.4) and 23.3% for beluga (*n* = 98; SD =17.4). For samples for which only the testes weight including the epididymides was obtained, 23.0% and 23.3% of the weight was subtracted from the total weight for narwhal and beluga, respectively. Testes mass was $\log_{10}$ transformed before analysis.

3.3.4.1.1 Beluga

An ANOVA was performed to test for variation in body length between the beluga stocks to ensure samples conformed to previous findings in variation in body length between beluga stocks (Heide-Jørgenson and Teilmann 1994; Stewart 1994). An ANOVA was also performed on body mass to compare body masses between stocks. Post-hoc Tukey tests were conducted to assess differences between stocks.

Differences in testes mass among stocks may be related to the timing of the hunts, as testes regress in mass in several species of cetacean outside the breeding season (Hohn, Chivers and Barlow 1985; Ferrero and Walker 1993; van Waerebeek and Read 1994; Connor, Read and Wrangham 2000; Mogoe et al. 2000; Neimanis et al. 2000; MacLeod 2010). Belugas in this study were harvested at various communities throughout the year, with the majority of the whales harvested during the summer months. A General Linear Model (GLM) tested for differences in log transformed testes masses within beluga stocks and across date of harvest; estimated body mass was included in the model to control for variation in body size. Only sexually mature whales were included in the GLM. Post-hoc Tukey tests assessed differences between stocks.

3.3.4.1.2 Narwhal

A GLM was also performed on physically and sexually mature narwhal testes masses to determine differences between narwhal stocks as well as evidence of testicular regression. The log value of estimated total body weight was used as the covariate. To establish the relationship between testes mass and tusk length, narwhal tusks and apical penis lengths were regressed on body length, and testes mass on body mass. A linear regression analysis was performed on the tusk and testes mass residuals to determine if tusk length was correlated with testes mass. Residuals were used for this analysis to control for effects of body size. A linear regression analysis was also performed on tusk length and body length to establish the tusk growth rate in both immature and mature whales. All data were $\log_{10}$ transformed before analysis. A two-factor ANOVA was performed to compare exposed tusk length for immature and mature narwhals between narwhal populations.

3.3.4.1.3 Interspecific Comparison

A GLM compared narwhal and beluga testes masses. Species, stock, and month of harvest were included as variables, as well as estimated body mass to control for variation in body size. Stock was nested within species. All narwhal samples were collected between June and October, beluga samples were limited to those collected between July and October to limit the effects of whales that were potentially breeding.

To estimate the potential for sperm competition in both species, a GLM was used to compare the allometric relationship between testes mass and body mass for both beluga and narwhal..

Apical penis length and female reproductive tract length of mature individuals were compared between species using an ANCOVA analysis with body length as the covariate to control for effects of body size. An ANCOVA was also performed to compare these measures between reproductive class (mature and immature) within both species using Systat and SigmaPlot (Systat Software 2004, 2008).

3.3.4.2 Females

Total female reproductive tract length was compared between stocks of beluga using an ANCOVA analysis with body length as the covariate to control for body size. Post-hoc Tukey tests were performed to assess differences between groups. Average uterine horn length was similarly compared between beluga stocks.

As there were no significant differences in beluga female reproductive tract length between beluga stocks, all mature beluga samples were pooled together (n=42), as were all mature narwhal samples (n=20). An ANCOVA analysis using body as the covariate was performed to assess differences between species.

3.4 Results

3.4.1 Males

3.4.1.1 Beluga

Samples were collected from 318 beluga males, of which 79 samples were eliminated from analyses due to missing body length data.

The total length of sexually mature whales harvested from different stocks differed significantly ($F_{(4, 207)}$=14.24, p<0.001, *n*=212) (Table 3.1). Whales from the Cumberland Sound stock were significantly longer than whales from the Eastern Hudson Bay (p<0.001) and Western Hudson Bay stocks (p=0.001). Whales from the High Arctic stock were significantly longer than whales from the Cumberland Sound (p=0.010), Eastern Hudson Bay (p<0.001) and Western Hudson Bay (p<0.001) stocks. The Beaufort Sea stock was not significantly different than the other stocks, though this may be a result of the small sample size.

The estimated mass of whales harvested from different stocks differed significantly ($F_{(4, 207)}$ =45.65, p<0.001, *n*=212) (Table 3.1). Whales from the High Arctic were the heaviest, and significantly heavier than whales from Cumberland Sound (p<0.001), Eastern Hudson Bay (p<0.001), and Western Hudson Bay (p<0.001). Whales from the Beaufort Sea were the next heaviest whales, significantly heavier than whales from Eastern Hudson Bay (p<0.001) and Western Hudson Bay (p<0.001), but were not significantly different than whales from Cumberland Sound. The Cumberland Sound stock was heavier than Western Hudson Bay whales (p<0.001) and Eastern Hudson Bay whales (p<0.001). There was no difference in mass between whales from the Eastern and Western Hudson Bay populations. Mean testes mass was 0.07 ±0.04 % (range = 0.003 – 0.231, *n* = 186) of total body mass in mature beluga. Testes masses are presented in Table 3.2.

A significant relationship between $\log_{10}$ testes mass and month of harvest was found ($F_{(1,180)}$=4.43, p=0.004). Beluga testes mass was greatest in April (n=1) and December (n=1), with masses declining sharply after June. The lowest mass was in July (Figure 3.1).

Log_{10} testes masses were significantly larger in May than testes masses in July (p=0.001), August (p=0.023) and October (p=0.005). No other significant differences between months were found.

The GLM results showed that relative testes mass also varied significantly by stock ($F_{(4,180)}$ =5.92, p<0.001; Figure 3.2). A *post-hoc* Tukey test found that belugas from the Cumberland Sound stock had significantly lower relative testes masses than whales harvested from the Eastern and Western Hudson Bay stocks (p<0.003, p=0.004). Whales harvested in Eastern Hudson Bay had thehighest average testes mass after controlling for body mass, then whales from Western Hudson Bay. Whales harvested in the Beaufort Sea had the lowest relative testes mass (Figure 3.2).

3.4.1.2 Narwhal

A total of 145 male narwhal samples were collected, of which nine were eliminated due to missing length data.

Narwhal testes was 0.05±0.02% (0.01 – 0.09, n=104) of narwhal body mass. Narwhal testes samples were collected between June and October and showed no significant difference in size among months ($F_{(1,94)}$=1.14, p=0.29) or between the two narwhal stocks ($F_{(1,94)}$=0.03, p=0.86). Narwhal testes data are summarized in Table 3.2.

Tusk length was recorded for 17 immature narwhal, two from the Northern Hudson Bay stock, and 15 from the Baffin Bay stock. The average exposed tusk length for immature narwhal was 98.8 ±49.68cm (range =10.16-180.38). The average total tusk length was 125.34 ±49.16cm (range =53.34-217.81). Exposed tusk length data was available for 101 sexually mature narwhal, 15 from the Northern Hudson Bay stock and 86 from the Baffin Bay stock. The average exposed tusk length was 155.38 ±39.59cm (range = 5.08 – 226.06). Tusk length was significantly longer in sexually mature narwhal than in immature narwhal ($F_{(1,112)}$=5.35, p=0.02). There were no significant difference in tusk length between narwhal stocks ($F_{(1,112)}$=2.126, p=0.15, n=116).

There was a significant, positive relationship between tusk length and body length in sexually mature narwhal (Exposed Tusk Length(cm) = -188.32 + (0.78 * Body Length (cm), R^2=0.32, p<0.001, n=98) (Figure 3.3), but no significant relationship between body length and tusk length for immature whales (Exposed Tusk Length(cm) = -12.33 + (0.31 * Body Length(cm), R^2= 0.04, p=0.49, n= 15) (Figure 3.3). There was a significant positive correlation between log testes mass and log tusk length in physically and sexually mature narwhal (Tusk Residual = 0.001 + (0.14 * Testes Residual), R^2=0.09, p=0.01, n=90); there was no significant relationship between log testes mass and log tusk length in immature males (Tusk Residual = -0.04 + (0.40 * Testes Residual), R^2=0.18, p=0.17, n=12) (Figure 3.4). No significant correlation was found between apical penis length and narwhal tusk length in mature whales (Tusk Residual = 0.032 - (0.38 * Apical Penis Residual) R^2=0.04, p=0.69, n=7).

3.4.1.3 Interspecific comparison

Comparing testes mass between species while controlling for body mass revealed significant effects of stock, and species; but no significant effect of month. Sexually mature belugas had significantly higher relative testes masses than mature narwhal ($F_{(1,263)}$=12.96,

$p<0.01$, beluga n=173, narwhal n=99), though the narwhal testes range did overlap with that of beluga (Figure 3.5).

Narwhal apical penis length ($\bar{x}$ =20.77cm, SD=2.12, n=7) was not significantly different than beluga apical penis length ($\bar{x}$ =17.65, SD=6.74, n=44) even while controlling for body size ($F_{(1,40)}$, $p=0.68$, n=43) . Testes mass was negatively allometric for immature beluga (Log Testes Mass (kg) = 0.53 + (0.49 * Log Body Length (cm), $R^2=0.08$, $p=0.05$, n=46), but not immature narwhal (Log Testes Mass(kg) = -5.57 + (3.30 * Log Body Length (cm)), $R^2=0.78$, $p<0.001$, n =23). Testes mass in sexually mature whales was positively allometric for both species, with narwhal showing a steeper slope (Log Testes Mass(kg) = -9.88 + (3.64 * Log Body Length) $R^2=0.19$, $p<0.001$, n=104) than beluga (Log Testes Mass (kg) = -1.25 + (1.36 * Log Body Length (cm), $R^2=0.25$, $p<0.001$, n=191). The difference between the slopes was significant ($F_{(2,355)}=154.26$, $p<0.001$) (Figure 3.6).

3.4.2 Females

3.4.2.1 Belugas

There was no significant difference in total reproductive tract length between beluga stocks ($F_{(3,33)}=1.99$, p=0.13), or in average uterine horn length ($F_{(3,100)}=1.53$, p=0.21). There was a significant difference in uterine horn length between mature and immature female belugas ($F_{(1,118)}=7.9$, $p=0.006$) after controlling for body length, with mature beluga having significantly longer relative uterine horn lengths. There was no significant effect of body length $F_{(1,118)}=0.40$, $p=0.53$ on uterine horn length.

3.4.2.2 Interspecific Comparison

There was no significant difference in average uterine horn length between mature belugas and narwhals ($F_{(1, 115)}$ = 2.46, p=0.12).

3.5 Discussion

Understanding beluga and narwhal mating systems is important in the conservation of these species (Côté 2003; Lee et al. 2011; Rowe and Hutchings 2003; Steyaert et al. 2012). This is the first study to document the variation in reproductive tract metrics between these two closely related arctic species, as well as between stocks within each species. The data collected provides rare insights into their mating ecology.

Overall, adult beluga males had significantly larger testes relative to body mass than adult male narwhal, suggesting that sperm competition may play a greater role in beluga mating systems than in narwhal mating systems. Numerous studies on the relationship between testes size and mating system have found that large testes masses were associated with increased sperm competition (Connor et al. 2000; McEachern et al. 2009; Pitnick et al. 2006; Rose et al. 1997; Schaeff 2007; Zenuto 1999). It is difficult to extrapolate from testes data alone the degree to which sperm competition may be important to beluga and narwhal reproductive ecology, though it may play a larger role in beluga mating ecology than narwhal mating ecology.

Testes mass for all stocks of beluga combined were greatest in May and declined throughout the summer. Previous studies have stated that beluga mating season occurs between the months of April and July (Heide-Jørgenson and Teilmann 1994; O'Corry-Crowe et al. 2003). The month with the largest average testes size was May, but the largest testes came from whales sampled in April and December, though only one whale was sampled in December, and one in April. Such samples cannot be used to infer the mating behaviour of the species or the stock. The majority of samples were collected between spring and fall and seem to support spring breeding in beluga. Testes were smallest in beluga in July, so July may not be within the mating season for the beluga examined, as previously suggested (Heide-Jørgenson and Teilmann 1994; O'Corry-Crowe et al. 2003).

Belugas from the Eastern and Western Hudson Bay stocks had relatively larger testes in general than whales harvested from any of the other beluga stocks sampled. The small sample sizes of whales harvested from the Beaufort Sea and the High Arctic may not accurately represent testes masses in these stocks relative to the other stocks.

There was no significant difference between relative testes mass attributable to any given month sampled, rather the testes from the Hudson Bay whales adjusted for body mass were larger overall. Whales from the Hudson Bay populations are smaller both in overall length as well as in girth (Sergeant and Brodie 1969). Mass was estimated for all whale stocks using regression equations as actual body mass was not measured; this method does not account for seasonal or annual variation in body weight for individual whales. All whales were, however, harvested during the open water season which limits the influence of seasonal variation. The use of regression equations to determine body mass potentially biases the measurement of relative testes masses between stocks, as it is calculated as relative to total body mass. Given the limited data available, the use of estimated body mass is the best option available to compare relative testes mass among beluga stocks and between species.

Belugas from the Hudson Bay stocks were significantly shorter and lighter than belugass in Cumberland Sound and the High Arctic. As a result of being the physically smallest whales, whales from the Eastern Hudson Bay stock had the largest relative testes masses, followed by whales from the Western Hudson Bay stock. The Eastern Hudson Bay stock is rated as 'endangered' by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC) (COSEWIC 2004). This population was subjected to higher than sustainable hunt pressures resulting from a combination of subsistence and intensive commercial whaling between the 1860's and 1900's (Reeves 1990; Reeves and Mitchell 1989; Richard and Pike 1993). Sexual selection pressure typically declines with decreased population density, as the number of competitors decreases (assuming an equal reduction in both sexes, without the creation of a skew towards the limited mate) (Kokko et al. 2006). One would predict, then, that the relative testes mass of whales from this stock should not differ from whales belonging to other stocks. The larger relative testes masses in the Hudson Bay stocks whales are potentially an artifact, as absolute testes masses between whale populations are fairly uniform.

Narwhal testes were also expected to regress after the mating season. Testes were collected between June and October, with no significant differences among months. The timing of narwhal ovulation is not as well understood as beluga ovulation, as narwhal have not been successfully bred in captivity, though they are likely seasonal breeders with a protracted breeding season (Best and Fisher 1974). It is difficult to pinpoint the timing of the narwhal mating season based on the testes samples collected in my study alone. An estimated breeding season between February and April (Best and Fisher 1974) or May-June (Heide-Jørgensen and Garde 2011) , could not be supported or refuted by the data. No samples were collected between February and April, and samples collected between June and October showed no significant differences between months. It may be that narwhal testes do not regress outside the mating season, mate year-round or that the earlier estimated mating season of February to April is accurate and all testes collected were already regressed.

Narwhal tusks were significantly correlated with testes mass for sexually and physically mature males, which suggests that the tusks may be honest advertisements of male quality, indicating male fertility (Gomendio et al. 1998; Vanpé et al. 2007). Larger males, with proportionately larger tusks, would be likely to successfully deter smaller males from engaging in conflict, or be more likely to be successful in an intraspecific contest for mates (Andersson 1994). Narwhal have been observed crossing their tusks and striking them together, and males have been found with tips of broken horn embedded in their jaws near the base of their own tusk (Best 1981; Silverman and Dunbar 1980). In addition to fighting with males, the tusk may also serve to attract females, as captive female narwhal have been observed becoming excited by the presence of a tusk-shaped object such as a pole or broom handle in their pool, butting the object and jockeying for position close to it (Best 1981). Wounds near the genital opening of female narwhal have been found infected with the parasite *Cyamus nodosus*. *C. nodosus* is typically present near the base of the narwhal tusk, provide further evidence that the tusk may have some purpose in intersexual interactions as well (Porsild 1922).

Both beluga and narwhal exhibited negative allometric testes mass while immature and positive allometric testes masses following sexual maturity. This testicular growth pattern is typical of promiscuous mating systems (Miller et al. 1998; Yurkowski et al. 2011). Belugas have been suggested to be a more promiscuous species than narwhal, as their relative testes mass increases by nearly three times following maturity. A significant increase in testes mass would indicate a requirement for large quantities of sperm (Miller et al. 1998).

For narwhal, the growth pattern is not typical of the polygynous mating system expected (see Yurkowski et al. (2011). The narwhal tusk length may be an important factor in establishing hierarchy within narwhal (Best 1981), and could be useful for mate-guarding post-copulation. In roe deer, *Capreolus capreolus*, antler size is correlated with testes mass, and males with large antlers win intraspecific competitions (Sempéré and Boissin 1981; Vanpé et al. 2010; Vanpé et al. 2007). Although the relative narwhal testes mass doubles following sexual maturity, this ratio is lower than the potentially more promiscuous beluga, whose relative testes mass increases by nearly 3 times. I found evidence that the narwhal tusk was related to testes mass, and therefore potentially plays a role in competition for females. Further research is needed to fully understand beluga and narwhal mating systems, including genetic research to determine if increased testes size, or in the case of narwhal, greater tusk length is related to increased fecundity.

Female adaptations to sperm competition include longer, more complex reproductive tracts to limit fertilizations by undesirable mates (Barrett and Warner 1997; Eberhard 1998; Gomendio et al. 1998; Hosken and Blanckenhorn 1999; Ishida et al. 2001; Klusmann

2006). There were no significant differences between the length of the uterine horns of beluga and narwhal, though the female reproductive tract data was limited by low sample numbers. This may also be because the measure used was the uterine horn, while the length of the entire reproductive tract, or the vagina in particular, may be the best measure. Sperm can be lost from the vagina either passively (Birkhead et al. 1999), removed by the penis or ejaculate of other males (Ginsberg and Huck 1989), or be destroyed in a hostile environment (Birkhead et al. 1999; Møller 1998), so the vagina may be 'stage' for sperm competition, and female adaptations to sperm competition may be most pronounced in the vagina.

If beluga are promiscuous, as these results suggest, their mating system may provide protection against rapid extinction via social disruption, as species with a more promiscuous mating system can survive at smaller population densities than those with monogamous or polyngynous ones (Dobson and Lees 1989). The skewed sex-ratio of the harvest of these two species may be an additive stress on the conservation of the population, especially for narwhal, as larger tusks seem to be favoured both by hunters, and potentially by female narwhal (Best 1981). A more complete understanding of the importance of the tusk to females is necessary to ensure hunting of large-tusked males does not disrupt narwhal mating ecology. Female choosiness may lead to extinction faster than random mating, as females may not mate with males with sub-standard features (Legendre et al. 1999). Though I have found limited evidence to support relatively greater promiscuity in beluga and polygyny in narwhal, further research is needed to fully understand the degree to which sperm competition is important in both species, and the importance of the tusk in narwhal.

Beluga	Reproductive Status (*n*)		Mean Length (cm) ± SD	Range	CV %	Mean Mass (KG)± SD	Range	CV%
Beaufort Sea	Immature Males	0	-	-		-	-	-
	Mature Males	6	412±26	366.-445	27	1015.94±158.64	741.53-1232.27	166.48
	Immature Females	0	-	-	-	-	-	-
	Mature Females	0	-	-	-	-	-	-
Cumberland Sound	Immature Males	12	290±74	86-372	47.17	520.24±155.84	275.98-780.05	99.01
	Mature Males	29	406±40	277-488	15.34	999.03±214.85	379.88-1510.25	76.18
	Immature Females	2	192±94	126-259	848.14	-	-	-
	Mature Females	18	224±36	268-414	17.93	-	-	-
Eastern Hudson Bay	Immature Males	15	274±35	181-287	20.45	281.17±105.71	96.97-496.76	67.16
	Mature Males	59	375±41	312-518	10.76	624.11±205.22	317.26-1451.74	50.45
	Immature Females	4	233±33	184-250	51.90	-	-	-
	Mature Females	38	344±34	285-419	11.06	-	-	-
High Arctic	Immature Males	7	338±31	290-373	28.29	626.07±127.79	433.90-781.83	118.19
	Mature	9	458±41	386±505	31.51	1268.96±240.89	845.20-1583.40	172.322

	Males							
	Immature Females	2	283±2	282-284	16.14	-	-	-
	Mature Females	6	370±37	236-437	39.03	-	-	-
Western Hudson Bay	Immature Males	19	284±25	239-310	11.97	349.28±64.54	234.65-421.88	30.20
	Mature Males	93	384±37	312-503	7.63	695.50±160.23	429.70-1254.22	32.13
	Immature Females	7	247±13	220-259	11.97	-	-	-
	Mature females	54	338±36	277-442	9.83	-	-	-
Narwhal								
Baffin Bay	Immature Males	18	318±57	160-381	28.15	577.91±204.00	90.44-836.55	93.31
	Mature Males	91	446±28	389-549	5.81	1258.43±215.05	880.12-2130.75	42.89
	Immature Females	3	295±42	251-335	104.508	-	-	-
	Mature Females	12	389±39	340-447	24.467	-	-	-
Northern Hudson Bay	Immature Males	2	379±8	373-384	67.47	823.93±41.88	794.32-853.54	376.25
	Mature Males	14	427±21	394-457	11.96	1124.16±139.12	909.92-1335.10	80.33
	Immature Females	1	279	-	-	-	-	-
	Mature	2	415±34	391-439	306.61	-	-	-

Females

Table 3.1 Descriptive statistics of lengths (cm)± standard deviation and estimated weights (kg) of beluga (*Delphinapterus leucas*) and narwhal (*Monodon monoceros*) sampled in the Canadian Arctic from 1989-2008. CV denotes the coefficient of variance.

Beluga	Reproductive Status (*n*)		Mean Mass (g)	Range	CV
Beaufort Sea	Immature	0	-	-	-
	Mature	6	562.36±269.61	71.02- 827.74	282.93
Cumberland Sound	Immature	12	161.84±287.58	13.88-818.439	182.72
	Mature	29	576.15±295.65	32.21-1093.7	112.46
Eastern Hudson Bay	Immature	14	209.06±261.99	14.70-648.99	151.266
	Mature	60	426.35±205.77	30.38-919.13	54.10
High Arctic	Immature	7	82.30±38.13	41.6-133.68	35.27
	Mature	9	1075.88±372.09	576.86-1663.88	286.01
Western Hudson Bay	Immature	20	109.60±145.38	22.24-455.85	72.30
	Mature	90	615.95±357.65	29.99-1663.88	79.59
Narwhal					
Baffin Bay	Immature	18	152.30±167.14	26.17-608.61	85.94
	Mature	110	647.89±253.30	73.16-1206.68	53.05
Northern Hudson Bay	Immature	2	299.76±203.53	155.85-443.68	1828.63
	Mature	14	514.77±186.71	163.49-768.81	107.80

Table 3.2 Descriptive statistics of testes mass (g) ± standard deviation (excluding the epididymus) for beluga (*Delphinapterus leucas*) and narwhal (*Monodon monoceros*) sampled in the Canadian Arctic from 1989 -2008. CV denotes the coefficient of variance.

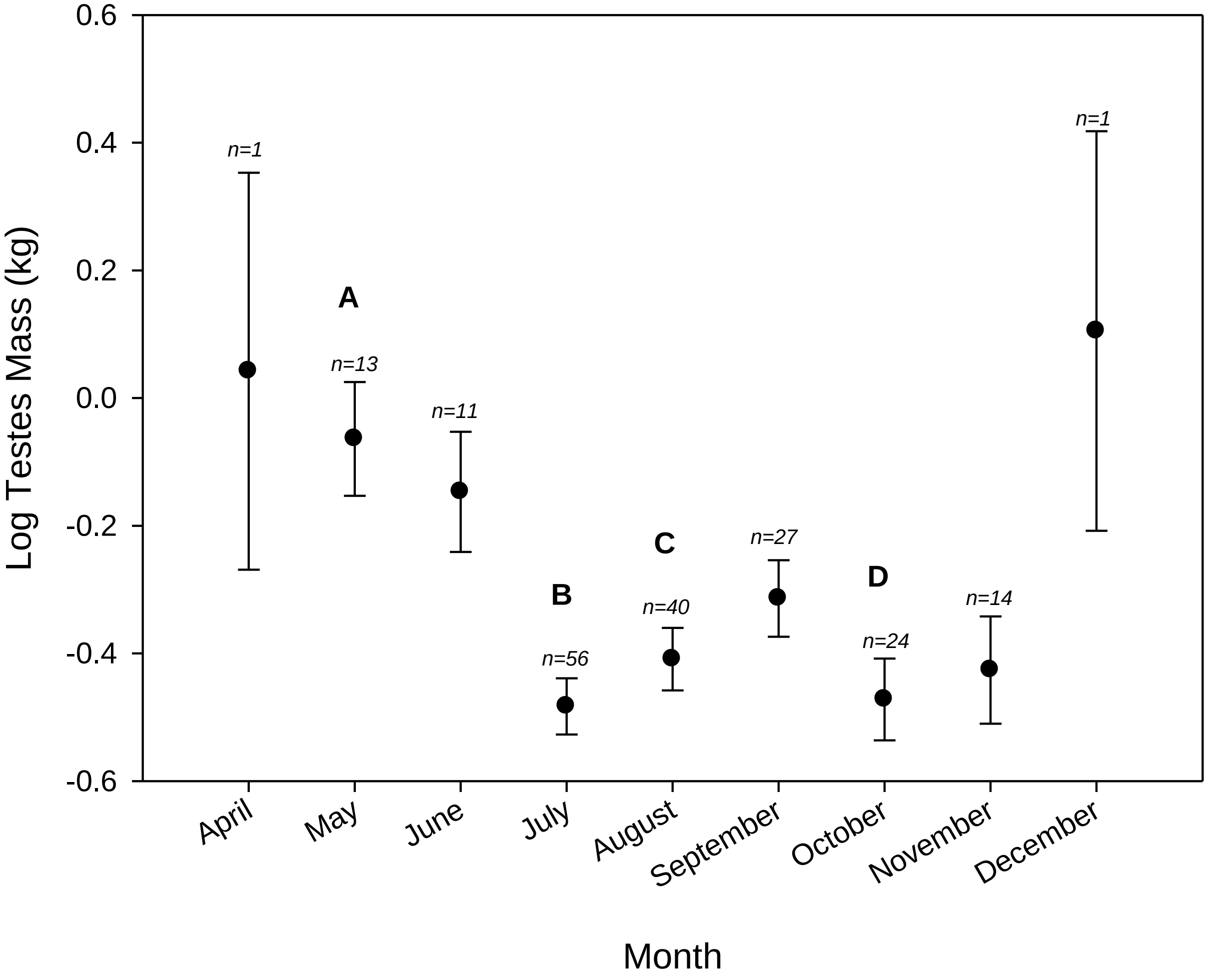

Figure 3.1 Least square mean of beluga relative testes mass by month. Log_{10} testes mass was highest in December and April, though sample size was low (*n*=1). Log_{10} testes masses were significantly larger in May than in July, August and October. Sample sizes for each month are April: *n*=1, May: *n*= 14, June: *n*=12, July: *n* =61, August: *n*= 52, September: *n* = 30, October: *n*=24, November: *n*= 15, December: *n* = 1. Error bars represent ±1 standard error.

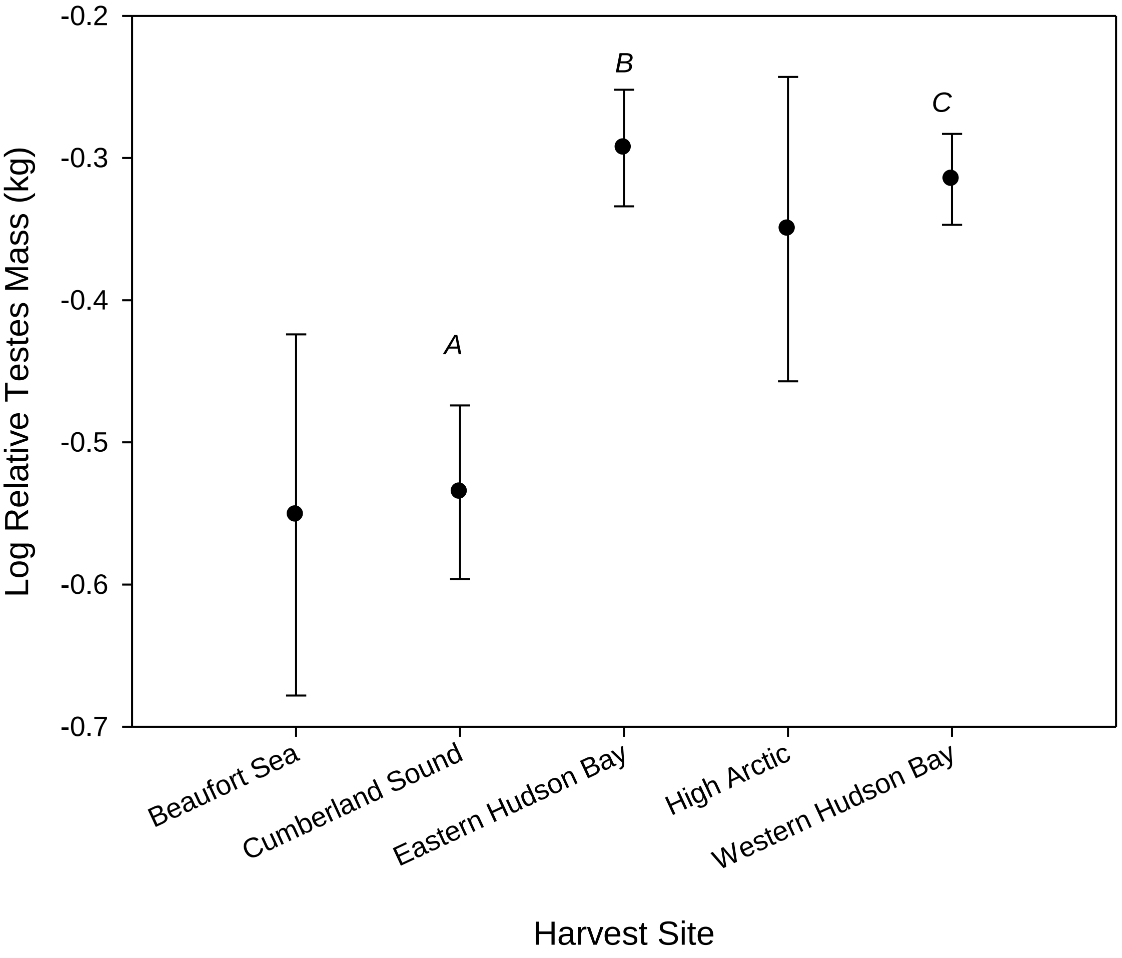

Figure 3.2 Least square means of log testes mass (kg) for beluga stocks. The Eastern Hudson Bay stock had the highest relative testes mass, followed by the Western Hudson Bay stock. Whales from the Beaufort Sea had the lowest relative testes mass of all the stocks sampled. There are 173 beluga (Beaufort Sea n= 6, Cumberland Sound n=28, Eastern Hudson Bay n = 49, High Arctic n=9, Western Hudson Bay n=81). Eastern and Western Hudson Bay whales had significantly larger relative testes masses than whales from Cumberland Sound. Error bars represent ±1 standard error.

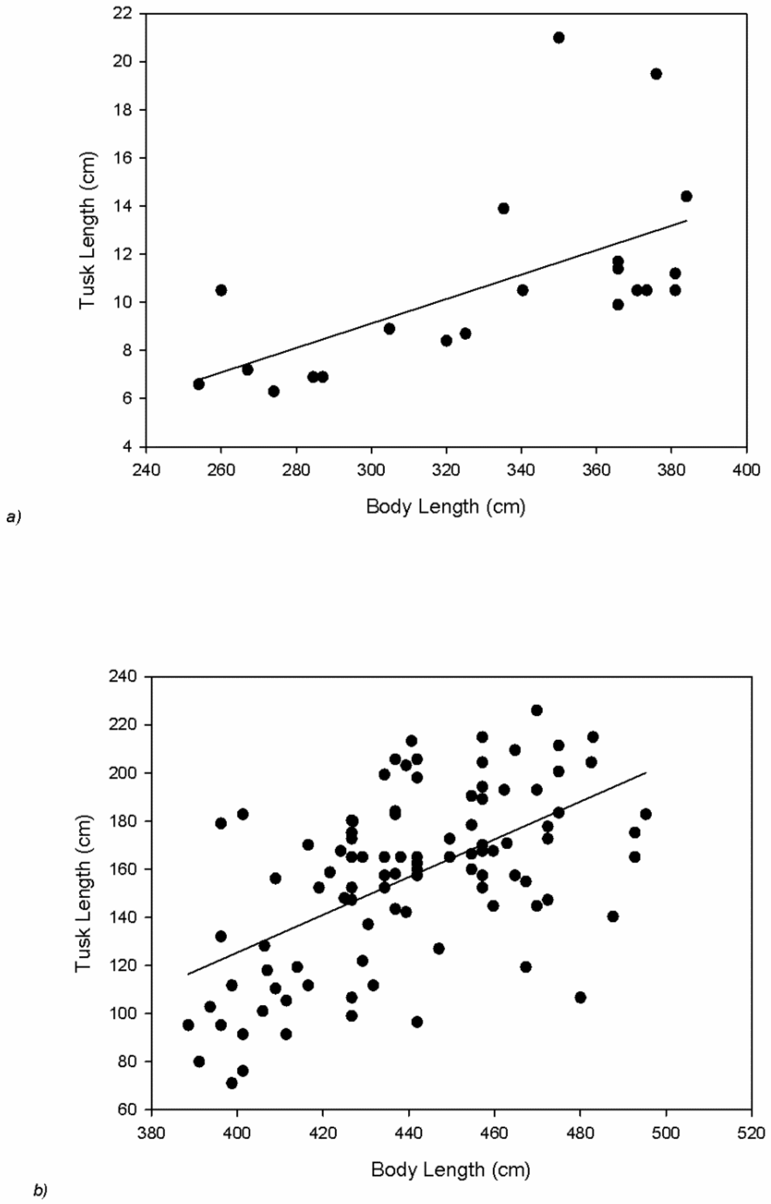

Figure 3.3 Narwhal tusk lengths plotted against narwhal body length for a. immature whales and b. mature whales. A significant positive correlation was found between body length and tusk length for mature whales, but not immature narwhals.

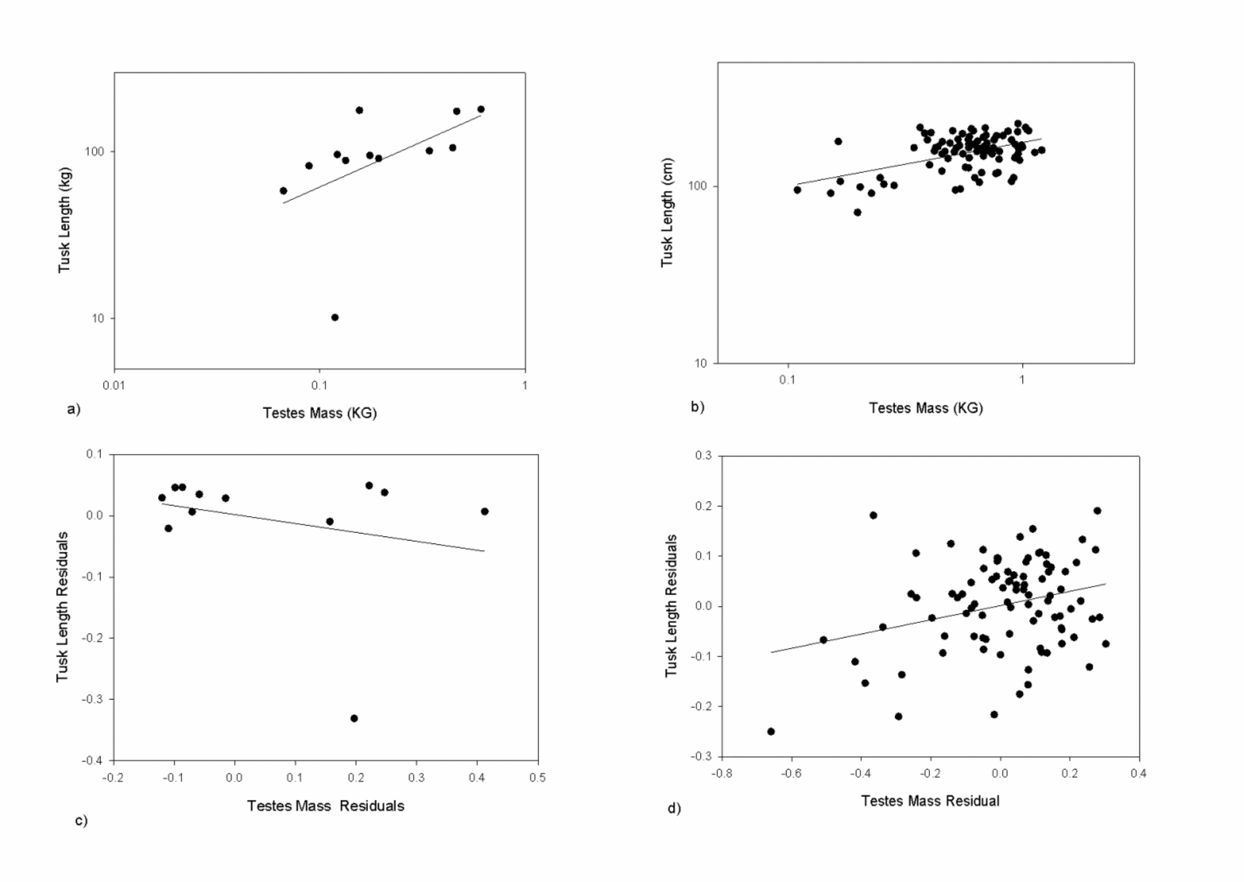

Figure 3.4. Narwhal tusk lengths plotted against testes masses (a. and b.) on a $\log_{10}$ scale, and residual tusk lengths plotted against residual testes masses (c. and d.). Data from immature narwhal is presented in a. and c.; data from mature narwhal is presented in b. and d.. Significant correlations were found for mature narwhal (n=91) (b. and d.) but not immature narwhal (n=12) (a. and c.).

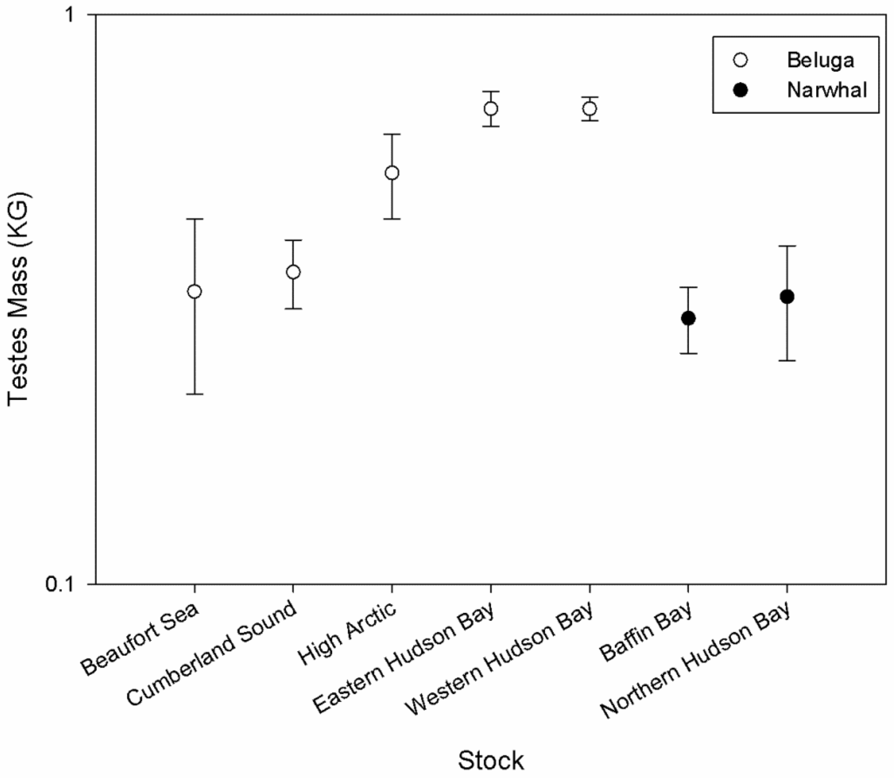

Figure 3.5 GLM Least Square means of relative testes mass (kg) for mature beluga and narwhal stocks on a $\log_{10}$ scale relative to body mass. Overall, beluga had significantly larger testes than narwhal ($p<0.001$) but the range of relative testes masses in beluga spanned those in narwhal. There are 173 beluga (Beaufort Sea n= 6, Cumberland Sound n=28, Eastern Hudson Bay n = 49, High Arctic n=9, Western Hudson Bay n=81) and 99 narwhal (Baffin Bay n=84, Northern Hudson Bay n = 15). Error bars represent ±1 standard error.

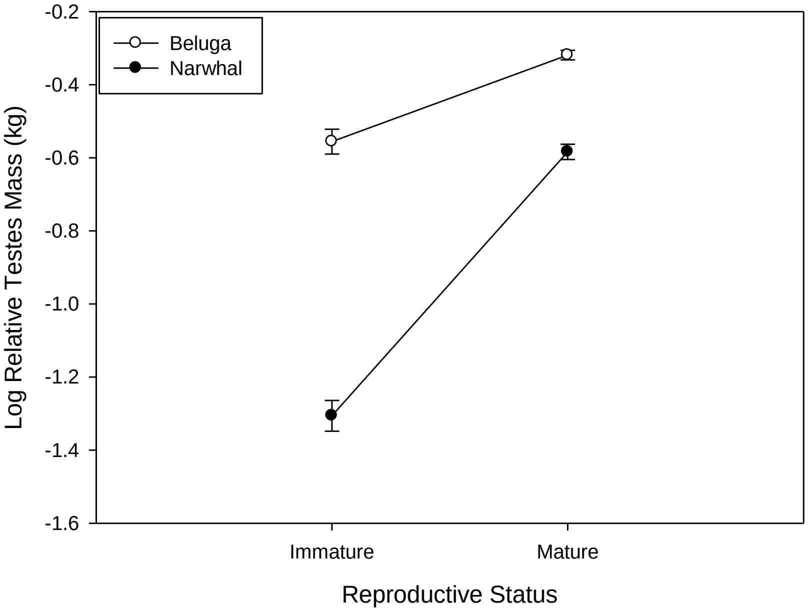

Figure 3.6 Least Square means of $\log_{10}$ beluga and narwhal testes mass by reproductive status. There was a significant difference between beluga and narwhal relative testes mass, with narwhal having a sharper increase in testes mass upon maturation than beluga. Error bars represent ±1 standard error.

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4 Stable Isotopes and Fatty Acids Differentiate Between Summer and Spring Feeding in Belugas (*Delphinapterus leucas*) in Hudson Bay

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4.1 Abstract

Hudson Bay beluga whales (*Delphinapterus leucas*) migrate into Hudson Bay during the spring, spend the summer near and in estuaries, and migrate back to Hudson Strait in the winter. Beluga use of estuaries during the summer is poorly understood, but a variety of theories have been advanced, such as predator avoidance, warm-water advantage, and prey movement. Estuaries flowing into Hudson Bay provide hydroelectricity, and it is important to understand beluga use of estuaries to mitigate the potential impact of hydroelectric development.

Beluga whale muscle, liver, and blubber samples were collected between 2003 and 2007 from belugas specimens harvested by two Inuit communities in Hudson Bay; Sanikiluaq in the Belcher Islands on the east coast of Hudson Bay (late winter-spring), and Arviat on the west coast of Hudson Bay (summer). Muscle and liver samples were analysed for δ^{13}C, δ^{15}N, and δ^{34}S stable isotopes and blubber samples were analysed for fatty acids.

Results show interannual variation in stable isotopes but not fatty acids, indicating fluctuating amounts of freshwater entering the Hudson Bay ecosystem. Tissue isotope turnover rates for muscle are estimated at 25-136 days, and blubber fatty acids are encorporated into marine mammal blubber approximately one month following consumption. Results showed significant differences between δ^{13}C isotopes in muscle and liver tissue in whales in Sanikiluaq, but not in Arviat, suggesting that the muscle δ^{13} C turnover rate may be substantially less than 136 days, especially in migrating whales. Results also indicate that belugas harvested in Sanikiluaq are feeding along the floe edge; significantly higher levels of the 20- and 22-carbon length monounsaturates in blubber indicate calenoid copepods in the food web, species that are associated with sea-ice algal diatoms. Sanikiluaq belugas were also δ^{13}C depleted relative to belugas harvested near Arviat. Belugas harvested near Arviat may be feeding preferentially in the energy-rich estuary plume. Blubber samples from whales harvested near Arviat were high in fatty acids typical of

pelagic feeding, as well as low amounts of fatty acids associated with dinoflagellates, zooplankton that do not inhabit turbid waters.

If belugas are preferentially feeding in estuary plumes, hydroelectric development may impact them if it alters the output of estuaries and affects primary productivity.

4.2 Introduction

Odontocetes are predominantly marine species and inhabit all of the oceans of the world. They are large bodied, long-lived species with low fecundity rates, making them particularly vulnerable to population declines through habitat loss and disruption (Pyenson 2009). Despite human interest in preserving species diversity, 14 odontocete species are considered to be at risk (five near-threatened, five vulnerable, two endangered, and two critically endangered)(Iucn 2008). Of those 14, four species are obligate freshwater species, and two are euryhaline species. Odontocetes associated with freshwater habitats may be particularly at risk, as increased boat traffic, fishing pressures, hydroelectric activities and pollution may negatively affect these species more intensively than oceanic species (Turvey et al. 2007). Beluga whales (*Delphinapterus leucas*) are a euryhaline species with a circumpolar distribution. They inhabit ice-covered oceans in the winter and estuaries and surrounding areas in the summer (Caron and Smith 1990; O'Corry-Crowe et al. 2003; Richard et al. 1990). In Canada, two populations of beluga are endangered (Eastern Hudson Bay and Ungava Bay), two are threatened (Cumberland Sound, St. Lawrence Estuary) and two are listed as special concern (Eastern High Arctic/Baffin Bay, Western Hudson Bay). Only the Beaufort Sea population is not at risk (COSEWIC 2004).

Beluga whales are a keystone species in the Hudson Bay marine ecosystem. Beginning with the sea-ice break up in Hudson Bay, Western Hudson Bay belugas leave their winter habitat in Hudson Strait and southwest Davis Strait and travel to their summer grounds (Caron and Smith 1990; Richard 1994, 2005; Richard and Pike 1993). By mid-July, belugas range across Hudson Bay along the eastern coast, into James Bay in the south, and up the west coast (Richard 1994). Summer congregations of beluga occur in estuaries (Caron and Smith 1990; Richard 1994, 2005; Richard et al. 1990) (Figure 4.1).

Estuaries are important areas for beluga. Beluga are a highly philopatric species that returns to the same estuaries every summer (Caron and Smith 1990; Richard 1994, 2005; Richard et al. 1990), despite hunting pressure from terrestrial predators (Hobbs et al. 2005; Watts et al. 1991). Estuary use by beluga is not well understood, though a number of hypotheses have been suggested, including prey movement (Kleinenberg (1969); but see Hobbs et al. (2005); Sergeant (1973)), calving and warm water advantage (Brodie et al. 1981; Sergeant and Brodie 1975) but see Watts et al. (1991)) and predator avoidance (Brodie 1971; Richard 2005). Rivers flowing to Hudson Bay have been dammed upstream to produce hydroelectricity (Stewart and Barber 2010). Reduced ice coverage in Hudson Bay may open the area for greater aquatic vessel traffic, and cause additional stress to beluga populations in the area (Tynan and DeMaster 1997). A better understanding of Hudson Bay beluga estuary use is essential in mitigating impact from hydroelectricity production as well as other anthropogenic effects. Increased understanding of this population of beluga may provide insights for managing vulnerable populations of beluga, as well as other vulnerable freshwater associated odontocete species.

Beluga feeding regimes in Hudson Bay are poorly understood. Research into potential beluga prey species in the late 1980s indicated that beluga in Hudson Bay may be feeding more regularly on capelin (*Mallotus villosus*) than on sea-ice associated species such as Arctic cod (*Boreogadus saida*) (Kelley et al. 2010). Capelin is a temperate cold-water fish species (Carscadden and Vilhjálmsson 2002) adapted to living at the edge of Arctic waters (Carscadden and Vilhjálmsson 2002; Vesin et al. 1981; Vilhjálmsson 2002) and using estuaries and warmer waters to spawn (Carscadden et al. 2001; Carscadden and Vilhjálmsson 2002; Vesin et al. 1981). Beluga from the Western Hudson Bay have also been observed consuming capelin in estuaries near Churchill (Watts and Draper 1986). Preferential feeding on capelin suggests beluga were feeding in or around estuaries. If belugas are using Hudson Bay estuaries as feeding grounds, industrial and hydroelectric development along the estuaries may cause significant changes or disruptions in estuary ecology (Drinkwater and Frank 1994; Edgar et al. 2000), and therefore beluga feeding regimes. Refutation of the hypothesis that beluga used estuaries is based on a lack of food found in harvested beluga stomachs (Fraker et al. 1979; Sergeant 1973), though stomach contents are biased towards recently consumed prey (Kelley et al. 2010; Loseto et al. 2009). The use of dietary biomarkers has enabled insight into the diets of animals without having to rely solely on stomach contents, and may provide insight into beluga use of estuaries.

Dietary biomarkers such as stable isotopes and fatty acids collected from beluga whales can indicate areas of energy acquisition and allow for discrimination between populations of beluga whales. Fatty acids and stable isotopes have been used in studies of marine mammals to determine diet composition, trophic relationships, foraging locations, and stock structure (Hussey et al. 2014; Iverson et al. 2004; Kelley et al. 2010; Kurle and Worthy 2001; Loseto et al. 2009; Olsen and Grahl-Nielsen 2003; Outridge and Stewart 1999). Fatty acids are incorporated into predator blubber in predictable ways, creating a fatty acid 'signature' that reflects the type of prey eaten, making them an ideal diet biomarker (Budge et al. 2006; Iverson et al. 1997; Kelley et al. 2010; Loseto et al. 2009). Stable isotope analyses are based on the relative ratios of carbon and nitrogen in prey incorporated into the tissues of higher trophic level organisms (Budge et al. 2008; Caut et al. 2011; Hobson and Clark 1992; Hobson et al. 1996; Hussey et al. 2014; Kurle and Worthy 2002; Young et al. 2010). This can be used to determine trophic interactions and migratory patterns, and ecosystems important in energy acquisition (Hobson 1999; Kurle and Worthy 2002). Trophic level can be determined between predator and prey using nitrogen because the enrichment of nitrogen is predictable, and higher level predators have the highest $\delta^{15}N$ levels (Cabana and Rasmussen 1996). Variations in carbon isotope ratios reflect different carbon sources of primary productivity originating in different food webs such as inshore versus offshore, benthic versus pelagic, and estuary versus oceanic, and terrestrial vs. marine (Budge et al. 2008; Hobson et al. 2002; Hobson et al. 1996; Michener and Kaufman 2007; Smith et al. 1996). Sulphur and nitrogen isotopes have also been used to elucidate feeding locations (Connolly et al. 2004; MacAvoy et al. 1998; Peterson and Fry 1987). Sulphur isotopes can be used to distinguish between terrestrial and marine food sources (MacAvoy et al. 1998; Peterson and Fry 1987). In addition to understanding recent feeding preferences, stable isotope analysis of multiple tissues can provide information on foraging over a range of timescales because of their different turnover rates providing information on different foraging locations of migratory species (Hobson 1999).

The primary aim of this chapter is to identify potential feeding areas of Hudson Bay belugas in spring when migrating and in the late summer; this chapter will test if fatty acids and stable isotopes can be used to distinguish between different feeding regimes exhibited in the same population of whales. Feeding behaviour in late winter and spring and summer is examined. Examining summer diet may elucidate important feeding areas for beluga and provide information on beluga estuary use. If beluga are feeding predominantly in estuaries, they should have fatty acid biomarkers indicative of freshwater feeding (Smith et al. 1996), as well as lower levels of $\delta^{13}C$ and $\delta^{34}S$ isotopes (MacAvoy et al. 1998) and higher levels of $\delta^{15}N$ (Connolly et al. 2009) relative to beluga harvested earlier in the year. Beluga may not be feeding in estuaries *per se*, but may feed offshore in the estuary plume at the interaction between freshwater and marine water (Smith 2007). If this is true, beluga blubber should be higher in fatty acids related to open-ocean pelagic feeding (Scott et al. 1999), and low levels of the fatty acids 18:4n3, and 22:6n3 as these fatty acids are characteristic of dinoflagellates (Graeve et al. 1994; Herzka 2005). Blubber should also have higher levels of 16:1n7, as low levels of this fatty acid are associated with food chains including dinoflagellates (Graeve et al. 1994). Dinoflagellates are commonly found in pelagic phytoplankton blooms, but are adapted to low-nutrient and low-turbidity conditions (Graeve et al. 1994; Rochon 2009) which are not characteristic of estuary plumes (Kuzyk et al. 2008). Higher levels of $\delta^{15}N$ isotopes, and lower levels of $\delta^{13}C$ and $\delta^{34}S$ isotopes indicative of terrestrial input, would also indicate freshwater feeding, though depending on where beluga are feeding along the freshwater-marine gradient, they may also be enriched in $\delta^{13}C$ (Connolly et al. 2009).

Estuaries may also be important areas for beluga for reasons other than feeding. Beluga may not be feeding in estuaries at all, but are instead feeding benthically along the coast. If this is true, beluga muscle and liver tissue should have higher levels of $\delta^{13}C$ and $\delta^{15}N$, in addition to decreased $\delta^{34}S$ isotopes (Connolly et al. 2004; Fry and Sherr 1984; Hobson et al. 2002; Peterson and Fry 1987; Young et al. 2010) relative to spring diets. Blubber tissue should have increased levels of the monounsaturates 20:1n7 and 22:1n7, which are both carbon-elongated forms of the fatty acid 18:1n7, a fatty acid elongated from 16:1n7 only in decapods (Graeve et al. 1994).

Additionally, beluga may be fasting in summer (Koski et al. 2002). Fasting whales are likely to have higher levels of triacylglycerols relative to wax esters in their blubber (Koopman 2006; Lockyer 1991), as they can be easily mobilized for energy (Debier et al. 2006). Monodontids (belugas and narwhal) have blubber composed only of triacylglycerols (Koopman 2006), indicating a potential need for rapid fat metabolism. Fasting whale muscle and liver tissue should be $\delta^{13}C$ depleted (Kurle and Worthy 2002; Young and Ferguson 2013) relative to whales that are feeding. They may also have $\delta^{15}N$ enriched tissue, a phenomena correlated with periods of fasting in ringed seals (*Phoca hispida*) (Young and Ferguson 2013), birds (Cherel et al. 2005; Hobson et al. 1993), though not in fin whales (*Balaenoptera physalus*) (Aguilar et al. 2014).

4.3 Methods

4.3.1 Sample Collection

Beluga muscle, liver, and blubber samples were collected from two Inuit communities in Nunavut, Canada; Sanikiluaq (56˚32’34”N 079˚13’30”W) , in the Belcher Islands on the east coast of Hudson Bay, and Arviat (56°32′34″N 079°13′30″W)., located on the west coast of Hudson Bay. All samples were collected from animals harvested during annual subsistence hunts. Sampling kits were mailed to hunters with instructions on sample tissues to collect, and how to collect them. Samples were collected in Arviat between the months of July and

August in 2003, 2005 and 2007. Samples were collected in Sanikiluaq between July and September in 2003, March – May in 2005, and in June in 2007.

A total of 99 individual whales were sampled; 17 from Arviat in 2003, 20 in 2005, and 14 in 2007; and 14 from Sanikiluaq in 2003, 19 in 2005, and 15 in 2007. The Arviat sample set was missing three liver samples and one muscle sample from 2003, and two blubber samples from 2005. The Sanikiluaq sample set was missing one muscle and one liver sample from 2003, four muscle samples and all blubber samples from 2005, and five muscle samples from 2007. Blubber samples were collected with both muscle and skin attached to the sample.

For the purposes of this study, all whale sexes were genetically determined using primers developed by Shaw et al. (2003): LGL331 (CAAATC ATG CAA GGA TAG AC) (described in (Petersen et al. 2012), with the exception of 2 whales, ARAR-xx-1065 (female) and ARAR-xx-1161 (male), for which hunter-assigned sex was used. Genetic sexing was completed by D. Tenkula at the Department of Fisheries and Oceans (DFO), Winnipeg, MB Canada. Whale age was determined by counting dentine layers in the teeth, as described in Stewart et al. (2006). Whale age and sex were compared between whales harvested to determine if age-sex composition of whales was similar.

4.3.2 Stable Isotope Analysis

Beluga muscle and liver samples were analysed for stable isotopes. Stable isotopes in liver samples are representative of prey that was more recently consumed, and isotopes from muscle samples represent prey that was consumed over a longer period of time (Bauchinger and McWilliams 2009; Buchheister and Latour 2010; Hobson and Clark 1992).

Muscle samples were analysed for $\delta^{13}C$, $\delta^{15}N$, and $\delta^{34}S$ and liver samples for $\delta^{13}C$ and $\delta^{15}N$. A 5 g piece of each tissue was sub-sampled from each field-collected sample, freeze dried, and then pulverized. Lipids were removed from 200 mg of the pulverized tissue by adding 5 ml of a solution of chloroform: methanol in a 2:1 ratio to the sample and refrigerating for 18 hours. The sample was then centrifuged at 2500 RPM for 10 minutes, and the solvent containing the lipid was removed. Five ml of fresh solvent was added to the tissue, followed by stirring for two hours at room temperature. The sample was again centrifuged and the solvent removed. Lipids were extracted a third time for one hour, the sample centrifuged at 2500 RPM and the solvent removed. The samples were dried on a nitrogen evaporator for one hour.

Dried samples were transferred to a plastic cryovial and sent to the Waterloo Environmental Isotope Laboratory (uwEILAB) for mass spectrometry analysis. Carbon and nitrogen isotopic analyses on the muscle and liver samples were accomplished by continuous flow, isotopic ratio mass spectrometry (CF-IRMS) using a Delta Plus Continuous Flow Stable Isotope Ratio Mass Spectrometer (Thermo Finnigan / Bremen-Germany) coupled to a Carlo Erba Elemental Analyzer (Model: CHNS-O EA1108 - Italy). Sulphur analysis was performed on an Isochrom Continuous Flow Stable Isotope Ratio Mass Spectrometer (GV Instruments / Micromass-UK) coupled to a Costech Elemental Analyzer (Model: CNSO 4010UK). Stable isotope ratios are expressed as delta (δ) and are measures of parts per thousand difference between the isotope ratios of the samples and those of a known international standard. Vienna PeeDee Belemnite (VPDB) was used for carbon isotopes, atmospheric nitrogen (AIR) was used for nitrogen isotopes, and Vienna Canon Diablo Troilite (VCDT) was used for sulphur isotopes.

4.3.3 Fatty Acid Analysis

A 1 g piece of blubber was sampled from the inner layer of the blubber sample, closest to the muscle, provided by the hunters. The inner layer is the most metabolically active layer, and represents fatty acids deposited by prey items most recently consumed (Loseto et al. 2009). The blubber sub-sample was added to a glass vial containing 21 ml of a 2:1 ratio mixture of chloroform: methanol. The tube was shaken and the blubber crushed with a glass rod to remove all of the lipid. A 0.7% aqueous sodium chloride mixture was added to the sample, and then centrifuged for 15 – 20 minutes, after which the solution in the test tube separated into two layers. The lower, lipid containing layer was removed and placed into a kimax tube with 5g of anhydrous sodium sulphate and shaken to remove impurities. The solution was removed and placed in a pre-weighed glass tube. A nitrogen evaporator was used to evaporate the solvent from the tube, leaving the extracted lipid. The mass of the lipid was determined by weighing the dry sample and subtracting the mass of the pre-weighed tube.

After lipid was extraction, 1.5 ml of hexane and 1.5 ml of Hilditch reagent (NH_2SO_4) was added to the sample. The samples was then flushed with nitrogen and heated at 100º C for one hour. The sample was allowed to cool to ambient room temperature and then mixed with 1 ml of hexane and 2 ml of water. It was then centrifuged it for 5 minutes, so that the solution separated into two distinct layers, with the lipid containing layer on the top. The lipid containing (top) layer was removed, and the hexane extraction procedure repeated twice more. After the hexane extraction, 2 ml of distilled water was added to the hexane extracts and it was shaken. The lipid containing solvent separated from and floated on top of the distilled water. The lipid layer was removed to a tube containing 5g of anhydrous sodium sulphate, then transferred to a pre-weighed test tube and evaporated using nitrogen. The sample was weighed to determine total mass of lipid removed before it was stored in hexane.

4.3.4 Gas Chromatography

The extracted lipids were placed in a gas chromatograph (GC) (Agilent Model 6890) following dilution in hexane in a 10:1 hexane:lipid ratio. The hexane and lipids volatilized into a continuous flow of helium gas flowing at 0.7 ml/min through the silica GC column (DB-23; 30m x 0.25mm ID; 0.25 micron film thickness), where volatile and semi-volatile lipids separated according to their different vapour pressures. A mass spectrometer (Agilent 5973 Mass Selective Detector) detected the compounds eluting from the GC column, and recorded and stored them in an MSD ChemStation data file. The mass spectrometer was operated in Selected Ion Monitoring (SIM) mode using ions and correction factors as outlined by (Thurnhofer and Vetter 2005). The inlet temperature was set at 260°C and the transfer line temperature was set at 300°C, and the following temperature program used: Start at 60°C with a 1 min hold; ramp up to 170°C at 15°C/min. Hold for 11 min, ramp up to 190°C at a rate of 3°C/min and hold for 7 min; ramp up to 200°C at a rate of 5°C/minute and hold for 2.5 minutes. The stored data was analysed using MSD ChemStation Data Analysis user interface.

Fatty acids were integrated by MSD ChemStation Data Analysis (Agilent 2003). The fatty acid quantities were corrected using correction factors determined by standards (Nu Chek Prep), and expressed as the mass percent of the total identified fatty acids. Fatty acids are expressed using the shorthand nomenclature A:BnX, where A represents the number of carbon atoms, B the number of double bonds, and X the position of the terminal bond closest to the methyl group(Loseto et al. 2009). Although a total of 65 fatty acids were identified, the analysis was confined to 29 fatty acids identified by Iverson et al. (2004) as known to transfer predictably from predator to prey.

4.3.5 Data Analysis

A chi-square test was performed to determine if the beluga harvested in Sanikiluaq and Arviat differed significantly in age-sex structure. A chi-square with Yates correction for continuity was used to test for the difference in the numbers of males and females harvested in each community. A Kolmogorov-Smirnov (K-S) two-sample test was performed to test for difference in age and length distribution between whales harvested at both sites. The purpose of these tests was to ensure that the populations were of similar composition, and that differences in fatty acid signatures and stable isotope ratios were indicative of differences related to feeding location, and not due to sex-age habitat selection (*sensu* Loseto et al.(2009).

To determine variation in the levels of carbon, nitrogen and sulphur isotopes between communities, ANOVA analyses were performed (Systat Software 2008). Analyses tested for differences between communities and between years. Post-hoc Holm-Sidak pairwise comparisons were used to test for the differences between communities within each individual year.

To determine the variation between nitrogen and carbon isotopes between tissues collected from the same community, a second ANOVA was performed using SYSTAT 11.0 (Systat Software 2008). The analysis tested for differences between mean isotope ratios found in liver and muscle tissue within each community and across years. Post-hoc Holm-Sidak pairwise comparisons were used to test for the differences between tissues within each individual year.

All fatty acid data was log transformed before principle component analysis (PCA). A standardized PCA was used to compare the variation in fatty acid composition of blubber between the two communities. This analysis provides qualitative information about individuals and their fatty acid profiles by providing a graphical representation of the proximity of individual whales to fatty acid biomarkers. Proximity of whales to other whales indicates relatively similar fatty acid profiles; proximity of whales to fatty acids indicates relatively high percent of those fatty acids in the whale's blubber (Dahl et al. 2000; Kelley et al. 2010; Loseto et al. 2009). This method cannot be used to make quantitative statements about prey consumption.

A second PCA was performed with the fatty acids described by Smith et al. (1996) (18.2n6, 18.3n3, 20.1n9, 20.4n6, 22.1n11, total saturate fatty acids, total monounsaturated fatty acids, total poluyunsaturated fatty acids, and omega-3:omega-6 ratio) to determine if fatty acid profiles characteristic of freshwater and marine feeding behaviour could distinguish between whales harvested in Sanikiluaq and Arviat. Running a PCA with the fatty acids reduced to the above 10 fatty acids and fatty acid groups increases the statistical strength of the analysis (Budge et al. 2006), as well as provides insights into the effects of these important fatty acids without results being confounded by fatty acids that appear significant due to low variability.

Twelve fatty acids that transfer predictably from predator to prey were compared, as well as provide fatty acid signals characteristic of primary productivity in specific marine areas (16:1n7, 16:4n1, 18:2n6, 18:3n3, 18:4n3, 20:1n7, 20:1n9, 20:5n3, 22:1n7, 22:1n9, 22:1n11) (Scott et al. 1999). A two-way ANOVA analyses was performed on total % fatty acids to compare the relative percent of those fatty acids between beluga sampled at both communities, as well as among years.

4.4 Results

4.4.1 Population Statistics

The chi-square test showed no significant differences between the proportion of males and females harvested in each community (p=0.91) (Table 1). Mean and median age and length were not significantly different between whales harvested in Arviat and whales harvested in Sanikiluaq. (Table 2).The K-S test found no significant difference in age structure of whales between the two sites (p=0.26), or in length (p=0.30). Results indicate that the harvests of whales from the two sites are similarly structured

4.4.2 Stable Isotopes

4.4.2.1 Carbon

There was a significant effect of year (p<0.001, df=2, F=16.43), harvest site (p<0.001, df=1, F=41.51) and an interaction effect between year and harvest site (p<0.001, df=2, F=11.92) for muscle tissue.

Beluga muscle tissue was most enriched in 2007 (-17.91‰ ± 0.11) and least enriched in 2003 (-18.46‰±0.1). Muscle tissue from whales sampled in 2005 was intermediate (-18.70‰±0.09). Post-hoc Tukey tests found significant differences in δ^{13}C enrichment between 2007 and 2005 (*p*<0.001), 2007 and 2003 (*p*<0.001). There was no significant difference between 2003 and 2005 (*p*=0.08).

Overall, muscle tissue from belugas sampled in Arviat were more enriched in δ^{13}C (-17.99‰±0.07) than belugas sampled in Sanikiluaq (-18.72‰±0.09). Muscle tissue was significantly more enriched in whales harvested in Arviat in 2003 (*p*=0.001) and in 2005 (*p*<0.001), but not in 2007 (*p*=0.67) (Figure 4.2).

There was a significant effect of year (p<0.001, df=2, F=12.46), harvest site (p<0.001, df=1, F=20.36), and an interaction between year and harvest site (p=0.05, df=2, F=3.14) for liver δ^{13}C.

Liver tissue was most enriched in 2007 (-17.80‰±0.08), followed by 2003 (-18.14‰±0.09), and least enriched in 2005 (-18.35‰±0.08). Post-hoc Tukey tests found significant differences between 2007 and 2005 (*p*<0.001), 2003 and 2007 (*p*<0.001). There was no significant difference between 2003 and 2005 (*p*=0.76).

Liver tissue from whales sampled from Arviat was more enriched in δ^{13}C (-17.89‰±0.07) than whales sampled in Sanikiluaq (-18.30‰±0.07). Liver tissue was significantly more enriched in whales harvested in Arviat in 2003 (*p*=0.03) and in 2005 (*p*<0.001) but not in 2007 (*p*=0.32) (Figure 4.2).

Post-hoc Tukey tests found no significant differences between years in muscle or liver tissue in whales sampled in Arviat. There were significant differences between years in whales sampled in Sanikiluaq between 2007 and 2005 (*p*<0.001), 2007 and 2003 (*p*<0.001) and 2003 and 2005 (*p*=0.003).

An ANOVA found no significant effect of year sampled (p=0.09. df=2, F=2.440), tissue type (muscle vs. liver) (p=0.29, df=1, F=1.116), or an interaction effect between the two (p=0.65, df=2, F=0.43) for whales sampled in Arviat (Figure 4.3)

There were significant effects of year sampled (*p*<0.001, df=2, F=34.49) tissue type (*p*<0.001, df=1, F=13.13) but no interaction effect between the two (*p* = 0.07, df=2, F=2.77) for whales sampled in Sanikiluaq (Figure 4.4). Liver was more enriched in δ^{13}C than muscle in 2003 (*p*=0.03) and 2005 (*p*<0.001), but not in 2007 (*p*=0.75).

4.4.2.2 Nitrogen

There was no significant effect of year (p=0.82, df=2, F=0.19) for muscle tissue, but there was a significant effect of harvest site (p<0.001, df=1, F= 25.71). There was no interaction effect between year and harvest site (p=0.09, df= 2, F=2.49).

Overall, muscle tissue from belugas sampled in Arviat were more enriched in δ^{15}N (16.41‰±0.08) than belugas sampled in Sanikiluaq (15.77‰±0.10). Muscle tissue was significantly more enriched in whales harvested in Arviat in 2003 (*p*=0.01) and in 2005 (*p*<0.001), but not in 2007 (*p*=0.16) ((Figure 4.2).

There was no significant effect of year (p=0.15, df=2, F=1.94) on beluga liver tissue, but there was a significant effect of harvest site (p<0.001, df=1, F=27.25). There was no interaction between year and harvest site (p=0.22, df=2, F=1.56) for liver δ^{15}N.

Liver tissue from whales sampled from Arviat was more enriched in δ^{15}N (16.97‰±0.08) than whales sampled in Sanikiluaq (16.35‰±0.08). Liver tissue was significantly more enriched in whales harvested in Arviat in 2003 ($p<0.001$), in 2005 ($p=0.003$) and in 2007 ($p<0.001$).

Post-hoc Tukey tests found no significant differences between years in muscle or liver tissue in whales sampled in Arviat or Sanikiluaq.

An ANOVA found significant effects of tissue type ($p<0.001$, df=2, F=18.13), but not for year sampled ($p=0.24$, df=2, F=1.47) or an interaction effect between the two ($p = 0.79$, df=2, F=0.24) for whales sampled in Arviat (Figure 4.3). Liver (16.97‰±0.09) was more enriched in $\delta^{15}N$ than muscle (16.41‰±0.09). Liver was more enriched in 2005 ($p=0.004$) and 2007 ($p=0.009$), but not in 2003 ($p=0.07$).

There were no significant effect of year sampled (p=0.24. df=2, F=1.47), but a significant difference in tissue type (p=0.29, df=1, F=1.116) in whales sampled in Sanikiluaq. There was no significant or an interaction effect between the two (p=0.79, df=2, F=0.24) (Figure 4.4). Liver (16.35‰±0.07) was more enriched than muscle (15.77‰±0.08, $p<0.001$), and was significantly more enriched in 2005 ($p<0.001$) and 2007 ($p=0.002$) but not in 2003 ($p=0.08$).

4.4.2.3 Sulphur

There was no significant effect of year (p = 0.15, df = 2, F = 1.93) or harvest site (p=0.19, df = 1, F=1.73) in sulphur isotopes. There was a significant effect interaction effect of harvest site and year (p<0.001, df =2, F=10.13). Whales harvested in Sanikiluaq had significantly higher levels of $\delta^{34}S$ than whales harvested in Arviat in 2003 (p<0.01), but significantly lower levels in 2005 (p=0.02). There was no significant difference in $\delta^{34}S$ between communities in 2007 (p=0.53).

No significant differences were detected between years in Arviat (Figure 4.3). Significant differences were found between 2003 and 2005 in Sanikiluaq ($p<0.001$) (Figure 4.4).

4.4.3 Fatty Acids

The PCA distinguished between whales harvested in Arviat and those harvested in Sanikluaq (Figure 4.5). The first axis of the PCA described 44.4% of the variation found between the beluga populations. The fatty acids that dominated the negative side of the X axis were 20- and 22-carbon monosaturates and polunsaturates, and the 18-carbon polyunsaturated omega-3 fatty acid 18:4n3. The positive side of the X-axis was dominated by the saturates 14.0, 16.0, and 17.0, and the 16-carbon length fatty acids 16.1n7, 16.4n3, and 16.2n6. The majority of the samples from Sanikiluaq were on the negative side of the X-axis. Arviat samples spread across both positive and negative sides of the X-axis, overlapping the Sanikiluaq samples, though there was a tendency for Arviat samples, especially samples from 2005, to be on the positive side of the axis. The Y-axis described 13.2% of the variation and was dominated by the polyunsaturates 18:3n3, 18:4n1 20:4n6 on the positive side, and the 22-carbon monounsaturates 22:1n7, 22:1n11, and 22:1n9 on the negative side.

The second PCA using only fatty acids associated with freshwater and marine feeding described 76.9% of the variation in fatty acids, with 51.1% described by the X-axis and 25.8% described by the Y-axis (Figure 4.6). Whales from Sanikiluaq were on the positive side of the graph, which was dominated by the fatty acids 22.1n11, 20.1n9 and the omega-3:omega-6 ratio. Whales from Arviat were on the negative side of the axis, and associated with the total monounsaturated and saturated fatty acids, the 18-carbon PUFAs and the fatty acids 18:2n6 and 18:3n3. The PCA distinguished between whales harvested at both sites, but there is substantial overlap between the two communities. The Y-axis was dominated by the monounsaturates 20:1n9 and 22:1n11 on the positive side, and the polyunsaturates 18:3n3 and 18:3n3 on the negative side.

There were no significant differences across years in any of the fatty acids tested. Significant differences were found for 10 of the 12 fatty acids testes between whales harvested in Sanikiluaq and Arviat. Sanikiluaq had significantly higher levels of 16:4n1, the 18-carbon omega 3 fatty acids 18:4n3, and the 20- and 22- carbon chain monounsaturates 20:1n7, 20:1n9, 22:1n7, 22:1n9, 22:1n11 22:6n3. Arviat had significantly higher levels of the fatty acids 16:1n7 and 18:2n6 (Figure 4.7).

4.5 Discussion

Given the times the whales used examined were harvested; it is most likely that whales harvested from Sanikiluaq were part of the migrating population. Consequently, fatty acid and stable isotope signatures from the whales used in this study represent beluga summer diets (Arviat) and late winter/spring diets (Sanikiluaq).

Stable isotope biomarkers are incorporated into tissues, but the rate of turnover varies depending on the tissue. Sampling a collection of tissues from an individual provides information on feeding both from prey consumed recently as well as long-term integrated prey (Caut et al. 2011; Kurle and Worthy 2002). Stable isotope turnover rates are dependent on protein metabolic rate (Kurle and Worthy 2002). Turnover rates have been determined for some marine mammal species. For instance, liver tissue has a high isotopic turnover rate with protein half-lives of approximately 1.9-6.7 days; skeletal muscle tissue has a longer isotope turnover rate with protein half-lives of 12.5-83.3 days in northern fur seals (*Callorhinus ursinus*) (Kurle and Worthy 2002).

Controlled feeding experiments detailing tissue turnover rates in have not been conducted on beluga, but tissue turnover rates have been determined in other cetacean species such as killer whales (*Orcinus orca*) and bottlenose dolphins (*Tursiops truncatus*) (Caut et al. 2011). Plasma and red blood cell isotope half-lives were 12.5-45.6 and 27.5 days respectively in *T. truncatus*, and 13.5-60.5 days for plasma in *O. orca*. Plasma and red blood cell half-lives were found to be longer in both species than the plasma and red blood half-lives found in black bears (*Ursus americanus*) (plasma: 4;red blood cells: 28 days) (Hilderbrand et al. 1996). This difference may be related to a larger body size in the odontocete species relative to the bear (Caut et al. 2011), or due to different fractionation rates related to physiological differences between aquatic diving and terrestrial mammals. Although the comparison of two piscivorous odontocete species to one terrestrial omnivorous animal is by no means a thorough examination of comparative fractionation rates, it suggests that odontocetes may have slower tissue turnover rates than terrestrial mammals and thus rates from terrestrial mammals should be applied to odontocetes with caution. Total isotopic integration of a diet item into tissue takes approximately 2-3 half-lives (Hilderbrand et al. 1996; Kurle and Worthy 2001, 2002). If belugas have isotopic fractionation rates similar to *T. truncatus*, (and blood plasma isotope half-life is used as a proxy for muscle tissue half-live, though blood has a somewhat more rapid turnover rate (Hobson and Clark 1992), muscle $\delta^{13}C$ turnover rates should be approximately 25- 136 days.

This study makes no definitive statements regarding turnover rates in beluga, as controlled studies have not been performed and are necessary to establish isotopic turnover rate in tissue (Hobson et al. 1993). There were significant differences in both $\delta^{13}C$ and $\delta^{15}N$ isotopes between liver and muscle tissues from whales harvested in Sanikiluaq. Sanikiluaq liver samples were $\delta^{13}C$ enriched relative to muscle samples, as well as $\delta^{15}N$. The significant difference in both of these tissues indicates that whales harvested in Sanikiluaq may have recently changed diet. The recently ingested dietary isotope signal, while being reflected in the liver, may not have been fully incorporated into the metabolically slower muscle tissue. Muscle tissue isotopes potentially represent feeding in wintering grounds in Hudson Strait, while liver tissues represent more recently incorporated prey items eaten while migrating. There were no significant differences in $\delta^{13}C$ levels between muscle and liver tissues sampled in Arviat; but liver tissue has significantly higher levels of $\delta^{15}N$ in relative to muscle samples. Variation between tissues for these samples may be attributable to changes in diet, but given that no significant differences in $\delta^{13}C$ were found between muscle and liver, the differences may be related to isotope assimilation in the tissues (Kurle and Worthy 2001, 2002), as the dominant proteins and their constituent amino acids may vary enough to influence a particular tissue's isotope ratio (Hare et al. 1991). Further, liver is enriched in $\delta^{15}N$ relative to other tissues in northern fur seals (*Callorhinus ursinus*) (Kurle and Worthy 2002). This suggests that stable isotopes from beluga harvested in Arviat are representative of dietary items consumed while on the summering grounds in Hudson Bay, and an estimate of 136 days for carbon isotope turnover rates (extrapolated from *T. truncatus*, Caut et al.(2011) may be an overestimation, at least for a migrating or recently migrated population of whales. Animals engaged in increased physical activity have increased metabolic rates, and therefore faster isotopic turnover rates (Hobson and Clark 1992), which may explain the similarity of carbon isotopes in liver and muscle tissue in beluga harvested in Arviat.

Fatty acid turnover rates are dependent on the rate of metabolism and deposition of fat, and vary between species (Berkley et al. 2013; Budge et al. 2006). Lower trophic level consumers incorporate fatty acids in a matter of hours to days, so algae and phytoplankton fatty acid signatures are rapidly reflected by their consumers (Budge et al. 2008; Graeve et al. 2005). Producer and prey species fatty acid signatures are likely incorporated into marine mammal tissue within a month (Iverson et al. 2002). The fatty acids in Arviat beluga, then, are likely representative of late summer (July-August) diets while the fatty acids in Sanikiluaq beluga are representative of spring (April-May) diets.

4.5.1 Interannual Variation

Interannual variation in fatty acid and stable isotope biomarkers may be reflective of either changes in prey consumed due to fluctuations in prey availability, or of environmental changes. There were no significant differences in fatty acids profiles between years, suggesting that belugas are not consuming different prey items in different years. There were, however, significant differences in some stable isotope levels among years, which may be reflective of changing environmental conditions in Hudson Bay. There was a significant decrease in $\delta^{13}C$ in both muscle and liver tissue in whales harvested in

Sanikiluaq in 2005. Samples from Arviat from 2005 were also carbon depleted relative to samples in 2007, but there was no significant difference between 2005 and 2003, or 2003 and 2007. The interannual fluctuation in $\delta^{13}C$ in Sanikiluaq may be related to an increase in freshwater into the Hudson Bay ecosystem, as there was a record amount of precipitation in the Hudson Bay basin in 2005 (Shein 2006), resulting in a 21% increase in stream flow into Hudson Bay (Déry et al. 2011). Freshwater has a depleted $\delta^{13}C$ ratio relative to marine water (Conkright and Sackett 1986; Michener and Kaufman 2007). Similarly, $\delta^{34}S$ in freshwater is depleted relative to marine water (MacAvoy et al. 1998). Whales sampled in Sanikiluaq showed a significant depletion of the $\delta^{34}S$ isotope in 2005 relative to 2003. Freshwater in Hudson Bay moves in a counter-clockwise direction from rivers and estuaries up the east coast and into Hudson Strait (Stewart and Barber 2010), so increased levels of freshwater entering Hudson Bay could affect stable isotope levels in whales in both their summering and wintering grounds.

Interannual fluctuations cannot be explained solely by higher levels of freshwater input, however. Though 2005 had the highest precipitation of any year included in this study, 2003 was an abnormally dry year (Smith 2007), and 2007 was intermediate between the two (Environment 2010). If levels of freshwater were the sole influence on interannual stable isotope variation, expect to find stable isotopes reflective of the change in freshwater input, i.e. lowest levels of $\delta^{13}C$ and $\delta^{34}S$ isotopes in 2005, highest levels in 2003, and intermediate levels in 2007 should also be observed. Sulphur isotopes in Sanikiluaq are the only isotopes, however, that correspond yearly with freshwater output. Freshwater input from rivers and estuaries travels faster and over a greater distance under sea ice in Hudson Bay than through open water (Kuzyk et al. 2008; Stewart and Lockhart 2004); it also impacts the timing of sea ice breakup (Stewart and Lockhart 2004). Rates of water flow from rivers controlled by hydroelectric dams, can vary between months and days even in high precipitation years, depending on hydroelectric requirements (Cushman 1985; Environment 2010). Upwellings of deeper, more nutrient rich water may also result from changes in turbulence from freshwater input (Michener and Kaufman 2007; Stewart and Lockhart 2004), and otherwise affect the nutrients and primary productivity in the region (Stewart and Lockhart 2004). Beluga may also alter their estuary use by utilizing areas further offshore in years of heavy precipitation (Smith 2007). The potential effects and interactions of all of these factors and their impact on the ecosystem and beluga feeding habits are not well understood for Hudson Bay.

4.5.2 Beluga Spring Diets

Belugas harvested in Sanikiluaq were significantly higher in fatty acids and stable isotopes indicating a sea-ice associated diet compared to belugas harvested later in the year in Arviat. They had significantly higher levels of the 20- and 22- carbon monounsaturates. These fatty acids are biomarkers for calanoid copepod as they are synthesized *de novo* in *Calanus* spp. (Budge et al. 2006; Budge et al. 2008; Falk-Petersen et al. 2009; Kelley et al. 2010; Loseto et al. 2009). Higher levels indicate feeding in a food web where *Calanus* transfers high levels of carbon from primary producers to higher trophic-level consumers (Falk-Petersen et al. 2009). High levels of calanoid copepod biomarkers indicate a food chain that includes *Calanus*, the pelagic copepod predator *Themisto libelulla* (Auel et al. 2002), and potentially Arctic cod (Kelley et al. 2010; Loseto et al. 2009). Calanoid copepods thrive on the sea-ice algae and phytoplankton blooms that accompany melting sea ice

(Falk-Petersen et al. 2009), and are indicative of a pelagic sea-ice associated feeding regime, as they thrive in open water between ice floes and the pelagic zone under ice (Scott et al. 1999). Belugas from the Beaufort Sea feeding offshore near sea ice were associated both with Arctic cod and copepod biomarkers (Loseto et al. 2009). Belugas harvested in Sanikiluaq were also significantly higher in the fatty acid 16:4n1, a fatty acid associated with ice algae (Scott et al. 1999). They also had higher levels of the polyunsaturated 22:6n3, which is typically higher in animals feeding on an open water rather than water covered in sea-ice (Scott et al. 1999); this suggests belugas may preferentially be feeding at open water close to the floe edge, rather than under sea ice.

Both muscle and liver tissue from whales harvested in Sanikiluaq were $\delta^{13}C$ depleted relative to belugas harvested later in the year in Arviat. Lower levels of $\delta^{13}C$ can be caused by offshore pelagic feeding, rather than inshore benthic feeding (Hobson 1999; Hobson et al. 2002). A pelagic feeding regime is further supported by depleted $\delta^{15}N$ (Young et al. 2010) and enriched $\delta^{34}S$ isotope levels (Connolly et al. 2004). Combined, fatty acid and stable isotopes indicate that open water between sea ice is an important feeding location for belugas in the spring, and it seems to be where they are consuming the bulk of their seasonal energy.

4.5.3 Beluga Summer Diets

During the summer, belugas are frequently seen in coastal waters and in the mouths of rivers that empty into Hudson Bay (Martin et al. 2001; Watts and Draper 1986; Watts et al. 1991). Fatty acid and stable isotope levels found in these whales are reflective of summer feeding regimes. These dietary biomarkers differentiated whales harvested in late summer from the whales harvested in the spring. The data did not support preferential feeding in estuaries. Due to the similarity in some of the biomarkers examined for discrimination between inshore benthic feeding and pelagic estuary plume, it may not be possible to state that belugas are preferentially feeding in one location compared to the other.

Belugas harvested in Arviat were more associated with the fatty acid 16:1n7 and the 16-carbon polyunsaturated fatty acids 16:4n3, and 16:2n6. Palmitoleic acid (16:1n7) is typical of open water (as opposed to sea ice covered) pelagic feeding regimes, as is it associated with pelagic herbivorous zooplankton feeding, as are high levels of C16 polyunsaturated fatty acids (Scott et al. 1999). Discrimination between summer diets and spring diets on the PCA were principally driven by these fatty acids, coupled with a low amounts of the 20- and 22-C monounsaturates representative of sea ice associated diets. This is expected as there is no sea ice present in Hudson Bay in late summer (Stewart and Barber 2010). This trend was also seen in the PCA using fatty acids indicative of freshwater and marine feeding. The 22:1n11 and 20:1n9 fatty acids were most associated with belugas harvested in Sanikiluaq, and least associated with belugas from Arviat. These differences may have been driving the separation between belugas harvested at both sites.

Belugas from Arviat were more associated with the total C18 polyunsaturates and specifically 18:2n6 and 18:3n3, but whales sampled from Sanikiluaq had significantly higher percentages of 18:3n3. Though belugas sampled in spring (Sanikiluaq) and summer (Arviat) separated with some overlap, there was no clear separation among fatty acids representative of freshwater and marine feeding. This may indicate that freshwater species are not important prey items for belugas.

Fatty acids may be helpful in differentiating estuary plume feeding from benthic feeding. Higher levels of 16:1n7 and low levels of 18:4n3 and 22:6n3 would indicate a food chain low in dinoflagellates, which would be expected in areas of high turbidity and nutrients (Graeve et al. 1994; Rochon 2009) like estuary plumes. Higher levels of 20- and 22-C monounsaturates are also expected in benthic food chains, as they are associated with benthic decapods (Graeve et al. 1994). Belugas in Arviat were significantly lower in the polyunsaturates 18:4n3 and 22:6n3, and higher in the monounsaturated 16:1n7, which would indicate lower abundance of dinoflagellates on the food web. Belugas harvested in Arviat also had significantly lower levels of 20:1n7 and 22:1n7. These results indicate that belugas in Arviat may have been feeding preferentially in the estuary plume. Further studies of the biomarkers of potential beluga prey species, as well as primary producers in the Hudson Bay and estuaries are necessary to corroborate these findings.

Summer diets were also distinguished from spring diets based on stable isotope ratios. Higher δ^{13}C and δ^{15}N isotope levels in summer indicate beluga were feeding in a different food web than in the spring, but cannot differentiate between inshore benthic feeding and feeding in the estuary plume. If belugas were feeding in the upper estuary, their δ^{13}C, δ^{15}N, and δ^{34}S would be depleted relative to their marine spring diet (Connolly et al. 2004; Hobson 1999). The belugas sampled were enriched in δ^{13}C and δ^{15}N, so they are likely not feeding far into estuaries. This supports the fatty acid data indicating little freshwater feeding. Enriched δ^{13}C and δ^{15}N are indicative of feeding benthically and inshore (Hobson 1999; Young et al. 2010). Conversely, they may reflect beluga feeding at a higher trophic level in summer as a result of greater biodiversity at highly productive estuary plumes (Lohrenz et al. 1990). Increased primary productivity has been detected in the lower St.

Lawrence Estuary, and marine mammals feeding in that region are C-enriched relative to mammals feeding in the Gulf (Lesage et al. 2001). Higher trophic levels are indicated by isotopically heavier $\delta^{15}N$ (Falk-Petersen et al. 2004; Hobson et al. 2002), and carbon isotope enrichment may also occur at a higher trophic level (Bocherens and Drucker 2003; Gaden and Stern 2010; Kelly 2000; Lesage et al. 2001). Both carbon and nitrogen isotopes are enriched at the mixing zone between freshwater and saltwater in estuary plumes (Connolly et al. 2009). Sulphur isotopes are depleted in both freshwater and benthic organisms, and without prey species sampled from both areas, they cannot be used to determine if belugas are feeding in the estuary or benthically (Connolly et al. 2004).

The summer estuarine habitat of belugas may offer a number of benefits. Belugas may move further into estuaries for a number of reasons unrelated to feeding, such as predator avoidance (Brodie 1971; Richard 2005) and seeking warmer waters to molt (St. Aubin et al. 1990). Though belugas have been observed feeding in estuaries (Watts and Draper 1986), results of this study indicate that upper estuaries may not be significant sources of energy for belugas in the summer. Behavioural observations have indicated that belugas move farther offshore at the mouths of estuaries during years of high river output, but do not move farther away at any other point along the coast (Smith 2007), suggesting beluga may be preferentially feeding in the estuary plume.

Belugas harvested in summer were $\delta^{15}N$ enriched relative to belugas harvested in spring, which may indicate fasting (Cherel et al. 2005; Hobson et al. 1993; Young and Ferguson 2013), but there was no observed depletion of $\delta^{13}C$ in summer harvested whales, which is also an indicator of fasting (Cherel et al. 2005; Hobson et al. 1993; Young and Ferguson

2013). Belugas harvested in summer were instead δ^{13}C enriched. Higher levels of Enrichment of tissue sin δ^{13}C, along with changes in fatty acid composition suggests a shift in diet composition from spring to summer diets.

Changes to estuary dynamics arising from the development of hydroelectric dams, may impact beluga feeding by altering estuary output, and therefore altering primary productivity at the estuary plume (Wang 2006). Belugas are a highly philopatric species, and return to the same estuaries despite pressures from hunters (Hobbs et al. 2005; Watts et al. 1991); they may not abandon estuaries as a result of reduced prey items. This may result in reduced body condition both in neonates and reproductive and lactating females, resulting in increased mortality and decreased fecundity, creating far-reaching impacts on the Hudson Bay ecosystem.

4.6 Conclusion

Here stable isotopes and fatty acids were used to examine dietary differences between the spring and summer diets of Hudson Bay belugas, as well as define important feeding areas for belugas in summer. There were significant differences between spring and summer diets which were predominantly characterized by the presence and absence of sea ice associated primary productivity. Dietary biomarkers were also used to determine important beluga feeding areas during the summer on the west coast of Hudson Bay. Fatty acids profiles of whales harvested in Arviat were not similar to those found in other marine mammals feeding on freshwater diets (Smith et al. 1996), and stable isotopes were not depleted relative to the marine spring diet (Connolly et al. 2004; Hobson 1999). This indicates that estuaries are likely not important feeding areas for belugas. Belugas may, however, be feeding preferentially in estuary plumes in summer.

Community		K-S Distance
Arviat	Age	
	$\bar{x}$ = 21.3, *SD* = 13.6, range = 3-61	0.10, p >0.20
	M = 19	
	Length	
	$\bar{x}$ = 321.4 *SD*=63.3, range = 127-35.3	0.13, p=0.05
	M=335.3	
Sanikiluaq	Age	
	$\bar{x}$ = 22.8, *SD*=11.2, range = 0 – 61	0.17, p=0.01
	M=23	
	Length	
	$\bar{x}$ =338.1, *SD*=55.7, range = 181.3 – 480.1	0.10, p >0.20
	M=346.7	

Table 4.1 Age (years) and length (cm) data for belugas harvested in Arviat and Sanikiluaq, and Kolmogrov-Smirnov (K-S) distances. Age was normally distributed in Arviat, but not in Sanikiluaq. Length was normally distributed in Sanikiluaq, but not in Arviat.

Subjects		Arviat	Sanikiluaq
Female	Count	19	16
	Expected Count	18.23	16.77
	Row %	54.29	45.71
	Column %	38.00	34.78
	Total	19.79	16.67
Male	Count	31	30
	Expected Count	31.77	29.23
	Row %	50.82	49.18
	Column %	62.00	65.22
	Total %	32.29	31.25

Table 4.2 Chi-square results comparing the proportion of male and female belugas harvested from each site. Results were not significant.

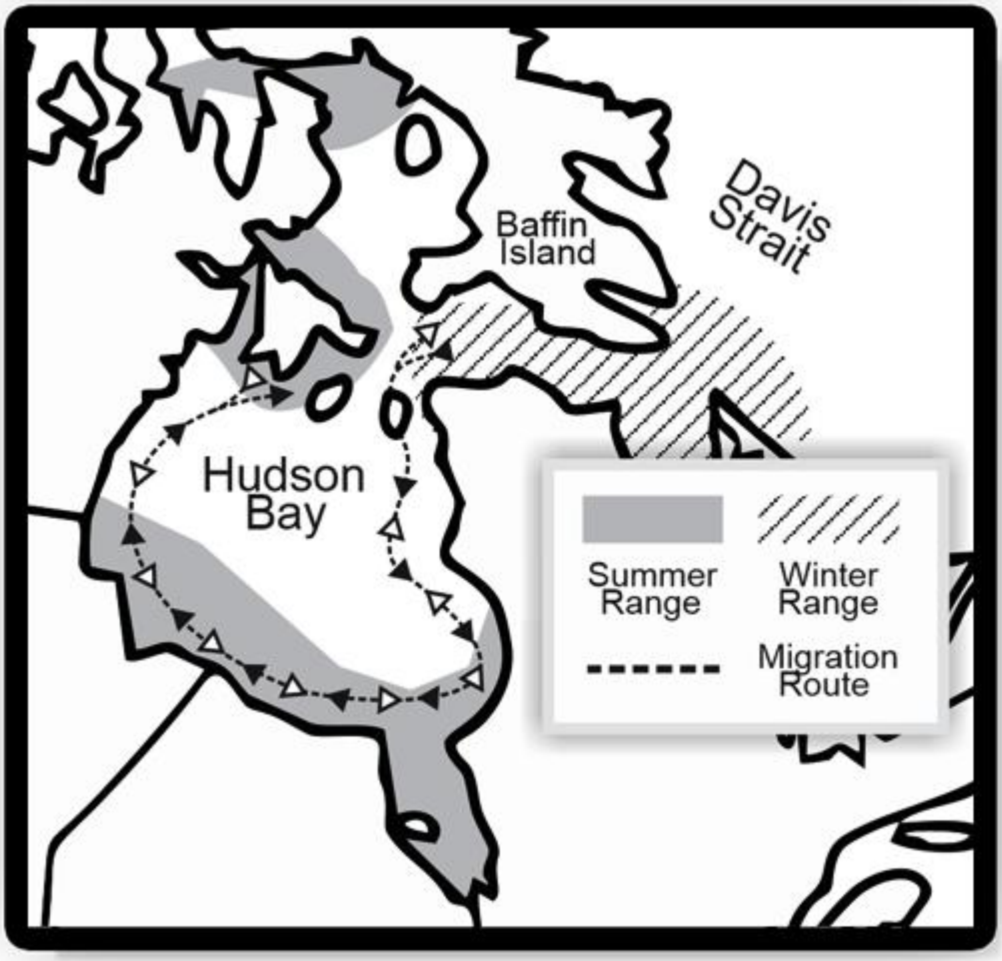


Figure 4.1 Map of Hudson Bay showing beluga migration routes and winter and summer ranges. Black triangles denote the direction of spring migration and white triangles denote the direction of autumn migration.

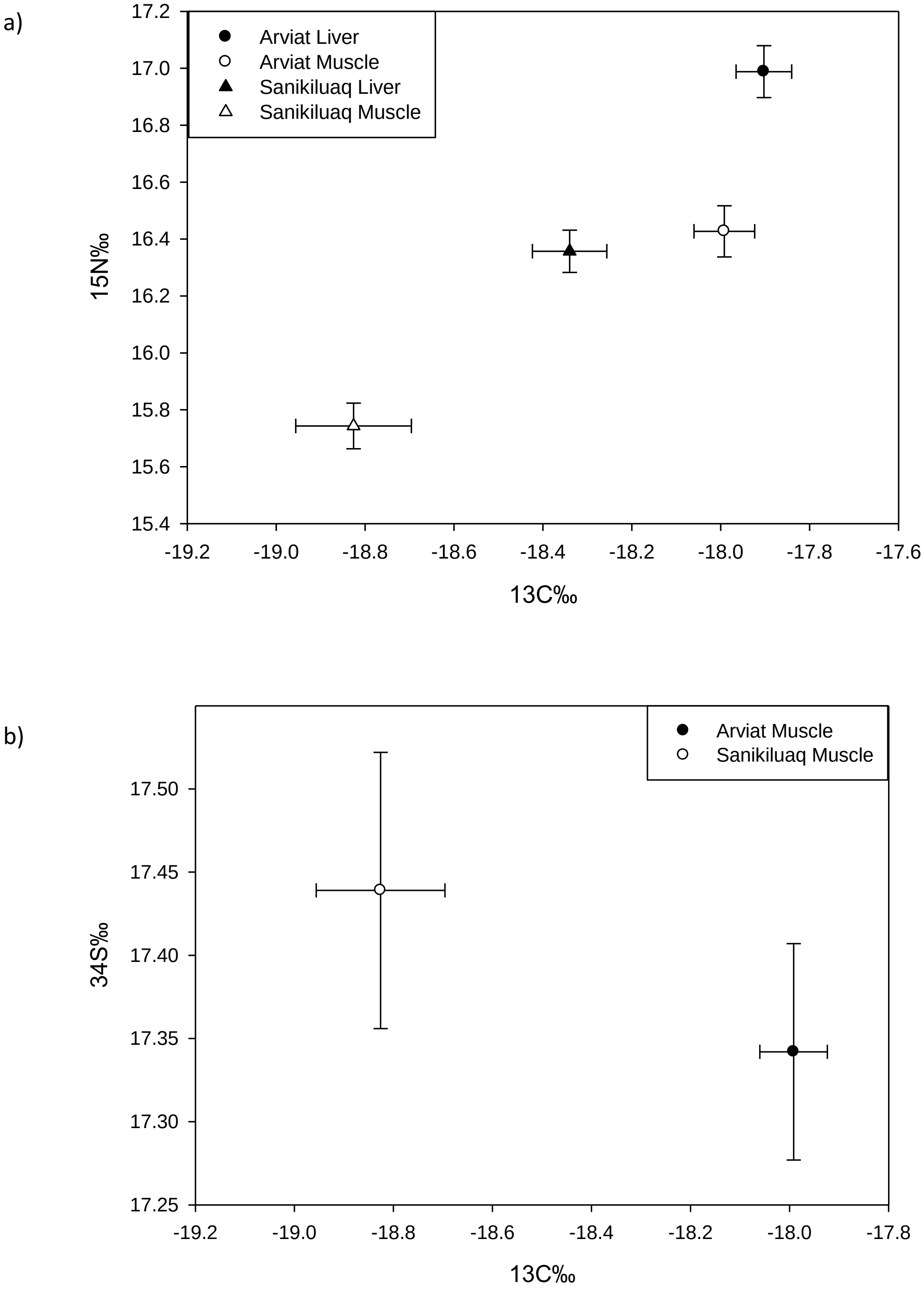
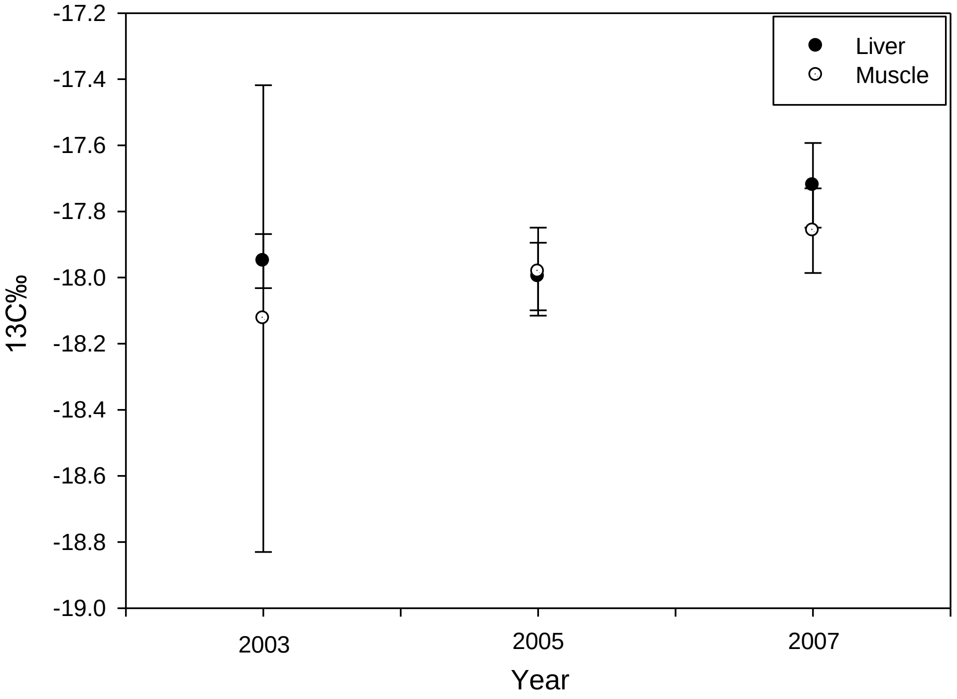
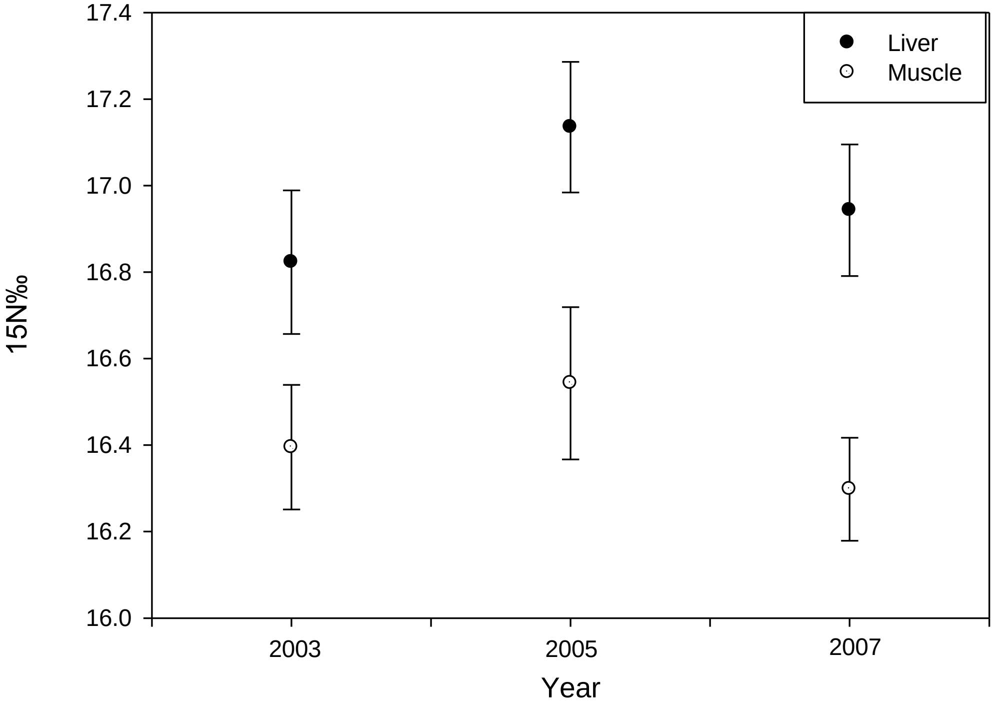


Figure 4.2. Carbon and nitrogen (a) and carbon and sulphur (b) isotope ratios in muscle and liver tissues in belugas harvested in Arviat and Sanikiluaq. Whales from Arviat are represented by circles that are open (muscle) or filled (liver). Whales from Arivat and Sanikiluaq are represented by open (muscle) or filled (liver) sircles and triangles, respectively. Error bars represent ± 1 standard error.

a)


-17.2
-17.4
-17.6
-17.8
-18.0
-18.2
-18.4
-18.6
-18.8
-19.0
13C‰
2003
2005
2007
Year
Liver
Muscle

b)


17.4
17.2
17.0
16.8
16.6
16.4
16.2
16.0
15N‰
2003
2005
2007
Year
Liver
Muscle

c)

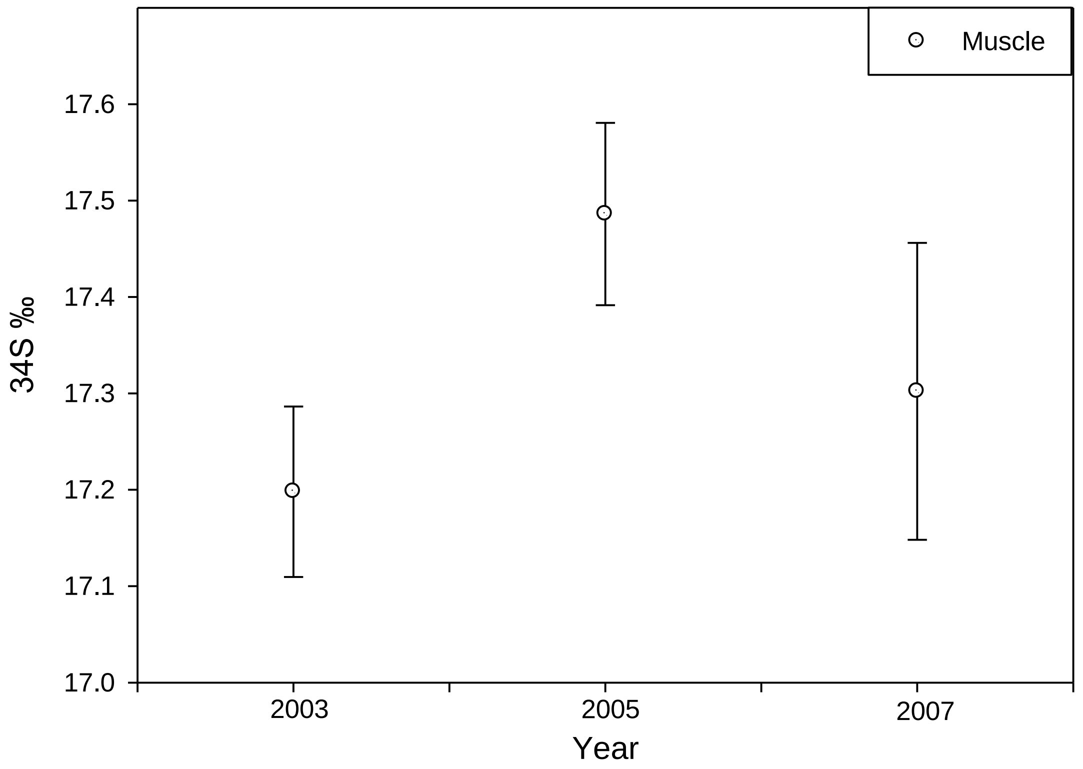
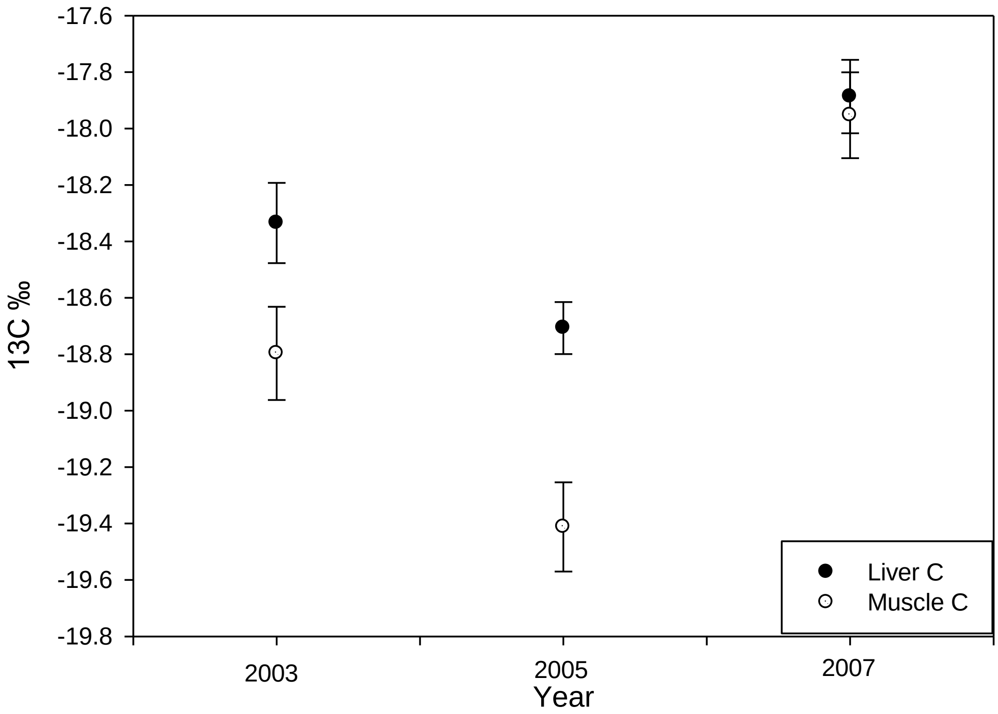
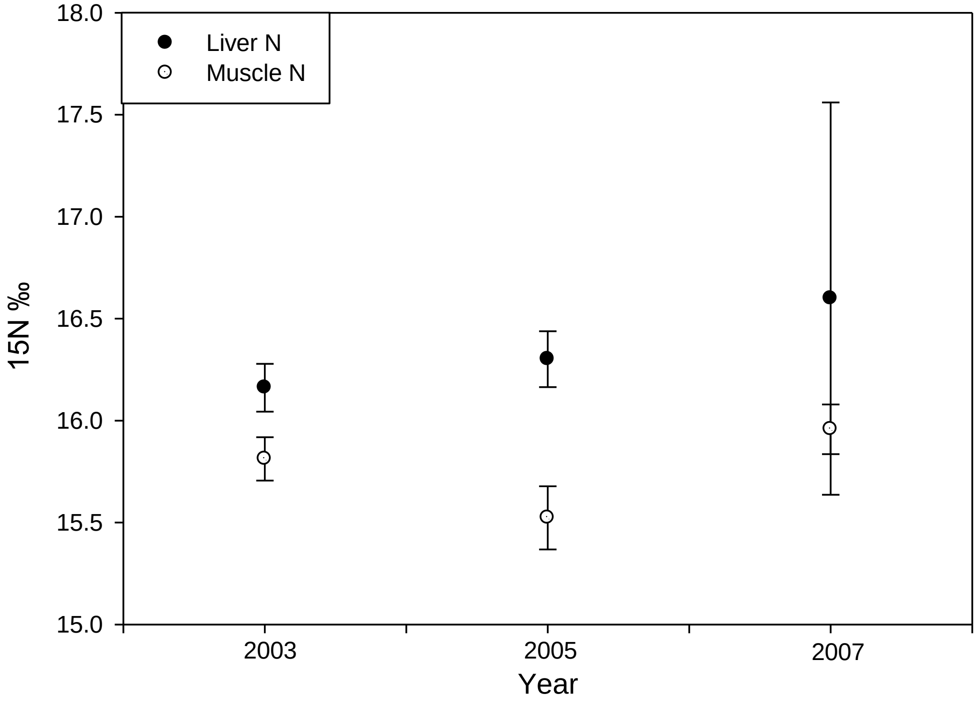


Figure 4.3 Mean isotope values for belugas harvested in Arviat on 2003, 2005 and 2007. Graphs represent a) δ^{13}C isotopes in muscle and liver tissue, b) δ^{15}N isotopes in liver and tissue, and c) δ^{34}S isotopes in muscle. Muscle tissue is represented by open circles while liver tissue is represented by filled circles. Error bars represent ±1 standard error.

a)


-17.6
-17.8
-18.0
-18.2
-18.4
-18.6
-18.8
-19.0
-19.2
-19.4
-19.6
-19.8
13C ‰
2003
2005
2007
Year
● Liver C
○ Muscle C

b)


18.0
17.5
17.0
16.5
16.0
15.5
15.0
15N ‰
2003
2005
2007
Year
● Liver N
○ Muscle N

c)

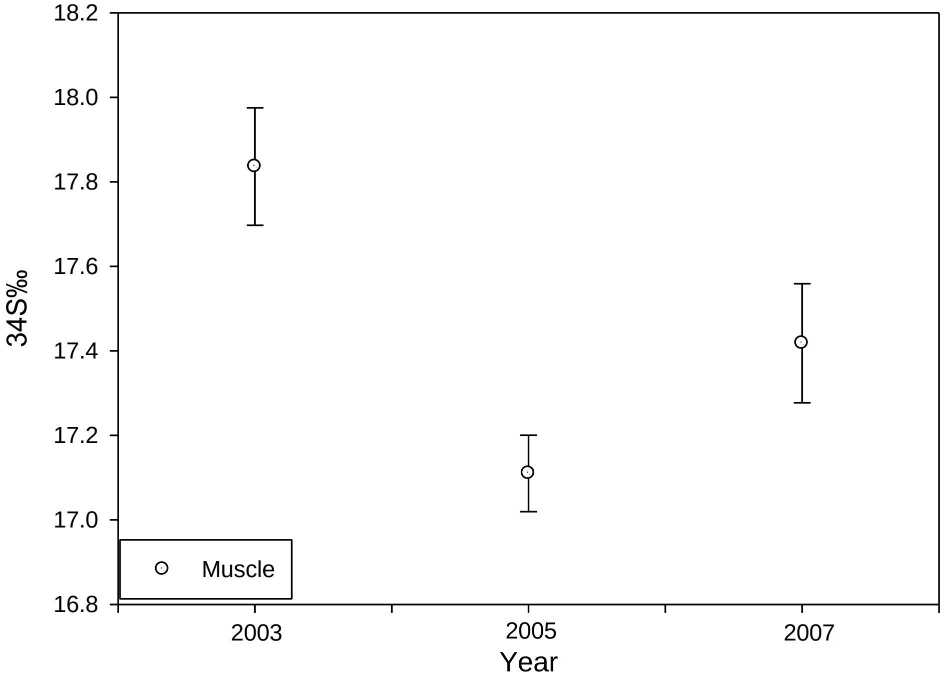


Figure 4.4 Mean isotope values for belugas harvested in Sanikiluaq in 2003, 2005 and 2007. Graphs represent a) $\delta^{13}C$ isotopes in muscle and liver tissue, b) $\delta^{15}N$ isotopes in liver and tissue, and c) $\delta^{34}S$ isotopes in muscle. Muscle tissue is represented by open circles and liver tissue is represented by filled circles. Error bars represent ±1 standard error.

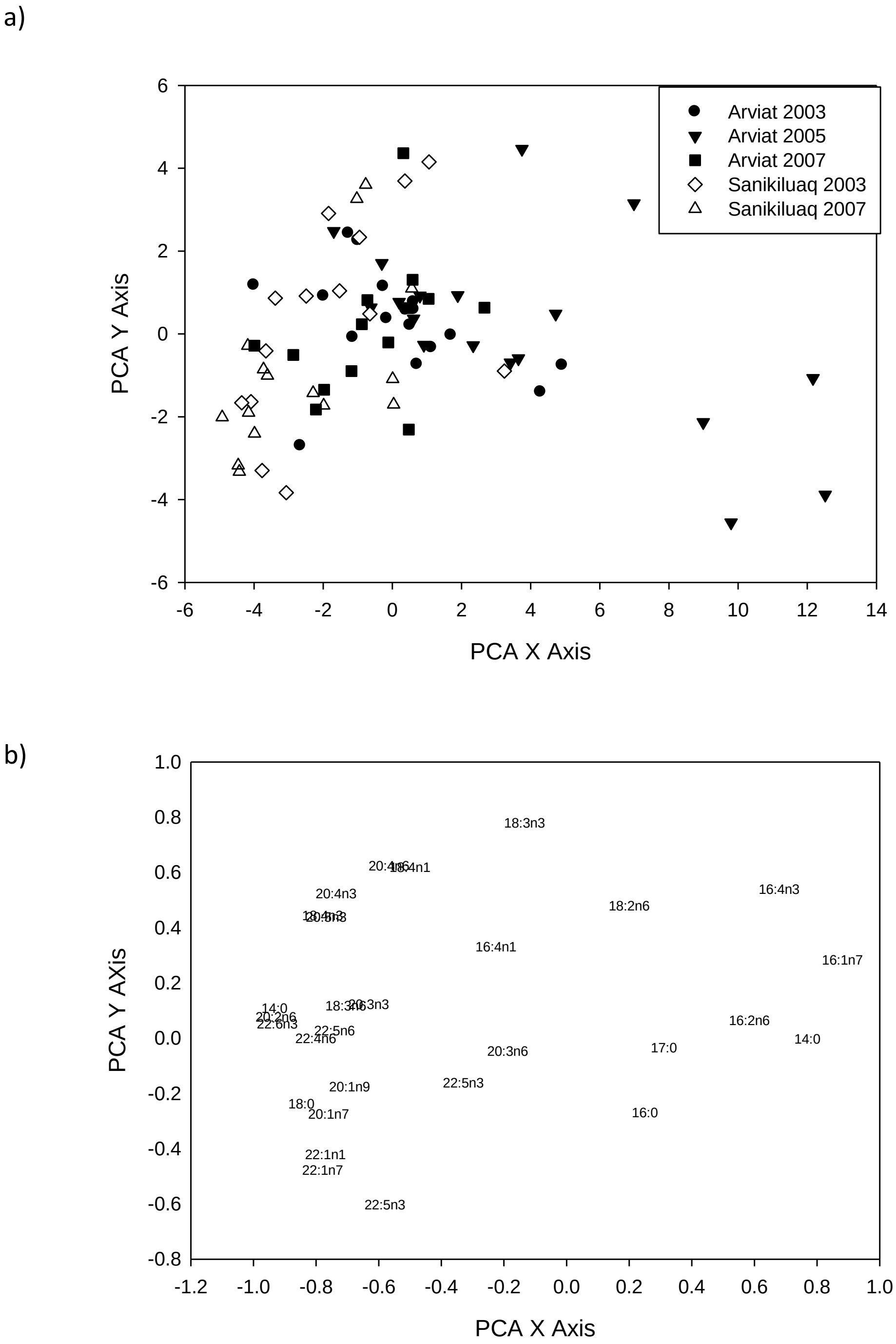


Figure 4.5 a) Principle Component Analysis plot of belugas harvested from Sanikiluaq and Arviat in 2003, 2005 and 2007; and b) the fatty acids contributing to the separation between sites. Fatty acids 14:0 16:1n7, 16:4n3 and 16:2n6 are associated with beluga from Arviat, and the 20 and 22 carbon monounsaturates and polyunsaturates are associated with belugas harvested in Sanikiluaq.

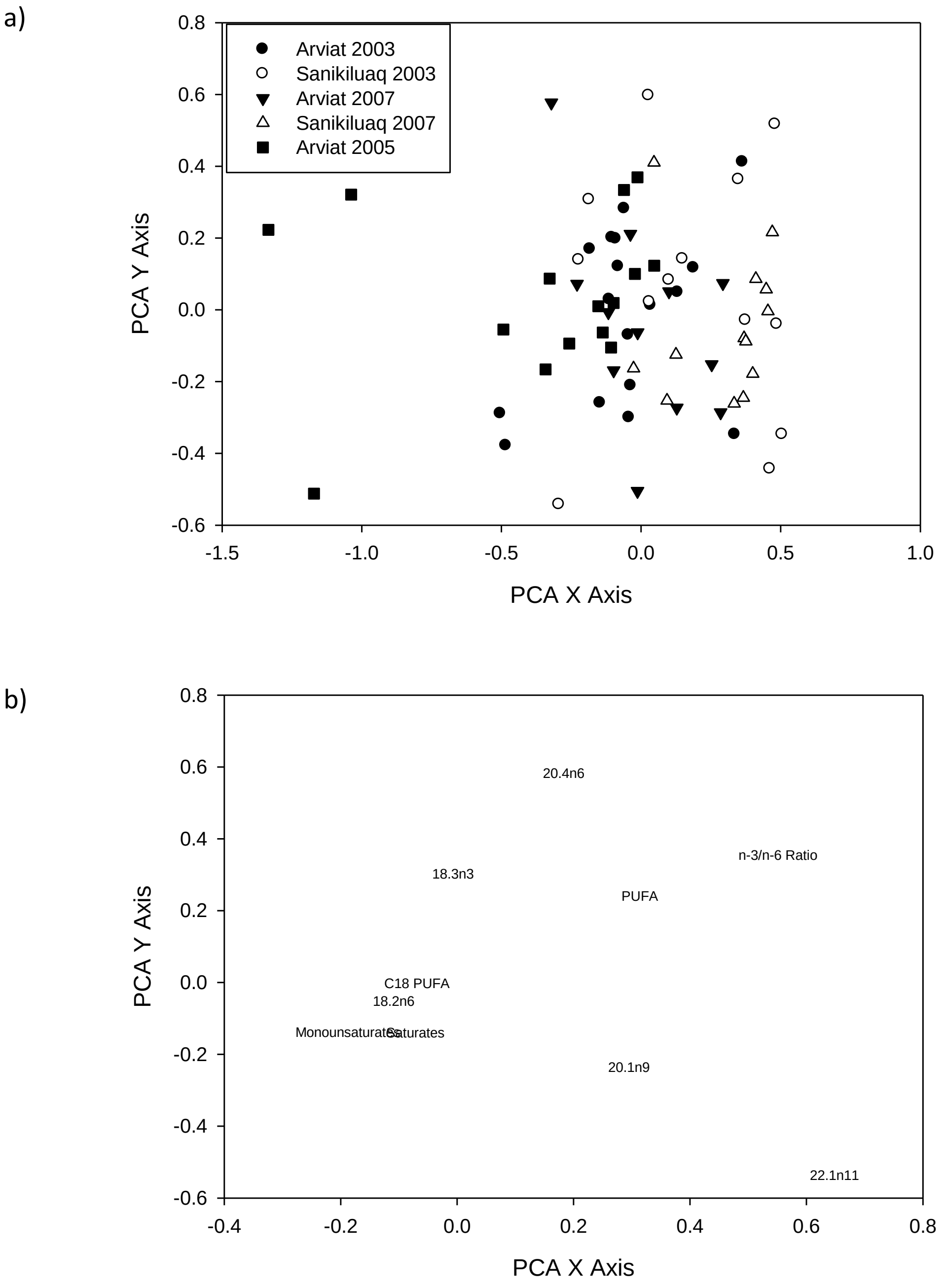


Figure 4.6 a) Principle Component Analysis plot of belugas harvested from Sanikiluaq and Arviat in 2003, 2005 and 2007; and b) a reduced set of fatty acids indicative of freshwater and marine feeding. Total saturates and monounsaturates, 18:2n6 18:3n3 and the 16-carbon length polyunsaturated acids are associated with whales harvested in Arviat, the 20 and 22 carbon monounsaturates 20:1n9 and 22:1n11 and the total omega-3 to omega 6 ratio are associated with whales harvested in Sanikiluaq.

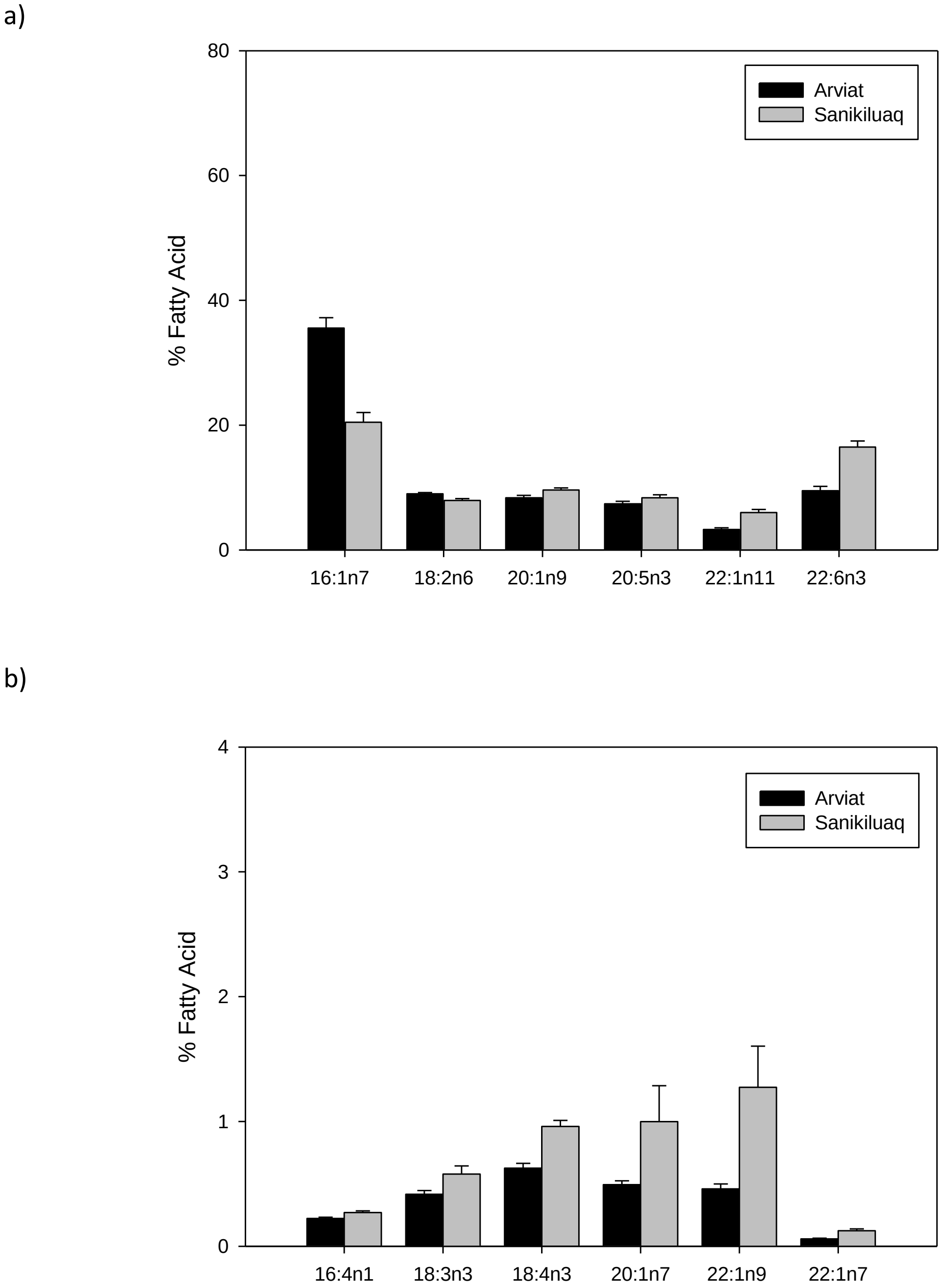


Figure 4.7 Variation in fatty acids in belugas harvested in Arviat and Sanikiluaq; fatty acids in a) are more than 1% of total fatty acids, and b) are less than 1% of total fatty acids. Error bars represent ±1 standard error.

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5 Sexual segregation in two closely related species: beluga whales (*Delphinapterus leucas*) and narwhal (*Monodon monoceros*)

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5.1 Abstract

Beluga whales and narwhals are important to the ecology of Arctic ecosystems, and the economy of Inuit. Despite being similarly-sized sister taxa ,they may exhibit different degrees of sexual segregation. Sexual segregation is the separation in social interactions, habitat use, or spatial use between sexes within a population. This may be a result of different energy requirements driven by sexual size dimorphism. Here stable isotopes and fatty acid analyses are used to determine if belugas harvested in Hudson Bay and narwhals harvested near Pond Inlet show different patterns of sexual segregation outside the mating season.

Beluga samples were collected from Inuit subsistence hunts in Arviat and Sanikiluaq between 2003 and 2007. Narwhal samples were collected from subsistence hunts in Pond Inlet between 2004 and 2006. Muscle and liver samples were analysed for $\delta^{13}C$ and $\delta^{15}N$ stable isotopes, and blubber samples were analysed for fatty acids. Tests were used to compare dietary biomarkers between males, females, and immature whales from each harvest site.

Tissues samples from belugas hunted in Sanikiluaq were sampled during the mating season and did not show sexual segregation in fatty acids or carbon isotopes, indicating that whales are feeding in the same location on similar prey species. Nitrogen isotope enrichment varied between sexes; this may be a result of larger males consuming larger prey items. Belugas from Arviat were harvested outside of the mating season and also showed no sexual segregation in diet. In contrast, significant differences in fatty acids between males and females and males and immature narwhal of both sexes harvested in

Pond Inlet indicate that males, females and immature whales are consuming different prey items. There were also significant differences between male and female and immature narwhal in carbon isotope enrichment, indicating that they are feeding in different locations. Males may be feeding more benthically, while females and immature whales may be feeding more pelagically near sea-ice. Implications are discussed.

5.2 Introduction

Beluga whales (*Delphinapterus leucas*) and narwhals (*Monodon monoceros*) are closely related top- level predators in the Canadian Arctic, and as such are a important species in the Arctic ecosystem, as the can influence food web structure and function (Brodie 1989). They also have a high cultural and economic importance to the Inuit, who rely on both whales as a food supply (Caulfield 1993; Hoover et al. 2013; Tyrrell 2007). The ecology of both species is not well understood, however, and some populations of whales are vulnerable (Cosewic 2004; Iucn 2008). Critical to any wildlife management plan is an understanding of where individuals in a population feed, which can be used to identify critical habitat (Rosenfeld and Hatfield 2006; Van Horne 1983). Critical habitat may be hard to define, however, as groups of individuals within a population may use habitats differently (Bowyer 2004; Kie and Bowyer 1999).

Sexual segregation is the separation, either in social interactions, habitat use, or spatial use between sexes (Ruckstuhl and Clutton-Brock 2005). Sexes may segregate for a variety of different reasons, including predator risk avoidance, variation in environmental conditions, and variation in forage selection requirements (Conradt 2005). Animals of different sexes and reproductive classes can require different types and amounts of food, either via variation in metabolic demands related to reproductive requirements (Staniland 2005; Stevick et al. 2002) or due to sexual size dimorphism (Ruckstuhl and Neuhaus 2002), which can lead to sexual segregation in habitat use. Sexual segregation has been reported in a number of odontocetes, including bottlenose dolphins (*Tursiops truncatus*) (Connor et al. 1999), sperm whales (*Physeter macrocephalus*) (Ohsumi 1966), botos (*Inia geoffrensis)*

(Martin and Da Silva 2004), harbor porpoises (*Phocoena phocoena*) (Read and Hohn 1995), dusky dolphins (*Lagenhorhynchus obscurus*) (van Waerebeek and Read 1994), striped dolphins (*Stenella coeruleoalba*) (Forcada et al. 1994), beluga (Loseto et al. 2006; Richard et al. 2001; Smith et al. 1994), and narwhal (Silverman 1979). Sexual segregation is more pronounced among more sexually dimorphic species (Mysterud 2000).

Sexual size dimorphism (SSD) is thought to arise as an adaptation to the physiological demands of each sex's respective reproductive roles (Fairbairn 1997). SSD can be related to mating systems, as species that rely on sperm competition over other types of competition tend to have smaller degrees of SSD, and species that rely more heavily on intrasexual physical contests tend to have greater SSD (Andersson 1994). The relationship between SSD and mating systems has been supported across a number of mammalian taxa, including odontocetes (Dixson 1998; Kenagy and Trombulak 1986; MacLeod 2010; Rose et al. 1997) (Chapter 2). In mammals, SSD is most frequently observed whereby males are the larger sex; increasing dimorphism is likely the result of increased sexual selection pressure on males in competition for females (Weckerly 1998). Females can also be the larger sex, which is likely the result of inter- and intra-annual cycles in resource acquisition necessary for pregnancy and lactation rather than mating system (Ralls 1976). Beluga whales and narwhal are the only members of the family Monodontidae (Nikaido et al. 2001). Both species exhibit some degree of SSD (Brodie 1989; Garde et al. 2007; Mansfield et al. 1975; Stewart 1994), and sexual segregation has been reported in some populations of both species (Caron and Smith 1990; Loseto et al. 2006; Silverman 1979).

Variation in environmental factors such as carrying capacity, population density, and food quality may result in different sexual segregation patterns between populations (Bowyer 2004). Sexual segregation in beluga populations in Hudson Bay was examined through visual observations and comparisons of harvested animals (Caron and Smith 1990; Sergeant 1973). Conflicting information about the ratio of males to females in and around Hudson Bay estuaries has been gathered through these methods. (Caron and Smith 1990) found higher neonate and female representation in estuaries on the east coast of Hudson Bay; Sergeant (1973) found a unified sex ratio in shot and netted whales on the west coast of Hudson Bay. Aerial surveys and surveys from shore found sexual segregation within narwhal populations in Pond Inlet, northern Baffin Island, and northwestern Greenland (Born et al. 1994; Kingsley et al. 1994; Silverman 1979). Observation-based assessments of herd composition may be biased towards immediately recognizable individuals, such as neonates, or males with large tusks (narwhal) (Born et al. 1994), and may not be the best method of establishing herd composition.

Alternately, dietary biomarkers can be used to establish what individual whales are feeding upon and therefore the habitat they are using (Hobson 1999; Hobson et al. 2002; Hobson et al. 1996; Kurle and Worthy 2001, 2002). Sexual segregation in habitat use in belugas observed by radiotelemetry data (Loseto et al. 2006), was supported by fatty acid and stable isotope dietary biomarkers in the Beaufort Sea population (Loseto et al. 2009; Loseto et al. 2008). Stable isotopes have also been used to examine sexual segregation in seals (Staniland 2005). Dietary biomarkers can be used to gain information on preferred foraging locations. Stable isotopes have been used to determine diet composition and foraging locations in marine mammals (Gaden and Stern 2010; Iverson et al. 2004; Kurle and Worthy 2001, 2002; Loseto et al. 2009; Olsen and Grahl-Nielsen 2003; Outridge and Stewart 1999). Carbon stable isotopes ($\delta^{13}C$) are only slightly enriched as they travel up trophic levels, and are good indicators of primary productivity (Budge et al. 2008; Lesage et al. 2001; O'Reilly et al. 2002; Young et al. 2010). Nitrogen stable isotopes enrich with increasing trophic level, and as such can be used to identify trophic level of target species (Cabana and Rasmussen 1996; Hobson 1999). Stable isotopes can moreover be used in coastal marine ecosystems to determine incorporation of freshwater prey into the diet (Connolly et al. 2009; Fry and Sherr 1984; MacAvoy et al. 1998), as well as benthic vs. pelagic feeding (Connolly et al. 2004; Peterson and Fry 1987; Young et al. 2010). Fatty acids from prey species are incorporated into predator tissue in predictable ways and can be used to determine predator diets as well as illustrate the effects of size and sex within species (Beck et al. 2007; Beck et al. 2005). Though beluga and narwhal are both size dimorphic species, narwhal exhibit greater SSD than do beluga (Chapter 3), and may also exhibit a greater degree of sexual segregation in habitat use. Fatty acid and stable isotope biomarkers may be useful to examine sexual segregation in feeding habits of beluga and narwhal in the eastern Canadian Arctic.

The majority of hypotheses developed to explain the factors that underlie sexual segregation have been developed and tested on social ungulates, though they may be extended to odontocetes (Michaud 2005). Three hypotheses proposed to explain sexual segregation that may be useful for understanding segregation in beluga and narwhal are the are the predation risk hypothesis, the forage selection hypothesis, and the scramble competition hypothesis (Ruckstuhl and Neuhaus 2000). In summary (adapted from Michaud (2005)): the predation risk hypothesis predicts females will remain in areas that provide the best protection from predators while with neonates, even if the food quality is poor, while males should select nutritionally rich feeding areas to remain in peak physical condition for mate competition; the forage selection hypothesis predicts that metabolic requirements vary with reproductive status, so groups with similar activity budgets will group together. Finally, the scramble competition hypothesis predicts sexual segregation based on food availability, with sexual segregation occurring only when food availability is low.

Dietary biomarkers were used to examine the differences among reproductive classes and between sex in narwhals and belugas. Beluga samples were collected both within and outside of the mating season, while narwhal samples were only collected outside the breeding season. Fatty acid and stable isotope biomarkers were used to attempt to determine if sexes were feeding in different locations as well as on different prey items. This information may be useful in establishing critical feeding locations for belugas and narwhals.

5.3 Methods

5.3.1 Sample Collection

All samples were collected from animals harvested during annual subsistence hunts in Nunavut, Canada. Sampling followed the same procedure outlined in Chapter 3. Narwhal ages were not determines as narwhal rarely have teeth in their lower jaws.

A total of 44 individual belugas were sampled from Arviat, a community in Hudson Bay that hunts the Western Hudson Bay beluga stock. Samples were collected between July and

September of 2003, 2005 and 2007. Two samples harvested from Arviat (one from 2005, one from 2007) were removed from analysis as they were missing data on length and sex. A total of 38 beluga samples collected from Sanikiluaq were used, the samples were collected between March and July. Blubber samples from all 19 belugas harvested in Sanikiluaq in 2005 were missing. Male belugas were categorized as sexually mature if they were a minimum of 310 cm long, and females as mature if they were at least 270cm long (Heide-Jørgenson and Teilmann 1994). All other belugas were categorized as immature.

A total of 35 individual narwhals were sampled from Pond Inlet, which represent the High Arctic stock (Richard 2010). Samples from Pond Inlet were collected between July and September in 2004, 2005, and 2006. Narwhal females that were at least 337 cm long were categorized as sexually mature, and males were similarly categorized if they were at least 388cm long (Garde et al. 2007). Five narwhal samples were removed from statistical analyses (two each from 2004 and 2006, and one from 2005) as they were missing data on length or sex.

Both belugas and narwhals are likely seasonal breeders with a protracted mating seasons (Best and Fisher 1974; Heide-Jørgenson and Teilmann 1994; O'Corry-Crowe et al. 2003). Belugas harvested in Arviat and narwhals harvested in Pond Inlet were both harvested outside the mating season, which is likely between April and July for belugas (Heide-Jørgenson and Teilmann 1994; O'Corry-Crowe et al. 2003; Chapter 3), and May to July for narwhals in the Baffin Bay area (Heide-Jørgensen and Garde 2011; Chapter 3). Belugas harvested in Sanikiluaq were harvested within the mating season.

5.3.2 Stable Isotope Analysis

Beluga muscle and liver samples were analyzed for $\delta^{13}C$ and $\delta^{15}N$ stable isotopes. Stable isotopes in liver samples are representative of prey that was more recently consumed, and isotopes from muscle samples represent prey that was consumed over a longer period of time (Hobson 1999; Kurle and Worthy 2002). All samples were freeze dried and delipified following the procedures outlined in Chapter 4 before being sent to the Waterloo Environmental Isotope Laboratory (uwEILAB) for mass spectrometry analysis; described in Chapter 4.

5.3.3 Fatty Acid Analysis

Lipid extraction and analysis followed that described in Chapter 4. To summarize, lipids were extracted from the innermost layer of blubber. Lipids were extracted from 0.5g of blubber using a 2:1 ratio of Chloroform: methanol. Extracted lipids were diluted and purified using hexane and Hilditch reagent (NH_2SO_4).

5.3.4 Data Analysis

A chi-square test was performed to determine if the narwhal and beluga populations had significantly different proportions of males to females, and mature and immature whales. Following the chi-square test, immature males and females were pooled into one group of immature whales within their population.

Two-way ANOVAS were used to test for differences between stable isotope enrichment levels in muscle and liver tissues between mature males and females, as well as for differences between reproductive class and year in both belugas and narwhals. ANOVAS followed with post-hoc Holm-Sidak tests were performed to test for pair-wise comparisons between reproductive classes. If significant differences between groups were found for one tissue but not the other, a post-hoc t-test was performed to compare the isotopes between tissue types within the reproductive class.

Principle Component Analysis (PCA) was used to determine whether beluga and narwhal sexes and reproductive classes were feeding on different prey items by analyzing differences in fatty acid profiles. Though this method cannot be used to make quantitative statements about whale diets, it can be used to perform qualitative investigation of diets (Dahl et al. 2000; Kelley et al. 2010; Loseto et al. 2009). Values from whales that plot close to each other on the PCA have similar fatty acid profiles relative to whales that plot farther away. Values from whales also plot close to fatty acids that make up a larger proportion of the whale's fatty acid profile.. PCA analyses were performed separately on whales harvested in Sanikiluaq, Arviat and Pond Inlet. All fatty acid data was $\log_{10}$-transformed before PCA analysis.

Of the 29 fatty acids known to transfer predictably from prey to predators (Iverson et al. 2004), 15 fatty acids that are useful in distinguishing between different potential feeding areas (open water, sea ice, and benthic areas (Scott et al. 1999)) were used to determine differences in feeding behaviour in narwhals and belugas. PCA analyses were followed by ANOVA tests on the selected fatty acids to determine if there was a statistically significant difference between the reproductive classes. All ANOVA tests were performed using SigmaPlot (Systat Software 2008). PCA analyses were performed using SYN-TAX (Podani 2001).

5.4 Results

5.4.1 Population Statistics

The chi-square test showed no significant differences between the proportion of mature males, mature females, immature males, and immature females harvested in each community (Chi square = 4.52, *p*=0.61) (Table 5.1) and justified pooling data by species.

5.4.2 Stable Isotopes

5.4.2.1 Narwhal

There was no significant difference in stable isotope values among years in any of the narwhal tissues tested. There were significant differences between sex and reproductive classes in δ^{13}C in narwhal samples. An ANOVA of δ^{13}C levels in muscle tissue among reproductive classes showed significant overall effects of reproductive class (*p*<0.001). In this regard, significant differences were found between mature males (-17.99‰±0.08) and mature females (-18.40‰±0.07; *p*=0.001), and mature males and immature whales (-18.45‰±0.08; p<0.001). Significant differences were found across reproductive classes in liver samples as well, with significant differences between mature males (-18.02‰±0.07) and mature females (-18.40‰±0.07; p=0.001), and between mature males and immature whales (-18.40‰±0.08; p=0.003) (Figure 5.1).

There were no significant differences in δ^{15}N isotope levels between male and female narwhals for muscle (males (m) =16.38‰±0.14, females (f) =16.31‰±0.162, p=0.08) or liver tissue (m=16.97‰±0.18, f=16.94‰±0.2, p=0.93) (Figure 5.2). There was no significant effect of reproductive class on δ^{15}N in muscle (p=0.02) and liver tissue (p=0.03). Mature males had significantly more enriched δ^{15}N muscle tissue (16.64‰±0.13) than immature males (15.98‰±0.17; p=0.006. Although there was a significant effect of reproductive class on liver tissue δ^{15}N isotope levels, post-hoc analyses showed no significant differences between any two groups. Stable isotope analyses are summarized in Table 5.2.

5.4.2.2 Belugas

There was no significant differences in δ^{13}C enrichment levels in muscle tissue sampled from belugas harvested in Arviat, either among reproductive classes or between sexes (p=0.80). There was also no significant difference in δ^{13}C levels in liver tissue between sexes or reproductive class (p=0.23) (Figure 5.1).

There was a significant difference in δ^{15}N in liver samples among reproductive classes (p=0.006). Mature male liver samples (17.31‰±0.13) were significantly more enriched than those of mature females (16.60‰±.02; p=0.004); immature whales were intermediate between the two (16.78‰±0.19) and were significantly different than mature males (p=0.03), but not mature females (p=0.51). A post-hoc t-test found a significant difference between muscle and liver δ^{15}N enrichment levels for mature male belugas (p=0.002).

Belugas harvested in Sanikiluaq showed no significant differences in δ^{13}C enrichment levels in liver or muscle tissues (Figure 5.1).

There was a significant difference in δ^{15}N enrichment levels in belugas harvested in Sanikiluaq. Mature males were significantly enriched in both muscle (15.95‰±0.40, p=0.004) and liver tissues (16.63‰±0.39, p=0.005) relative to mature females (muscle = 15.32‰±0.58, liver = 16.10‰±0.45). Immature whales were intermediate between the two, and had significantly depleted liver δ^{15}N levels compared to mature males (16.04‰±0.40, p=0.005), but showed no significant difference in muscle δ^{15}N levels (15.79‰±0.44) (Figure 5.1). All beluga stable isotope analysis results are reported in Table 5.2.

5.4.3 Fatty Acids

5.4.3.1 Narwhal

The PCA for narwhal harvested in Pond Inlet explained 65.15% of the variation in narwhal fatty acid profiles, with 46.92% explained by the X-axis and 18.23% explained by the Y-axis. The majority of the mature female narwhals were on the positive side of the X-axis. There was overlap between all of the reproductive classes. The positive side of the X-axis was dominated by 16-and 18-carbon chain polyunsaturates; the negative side was dominated by the fatty acids 20 and 22-carbon mono and polyunsaturates (Figure 5.2). Significant differences were found between reproductive classes in the polyunsaturates 16.4n1, 18.4n3, and 20.5n3. Results are summarized in Table 5.2.

5.4.3.2 Beluga

The PCA for belugas harvested in Arviat explained 73.96% of the variation; 54.89% was explained by the X-axis, and 19.06% was explained by the Y-axis. There was no clear separation in fatty acid profiles between whales harvested in Arviat. Four mature males and one immature whale are separated from the other whales on the positive side of the X-axis and were associated with the palmitoleic acid (16:1n7); the rest of the whales clustered together with little differentiation between reproductive classes. There was no significant difference in the proportion of any of the fatty acids examined (Figure 5.3).

The PCA for belugas harvested in Sanikiluaq explained 64.82% of the variation; 40.45% was explained by the X-axis and 24.37% was explained by the Y-axis. There was no clear separation between males, females, and immature belugas in Sanikiluaq. The negative side of the PCA X-axis was predominated by the 20-and 22-carbon monounsaturates, and the positive side was predominated by the 16 and 18-carbon length mono- and polyunsaturates. There was no significant difference in the proportion of any of the fatty acids examined (Figure 5.4).

5.5 Discussion

Sexual segregation is the segregation between sexes outside the mating season (Barboza and Bowyer 2000). In order to establish sexual segregation in a population, it is necessary to assess space, habitat, and diet, as segregation can occur spatially, and through different habitat and prey selection (Bowyer 2004). Visual observations of belugas and narwhals in Hudson Bay and northern Baffin Bay have reported sexual segregation in both species (Caron and Smith 1990; Kingsley et al. 1994). Few studies have examined segregation in feeding behaviour in monodontids (Laidre and Heide-Jørgensen 2005; Loseto et al. 2009; Watt et al. 2013), however, results from this study indicate little sexual segregation in feeding for beluga, and provide some support for sexual segregation in narwhal.

There is some evidence for sexual segregation in feeding regimes in narwhals from Pond Inlet. Mature males were significantly more enriched in δ^{13}C in both muscle and liver tissue than females and immature narwhals, indicating that males may be feeding in a different carbon region than the other reproductive classes. Enrichment of carbon isotopes can be the result of inshore, benthic feeding regimes, relative to offshore pelagic feeding (Hobson

1999). Benthic feeding regimes often have high levels of fatty acids 20:1n7 and 22:1n7, as these are elongated from the fatty acid 18:1n7, a process that only occurs in benthic decapods (Graeve et al. 1994). Although seven males (six mature and one immature) were more associated with the fatty acids 20:1n7 and 22:1n7 on the negative side of the PCA, an equal number of males were on the positive side of the PCA and there was no statistically significant difference between mature males and other reproductive classes for either 20:1n7 or 22:1n7. Higher proportions of these fatty acids would be expected if males were feeding more heavily at the benthos than females, however decapods may not be predominant in the environment (Graeve et al. 1994). Male narwhal are known to swim in a supine position, which may make benthic feeding more efficient (Dietz et al. 2007). They are also larger, and therefore have the ability to dive deeper than females (Watt et al. 2013). However there is conflicting evidence supporting increased diving depths in male narwhals (Heide-Jørgensen and Dietz 1995; Laidre et al. 2003).

Carbon isotope enrichment may also be the result of higher levels of marine prey species abundance in the diet relative to freshwater prey species (MacAvoy et al. 1998). Female narwhals and calves have been reported more frequently in sheltered waters within bays and fjords (Born et al. 1994; Kingsley et al. 1994). If narwhal females are selecting protected near shore areas with higher freshwater input relative to open ocean habitats, their tissues may be carbon depleted relative to males that were feeding offshore. This behaviour may be an adaptation to evade predators such as killer whales (Sergeant and Brodie 1969; Shelden et al. 2003; Smith 2007). Feeding near sea ice may also provide some protection from killer whales, as killer whales typically avoid ice-heavy areas (Higdon and Ferguson 2009). Female narwhal had significantly higher levels of the fatty acids 16:4n1, and 20.5n3; both are fatty acids associated with sea-ice algal diatoms (Scott et al. 1999). Immature narwhal also had significantly higher levels of these fatty acids compared to males, as well as higher levels of 18.4n3, a fatty acid associated with open-water pelagic feeding in a food web that includes flagellates (Scott et al. 1999). All of these fatty acids are indicative of offshore pelagic feeding. The majority of examined fatty acids were not significantly different between groups, however, so there may be substantial overlap between male, female, and immature whale feeding regimes.

There were no significant differences found between sex in $\delta^{15}N$ in either muscle or liver in narwhal, but there was a significant difference between $\delta^{15}N$ enrichment between mature males and immature whales, which may be the result of larger bodied whales consuming larger prey items (Laidre et al. 2003; Watt et al. 2013). These dietary biomarkers indicate that male and female narwhal may be feeding at similar trophic levels. These findings support those by Finley and Gibb Finley and Gibb (1982), who found similar prey species in the stomachs of both male and female narwhal, and (Watt et al. (2013), who found similar feeding between males and females, as well as an increased $\delta^{13}C$ value for male narwhal. The increase in $\delta^{13}C$ indicates that mature male narwhal may be feeding in a different location than all other reproductive classes, but not on different prey items or at different trophic levels. Further studies are needed to elucidate the potential effect of isotopic fractionation and routing between sexes (Gannes et al. 1998; Gannes et al. 1997), to determine if the intersexual difference in $\delta^{13}C$ is the result of different feeding habitat selection or a difference in physiology.

Mature male belugas harvested in Arviat had significantly higher ratios of $\delta^{15}N$ isotopes relative to mature females in liver tissue only. The $\delta^{15}N$ enriched liver tissue may be the result of a recent shift to a higher trophic level, as liver tissue has a higher turnover rate than muscle tissue (Kurle and Worthy 2002). It may also be an artifact of the interannual variation in liver $\delta^{15}N$ relative to muscle. In 2005, belugas harvested in Arviat had significantly $\delta^{15}N$ enriched liver tissue relative to muscle; and there were only 2 mature females harvested in that year, compared to 10 mature males. Males were significantly enriched in $\delta^{15}N$ in muscle tissue in belugas harvested in Sanikiluaq as well, but nitrogen isotope levels were significantly different between muscle and liver tissue in all whales harvested in Sanikiluaq (Chapter 4).

Belugas would not be expected to exhibit sexual segregation inside the mating season, as males and females need to stay around each other to optimize their mating success (Ruckstuhl and Clutton-Brock 2005). There were no significant differences in either $\delta^{13}C$ in muscle or liver tissues or fatty acids between sexes or among reproductive classes for belugas harvested at either site. This indicates that belugas in the Western Hudson Bay population do not appear to exhibit sexual segregation outside the mating season, unlike belugas in the Beaufort Sea (Loseto et al. 2006; Richard et al. 2001), or the Eastern Hudson Bay population of belugas (Smith et al. 1994). Belugas in the Beaufort Sea have access to a different variety of feeding locations than do belugas from Hudson Bay. The Beaufort Sea retains some sea ice cover throughout the summer (Loseto et al. 2006), while sea ice is completely melted in Hudson Bay by late July (Stewart and Barber 2010). Belugas in the Beaufort Sea feed in nearshore, offshore, and sea-ice habitats (Loseto et al. 2009). With the absence of sea ice, belugas in Hudson Bay do not have the same potential variety in feeding areas. In western Hudson Bay, the most energetically productive area in the summer may be the estuary plume (Chapter 4). If all belugas feed in estuary plumes, they would be expected to show similar dietary biomarkers. There have been no diet studies on the Eastern Hudson Bay beluga population, and segregation within this population has only been found using visual observations (Caron and Smith 1990) which may be biased towards immediately recognizable individuals, so it is unclear as to whether this population exhibits sexually segregated feeding. Colbeck et al. (2012) found Eastern and Western beluga populations in Hudson Bay travelled in related, mixed-sex groups.

Lack of significant differences in feeding behaviour between male and females belugas does not remove the potential for sexual segregation in the Western Hudson Bay population, however, especially as it has been observed in other populations. The scramble competition hypothesis predicts that sexual segregation will only occur if food availability is low (Ruckstuhl and Neuhaus 2000). Belugas preferentially feed on prey items that are high in energy or occur in high densities (Bluhm and Gradinger 2008). If Western Hudson Bay belugas are feeding at highly productive estuary plumes (Chapter 4), they may be feeding in areas nutritionally dense enough to not necessitate sexual segregation; whereas the Mackenzie River estuary (Beaufort Sea) mouth is less productive than the offshore areas during the summer (Parsons et al. 1988) requiring belugas to segregate. Male belugas in the Beaufort Sea feed in the more productive areas offshore, however, which is the opposite of what is predicted by the scramble competition hypothesis.

Males would be predicted to feed in higher quality forage areas, however, under the predation risk hypothesis (Ruckstuhl and Neuhaus 2000). One explanation for beluga use of estuaries and near shore areas is these areas provide protection from predators such as killer whales (Brodie 1971; Hobbs et al. 2005; Richard 2005; Smith 2007). However, killer whales were uncommon predators of marine mammals in Hudson Bay or the Canadian Arctic until the mid-1900s (Ferguson et al. 2010; Higdon and Ferguson 2009), and Inuit traditional knowledge indicates belugas have frequented estuaries before the 1900s (Huntington 2000); suggesting estuary use may have a species-level adaptive function that is not related to specific predation pressues.

Estuary use also puts belugas at risk of predation by land-based predators such as humans (Finley et al. 1982). The predation risk hypothesis predicts that females will frequent areas that provide protection, while males will select feeding areas with the best quality prey. While this may seem true for belugas in the Beaufort Sea population, male belugas in Hudson Bay show no sign of feeding on higher quality food, as their fatty acid profiles indicate they are feeding on the same prey species as females. Fatty acid profiles of male and female narwhals also indicated that they are consuming the same prey items, so the predation risk hypothesis does not apply to either belugas or narwhal.

When segregation is driven by body size and one sex is larger than the other, sexual segregation will occur even if there are no underlying reproductive strategies driving the segregation (Croft et al. 2006). Larger males may need to feed more often or on more nutritionally dense prey in order to maintain body mass (Boyd et al. 1997). The activity budget/forage selection hypothesis predicts that body size and reproductive status dictates energy requirements (Ruckstuhl and Neuhaus 2000). If male narwhals are feeding benthically, they may be acquiring more prey than pelagically feeding females. There was a qualitative association between a group of male narwhals and the 20 and 22 carbon length monounsaturates, fatty acids associated with feeding on Arctic cod (*Boreogadus saida*) at sea-ice edges (Kelley et al. 2010; Loseto et al. 2009). Belugas in the Beaufort Sea fed preferentially at the sea-ice edge (Loseto et al. 2006; Loseto et al. 2009); it may be that it is a highly productive area large males require for maintaining body mass, and for growing and maintaining a tusk as a secondary sexual characteristic. However, there were an equal number of narwhal associated with the 16-and 18- carbon polyunsaturates; fatty acids associated with pelagic herbivorous zooplankton (Scott et al. 1999).

There was no evidence of dietary sexual segregation in beluga in Western Hudson Bay collected in Arviat. There was some evidence of sexual segregation in Eclipse Sound narwhal collected in Pond Inlet, however, none of the hypotheses proposed to predict the conditions underlying sexual segregation appear to fit narwhal ecology. (Ruckstuhl and Neuhaus 2002) developed a model in which sex differences in activity budget explain sexual segregation, with the range of spatial segregation explained by additional factors such as differences in predation risk. There is currently little evidence to suggest that differences in body size between sexes prevents activity synchronization (Michaud 2005), especially considering that both narwhals and belugas, as well as many other odontocetes, hunt socially (Connor 2000).

Subjects		Pond Inlet Narwhal	Arviat Belugas	Sanikiluaq Belugas
Immature Males	Counts	6	8	2
	Expected Counts	4.07	6.64	5.29
	Row %	37.5	50.0	12.5
	Column	20.0	16.33	5.13
	Total	5.09	6.78	1.70
Immature Females	Counts	3	5	5
	Expected Counts	3.305	5.40	4.30
	Row %	23.08	38.46	38.46
	Column	10.0	10.20	12.82
	Total	2.54	4.24	4.24
Mature Males	Counts	12	23	22
	Expected Counts	14.49	23.67	18.84
	Row %	21.05	40.35	38.60
	Column	40	46.94	56.41
	Total	10.17	19.49	18.64
Mature Females	Counts	9	13	10
	Expected Counts	8.14	13.29	10.58
	Row %	28.13	40.63	31.25
	Column	30.0	26.53	25.64
	Total	7.63	11.02	8.48

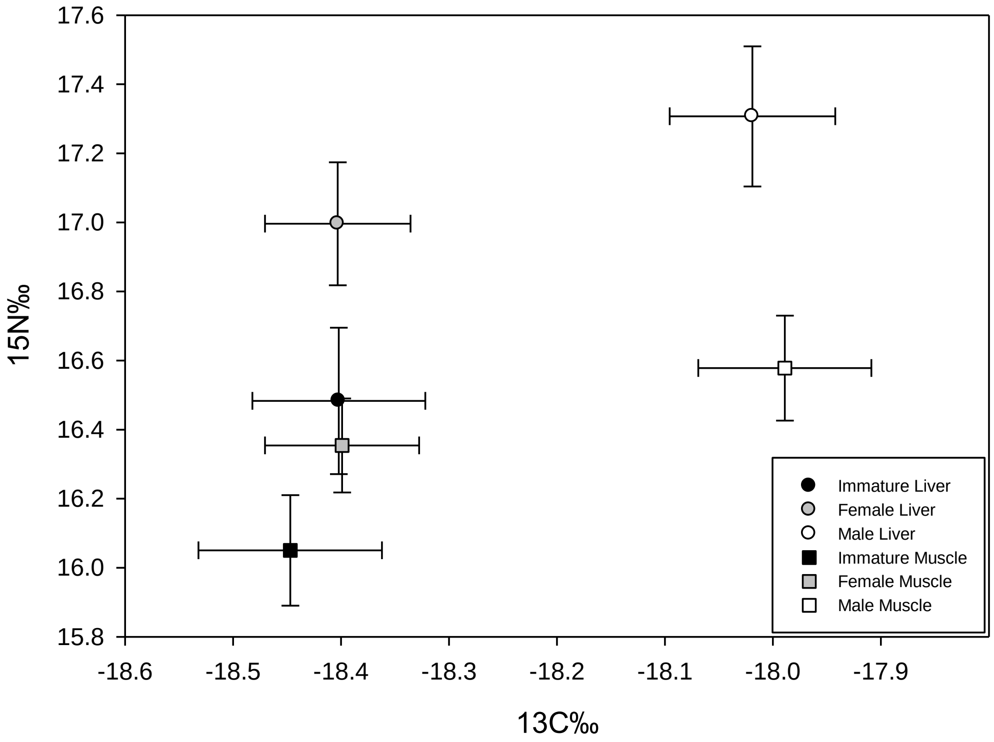
Table 5.1 Chi-square table showing the proportion of immature and mature male and female narwhal harvested in Pond Inlet, and beluga harvested in Arviat and Sanikiluaq. The proportions were not significantly different between groups of whales.

Pond Inlet Narwhal

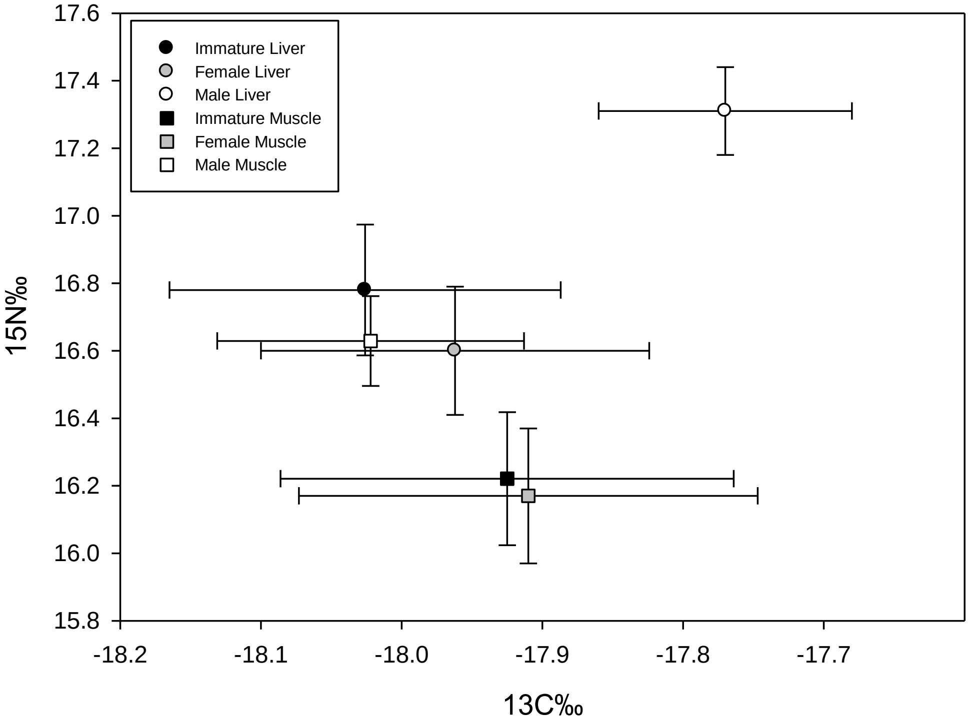
	Immature	Females	Males	Significant Differences
Stable Isotopes				
Muscle Carbon	-18.45‰±0.08	-18.40‰±0.07	-18.45‰±0.08	m vs. i: p<0.001 m vs. f: p=0.001
Muscle Nitrogen	16.05‰±0.16	16.35‰±0.14	16.58‰±0.15	none
Liver Carbon	-18.40‰±0.08	-18.40‰±0.07	-18.02‰±0.08	m vs. i: p=0.003 m vs. f: p=0.001
Liver Nitrogen	16.48‰±0.21	16.97‰±0.18	17.31‰±0.28	m vs. i: p=0.01
Fatty Acids				
16.4n1	0.16±0.04	0.16±.0.5	0.09±0.04	m vs. i: p<0.001 m vs. f: p=0.001
18.4n3	0.04±0.12	0.35±0.50	0.27±0.09	m vs. i:p<0.001 m vs. f: p=0.05
20.5n3	6.24±1.05	5.10±1.60	3.60±1.56	m vs. i: p<0.001 m vs. f: p=0.02
Arviat Beluga				
Stable Isotopes				
Muscle Carbon	-17.93‰±0.16	-17.9‰1±0.16	-18.82‰±0.11	none
Muscle Nitrogen	16.22‰±0.20	16.17‰±0.20	16.63‰±0.13	none
Liver Carbon	-18.03‰±0.14	-17.96‰±0.14	-17.77‰±0.90	none
Liver Nitrogen	16.78v±0.19	16.60‰±0.19	17.31‰±0.13	m vs. i: p =0.025 m vs. f: p=0.004
Sanikiluaq Beluga				
Stable Isotopes				
Muscle Carbon	-18.65‰±0.08	-19.24‰±0.68	-18.49‰±0.87	none
Muscle Nitrogen	15.79‰±0.44	15.32‰±0.58	15.95‰±0.43	m vs. f: p=0.005
Liver Carbon	-18.38‰±0.63	-18.60‰±0.37	-18.19‰±0.60	none
Liver Nitrogen	16.04‰±0.59	16.10‰±0.45	16.63‰±0.40	m vs. i: p=0.005 m vs f: p=0.004

Table 5.2 Summary of stable isotopes for all tissues examined in whales from all sites, and fatty acids that differed significantly within narwhal. None of the examined fatty acids were significantly different within belugas sampled from either site, and are not listed.

a)


17.6
17.4
17.2
17.0
16.8
16.6
16.4
16.2
16.0
15.8
15N‰
-18.6
-18.5
-18.4
-18.3
-18.2
-18.1
-18.0
-17.9
13C‰
● Immature Liver
● Female Liver
○ Male Liver
■ Immature Muscle
▨ Female Muscle
□ Male Muscle

b)


17.6
17.4
17.2
17.0
16.8
16.6
16.4
16.2
16.0
15.8
15N‰
-18.2
-18.1
-18.0
-17.9
-17.8
-17.7
13C‰
● Immature Liver
● Female Liver
○ Male Liver
■ Immature Muscle
▨ Female Muscle
□ Male Muscle

c)

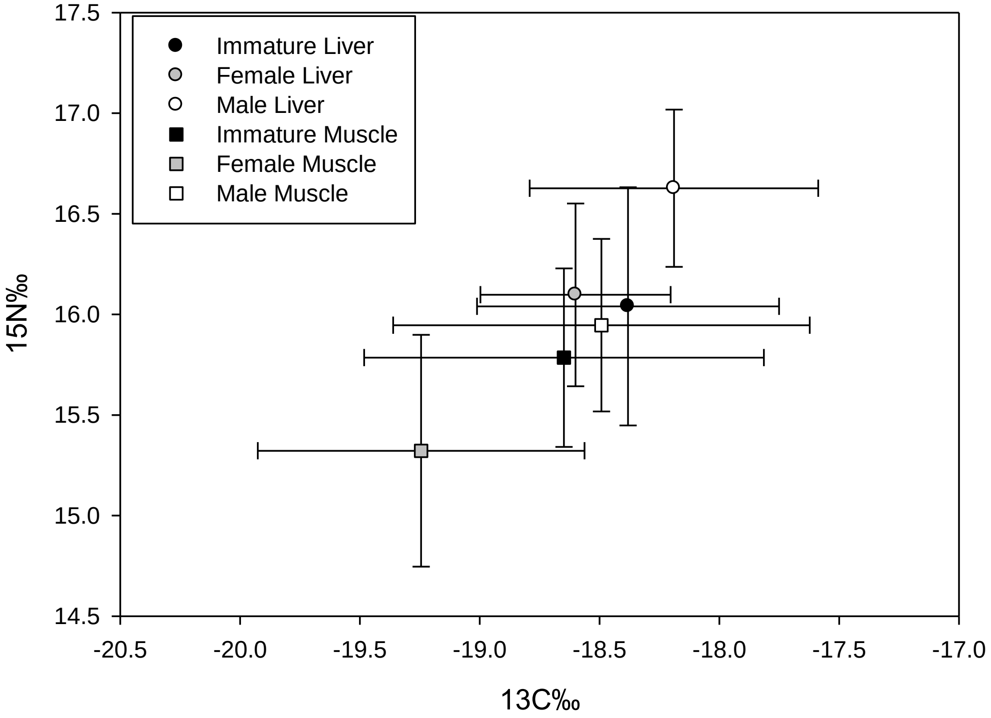
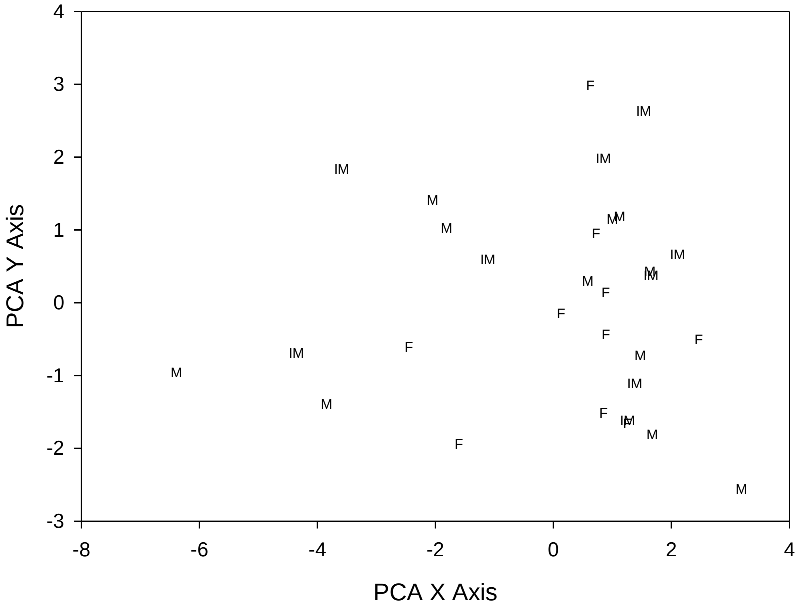


Figure 5.1 Distribution of mean δ^{13}C (X-axis) and δ^{15}N(Y-axis) in muscle and liver tissues in all reproductive classes of a) narwhal harvested in Pond Inlet, b) belugas harvested in Arviat, and c) belugas harvested in Sanikiluaq. Circles represent liver tissues, and squares represent muscle tissues. Immature whales are denoted by solid black shapes, females denoted in grey, and males denoted in white. Error bars represent ± 1 standard error.

a)



b)

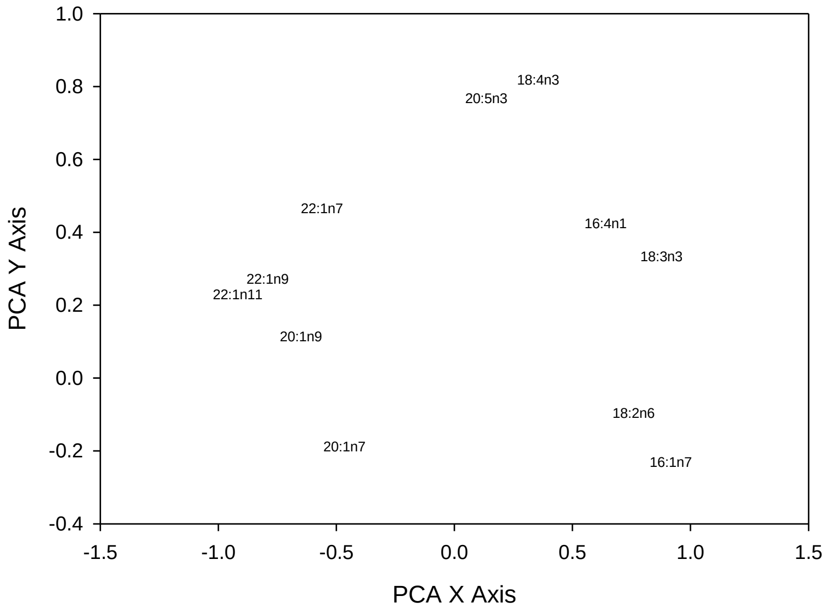


Figure 5.2) Principle Compnent Analysis plot of a) male (M), female (F) and immature (I) Pond Inlet narwhals and b)the fatty acids that drive the placement of the narwhal samples. Mature females tend to cluster on the positive side of the X axis and the negative side of the Y axis, close to the fatty acids 18.2n6 and 16.1n7. The negative side of the x-axis is dominated by the 20-and 22- carbon chain length monounsaturated fatty acids.

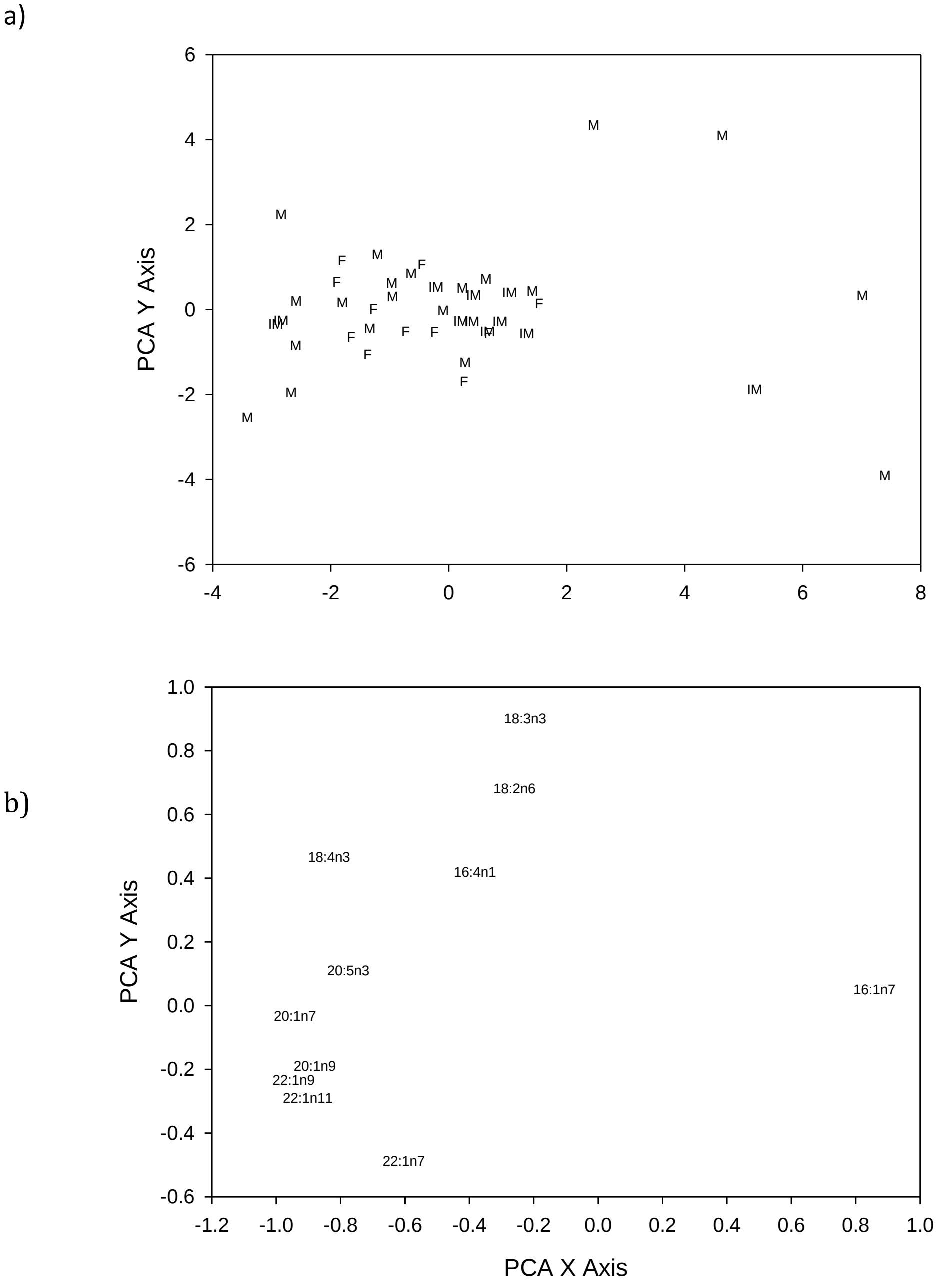
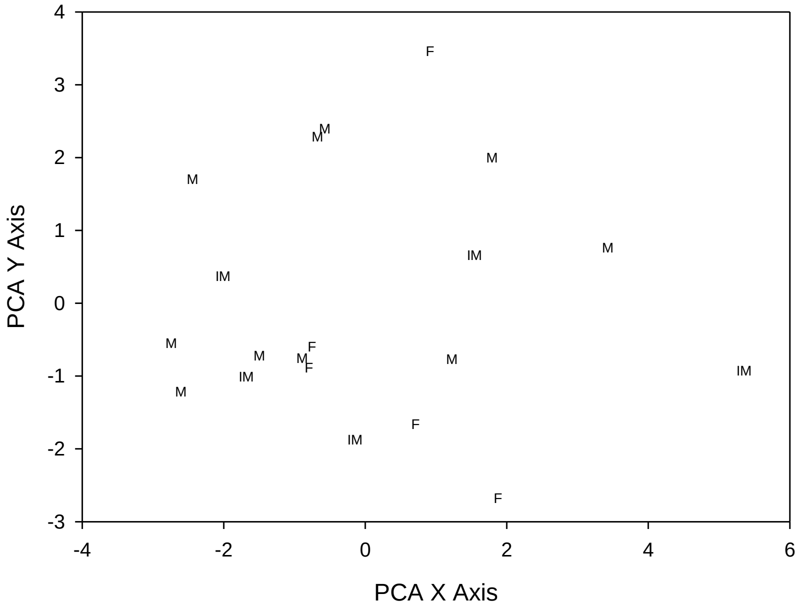


Figure 5.3 Principle Compnenet Analysis coordinates of a) male (M), female (F) and immature (I) belugas harvested in Arviat and b) the fatty acids that drive the distribution of beluga samples. The fatty acid 16:1n7 dominated the positive side of the X axis and was associated with 4 males. There is no other clear separation between reproductive classes.

a)



b)

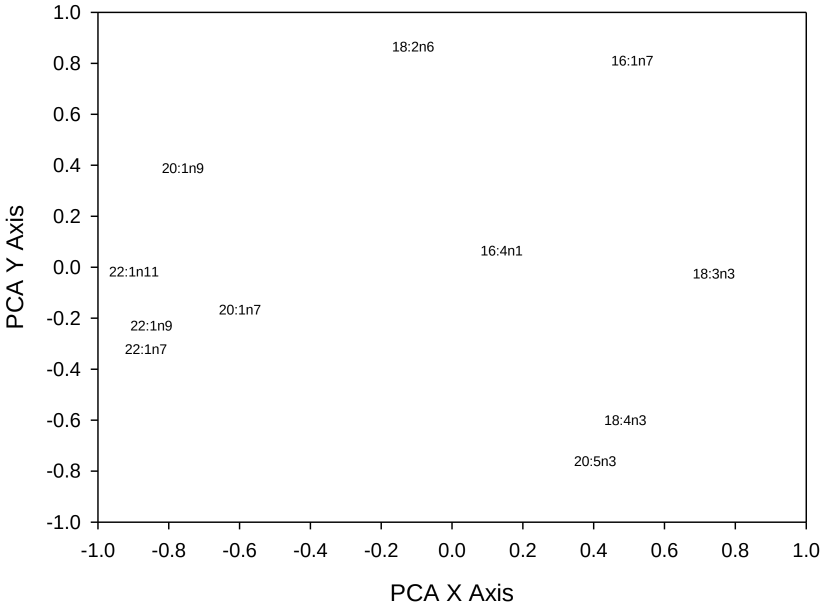


Figure 5.4 Principle Component Analysis plots of a) male (M), female (F), and immature (I) belugas harvested in Sanikiluaq and b) the fatty acids contributing to the segregation. No clear separation between reproductive classes based on fatty acids was evident. The negative side of the PCA is dominated by the 22- and 20- carbon chain length

monounsaturates, while the positive side is dominated by the 16-and 18-carbon length polyunsaturates.

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6 Conclusion

6.1 Linking Mating System and Feeding Ecology

Information on mating systems in marine species such as belugas and narwhals is difficult to obtain due to the logistics of observing the behaviour of underwater mammals with large home ranges, such as long periods of darkness in arctic winters, individuals travelling far offshore, and habitats covered in sea ice. Relative testes size and sexual size dimorphism (SSD) have been linked to mating systems in terrestrial mammals (Dixson 1998; Harcourt et al. 1995; Heske and Ostfeld 1990; Møller 1989; Rose et al. 1997; Zenuto 1999), and can be used to infer mating systems in species for which detailed behavioural observations are not possible (Connor et al. 2000; Kenagy and Trombulak 1986).

Though numerous studies have examined the relationship between (SSD), testes mass, and mating system, no study has focused exclusively on odontocetes; this is likely a result of a paucity of data. Chapter 2 examined the relationship between SSD, relative testes mass, and relative brain mass in 40 species of odontocetes and found a significant, negative relationship between SSD and testes mass. The results of Chapter 2 provided the basis for using relative testes mass to estimate beluga and narwhal mating systems.

Reproductive tracts collected from belugas and narwhal harvested over 20 years across the Canadian Arctic were used to estimate mating systems in both species. Chapter 3 is the first study to compare reproductive tract differences between belugas and narwhals, as well as among populations within each species. The results of this chapter indicate that belugas have larger testes masses relative to their body mass compared to narwhal, a characteristic indicative of a more polygynandrous mating system featuring post-copulatory sperm competition. Belugas were also less sexually dimorphic in size than narwhals, lending further support to a greater degree of sperm competition. Narwhal are known to exhibit SSD, as well as dimorphism in weaponry. Narwhal tusk length was correlated with testicle size; longer tusks were found on males with larger testes (Chapter 3). This suggests that the tusk may be an indicator of male quality and fertility (Johnson et al. 1993; Vanpé et al. 2010; Vanpé et al. 2007). Male narwhals have been observed both fencing with their tusks and with injuries from the tusks of other narwhals (Best 1981; Silverman and Dunbar 1980). If competition for mates in narwhal is associated with use of the tusk, narwhals are more likely have a polygynous mating system.

A mating system predominated by sperm competition may exhibit a lack of segregation between sexes outside the breeding season. Sexual segregation is most pronounced in species with significant levels of SSD (Mysterud 2000), and SSD is negatively correlated with testes size (indicative of sperm competition) in mammals (Kenagy and Trombulak 1986), including odontocetes (Chapter 2). There was no evidence of segregation in belugas either inside or outside the breeding season in fatty acid or $\delta^{13}C$ biomarkers. Belugas appeared to be eating similar prey items in similar locations, though males may have been consuming larger prey items from a higher trophic level than females or immature whales. Mate guarding is an important male strategy in polygynandrous mating systems, as it allows males to exclude other males from mating (Gomendio et al. 1998; Gomendio and

Roldan 1993; Preston et al. 2003). This behaviour may continue outside the mating season. Further, if males are not physically competing for females (an energetically costly activity), they may not have significantly different energy requirements and individuals of both sex can occupy the same territory (Ruckstuhl and Neuhaus 2000). Conversely, males in possession of a 'harem' in a polygynous mating system may be energetically disadvantaged as they must both fight off rival males, and restrict their feeding to be in close proximity to the females in their harem (Ruckstuhl and Clutton-Brock 2005; Ruckstuhl and Neuhaus 2000). The potential for narwhal to hold harems by fighting off other males has been reported (Nowak 1991), and results from Chapter 3 suggest a polygynous mating system.

Dominant males in a hierarchical polygynous mating system may be energetically disadvantaged as they must both fight off rival males, and restrict their feeding to be in close proximity to the females (Ruckstuhl and Neuhaus 2000). The potential exists for the narwhal tusk to be a non-violent indicator of status for males, or as a weapon (Nowak 1991), and results from Chapter 3 suggest a polygynous mating system. Chapter 5 provides some evidence of sexual segregation in narwhals in both prey items (fatty acids) and feeding location ($\delta^{13}C$), and narwhal exhibit more sexual segregation in feeding than do belugas in Hudson Bay.

The Arctic marine ecosystem is characterized by sea ice, which influences primary productivity via ice-associated diatoms (Horner et al. 1992). The presence of sea ice in an area provides a feeding area that is distinct and recognizable in dietary biomarkers such as fatty acid and stable isotopes, which have been shown to be useful in distinguishing whales feeding at ice floe edges and whales feeding near shore and in the benthos (Loseto et al.

2009; Loseto et al. 2008). Marine mammals in the High Arctic, such as narwhals harvested in Pond Inlet, have access to sea ice habitats throughout the year, while marine mammals in Hudson Bay forage in an ice-free habitat during the summer (Gagnon and Gough 2005; Stewart and Barber 2010).

Belugas in Hudson Bay do not have access to sea ice during the summer, and may be feeding pelagically in the estuary plume (Chapter 4). The resource-rich estuary plumes may be considered a resource dense area, and feeding in these regions may partially explain beluga site philopatry (Caron and Smith 1990).

The extent to which summer feeding is an important contribution to annual energy budgets in beluga and narwhal is unclear. Rorquals (Family: Balaenopteridae) fast annually, and their blubber is composed of higher levels of triacylglycerols relative to toothed whales (Lockyer 1991). Higher levels of triacylglycerols are also found in seasonally fasting phociid species that mobilise blubber reserves for energy (Bradshaw et al. 2003). Monodontid blubber is composed entirely of triacylglycerols (Koopman 2006), which may have arisen as an adaptation to the requirement to utilise stored energy. Belugas did not show depletion in $\delta^{13}C$ expected in fasting whales, however, so they may be feeding throughout the year. Neonates are reported in the herd composition of summering narwhal (Born et al. 1994), and belugas (Caron and Smith 1990). Lactation is an energetically demanding activity, and whales may require a substantial amount of energy during the summer. The seasonality exhibited in both beluga and narwhal migrations likely limit the length of the mating season (Van Hooff and van Schaik 1992; Whitehead and Mann 2000).

6.2 Conservation Implications

Belugas and narwhals are both at risk of habitat loss via decrease in sea ice caused by climate change (Laidre et al. 2008). Hudson Bay beluga stocks rely on sea ice throughout the winter for protection from predators (Heide-Jørgensen and Laidre 2004), as well as for feeding (Chapter 4). Narwhal are associated with sea ice throughout the year, and it may be an important feeding area in the winter (Laidre et al. 2003; Laidre et al. 2004; Laidre and Heide-Jørgensen 2005). Ice floe edges may also be an important feeding area for immature and female narwhals during the summer (Chapter 5), as they provide ice-algal diatom-based food webs (Scott et al. 1999). A reduction in the amount of sea ice present in narwhal summering grounds may negatively impact females and immature whale summer energy acquisition. This may reduce female ability to reproduce, and reduce juvenile survival.

Though belugas in Hudson Bay experience a sea-ice free habitat during the summer, sea ice is still an important regulator of the Hudson Bay ecosystem. As sea ice melts, it exposes the water column to sunlight which provides the conditions necessary for an increase in primary productivity (Bluhm and Gradinger 2008). The energy contained in the resulting phytoplankton bloom is transferred to higher trophic levels via grazing zooplankton (Bluhm and Gradinger 2008; Pershing et al. 2004). An alteration in the timing of solar energy may encourage a premature phytoplankton bloom that cannot be consumed by zooplankton that ascend the water column at specific times of the year to feed (Hansen et al. 2002; Hunt Jr et al. 2002; Winder and Schindler 2004). This may reduce the productivity of the estuary plume as well. Nutrient density in estuary plumes is in part the result of the upwelling of sunken detritus that is then fed on by zooplankton (Connolly et al. 2009). If estuary plumes are important summer feeding grounds for belugas as the results from Chapter 4 suggest, alterations in the trophic coupling of primary production and zooplankton may reduce the density of prey species available and therefore reduce the energy available to belugas.

Anthropogenic effects resulting from decreased sea ice cover include increased shipping and the potential for increased off-shore oil and gas exploration (Huebert 2011). Offshore oil and gas development can encroach on marine mammal territories, and the sound produced by drilling rigs may further disturb marine mammal populations (Hovelsrud et al. 2008). Habitat fragmentation and acoustic disturbances may reduce population density (and therefore potential mates), skew sex ratios, increase genetic drift, and reduce synchronicity in reproductive activated in populations (Banks et al. 2007). If narwhals have a polygynous mating system as suggested in Chapter 3, they may be more robust to the impacts of reduced population density and skewed sex ratios, as polygynous species do not exhibit the same inbreeding avoidance responses (refusal to mate) as monogamous species do (Berry 2006; Bessa-Gomes et al. 2003). If belugas have a more polygynandrous mating system, they may be robust to decreased population density, as population bottlenecks may increase female promiscuity (Michalczyk et al. 2011). Reduced synchronicity in reproductive activities may negatively impact belugas, however, as they undergo testicular regression, a marker of reproductive seasonality (Chapter 3).

Hunting pressures on narwhal are biased towards males (Petersen et al. 2012), as there is an economic benefit to harvesting tusked whales (Hoover et al. 2013). Hunting pressures on narwhal are likely to increase in the future (Petersen et al. 2012), which may lead to a population decline if the narwhal tusk is an honest advertisement of male fitness as suggested in Chapter 3. Selective harvesting of male narwhals, particularly males with large tusks, may contribute to the reduction of large tusks as these males are effectively selected out of the breeding population. Reduction in horn size has been observed in bighorn sheep (*Ovis canadensis*) where larger 'trophy rams' are preferentially hunted (Coltman et al. 2003), as well as in African elephants (*Loxodontia africana*) (Dobson 1998). If females show strong preferences for physical traits, conception rates may be limited (Côté 2003; Dobson 1998). Further, the results from Chapter 3 did not exclude the possibility of sperm competition in narwhals. Narwhals may be described as a 'weakly' polygynous species, (as compared to species in which one dominant males mates with many females) as complete isolation of females during the breeding season is likely impossible when whales can move in a 3-dimensional environment. Weakly polygynous species are more heavily affected than strongly polygynous species are more susceptible to the culling of males (Côté 2003).

Conservation plans for narwhals and belugas may be different. Belugas in Hudson Bay appear to be polygynandrous and exhibit low sexual segregation outside the mating season. Narwhals are potentially more polygynous, and exhibit sexual segregation outside the mating system, as inferred by dietary biomarkers. Critical habitats for beluga may be easier to establish, as all members of the Hudson Bay population seem to be utilising habitats in the same way. Narwhal conservation programs would need to protect both benthic habitats and areas with sea ice in order to protect feeding habitat of all whales within a population.

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7 Appendix

Species	Combined Testes Mass (g)	Sample Size	Epididymides	Reference	Male Mass (g)	Sample Size	Reference	Female Mass (g)	Sample Size	Reference	Brain Mass (g)	Reference
Kogia breviceps	2960	n=1	not stated, assumed included	(Harms et al. 2004)	40800	-	(Caldwell and Caldwell 1989)	40800	-	(Caldwell and Caldwell 1989)	1012	(Marino et al. 2006)
Kogia sima	4700	n=1	not included, added 17.5%	(Harrison et al. 1972)	27200	-	(Caldwell and Caldwell 1989)	27200	-	(Caldwell and Caldwell 1989)	622	(Marino et al. 2006)
Physeter macrocephalus	12000	averaged across populations	not stated, assumed included	(Rice 1989)	26939000	-	(Trites and Pauly 1998)	10098000	-	(Trites and Pauly 1998)	8028	(Marino et al. 2006)
Platanista gangetica	278.4	n=1	not stated, assumed included	(Harrison 1972)	90000*	n=16	(Harrison 1972; Perrin et al. 2005)	8500*	n=16	(Harrison 1972; Perrin et al. 2005)	295	(Marino et al. 2006)
Ziphius cavirostris	7900	n=2	not stated, assumed included	(Omura et al. 1955)	2250000	n=3	(Heyning 1989)	2030000	n=3	(Heyning 1989)	2004	(Marino et al. 2006)
Inia geoffrensis	914.29	n=7	not stated, assumed included	(Best and da Silva 1984)	125950	n=6	(Best and da Silva 1984)	79350	n=4	(Best and da Silva 1984)	6627.8	(Marino et al. 2004)
Lipotes vexillifer	36.33	n=3	not stated, assumed included	(Chen et al. 1984)	87500	n=3	(Chen 1989)	153900	n=5	(Chen 1989)	510	(Marino et al. 2006)
Phocaena sinus	934.44	n=6	not included, added 17.5%	(Vidal et al. 1999)	42500	n=1	(Vidal et al. 1999)	44000	n=1	(Vidal et al. 1999)	1999	(Marino et al. 2006)
Phocaena spinipinnis	483.6	n=9	included	(Reyes and Van Waerebeek 1995)	61333.33	n=10	(Reyes and Van Waerebeek 1995)	63818.2	n=11	(Reyes and Van Waerebeek 1995)	597	(Marino et al. 2006)
Phocaena phocaena	1971	n=26	included	(Fisher and Harrison 1970)	42000	n=26	(Fisher and Harrison 1970)	52500	n=2	(Fisher and Harrison 1970)	540	(Marino et al. 2006)

Phocaenoides dalli	244.45	n=29	not stated, assumed included	(Subramanian and Tatsukawa. 1987)	140931	n=29	(Subramanian and Tatsukawa 1987)	128625	n=8	(Subramanian and Tatsukawa 1987)	866	(Marino et al. 2006)
Neophaecana phocaenoides	1094.39	n=14	not included, added 17.5%	(Shirakihara et al. 1993)	50835.71	n=10	(Shirakihara et al. 1993)	44675	n=14	(Shirakihara et al. 1993)	466.3	(Kasuya 1999)
Delphinapterus leucas	664.89	n=187	included	Chapter 3	666770*	n=163	Chapter 3, (Doidge 1990; Stewart 1994)	543140*	n=92	Chapter 3, (Doidge 1990; Stewart 1994)	2083	(Marino et al. 2006)
Monodon monoceros	814.91	n=113	included	Chapter 3	12418000*	n=113	Chapter 3 (Hay and Mansfield 1989)	795498.67*	n=12	Chapter 3, (Hay and Mansfield 1989)	2997	(Marino et al. 2006)
Cephalorhynchus commersonii	1220	n=1	included	(Collet and Robineau 1988)	78000	n=1	(Collet and Robineau 1988)	76250	n=5	(Collet and Robineau 1988)	-	-
Cephalorhynchus hectori	709.5	n=8	included	(Slooten and Lad 1991)	36850	n=6	(Slooten and Lad 1991)	44360	n=5	(Slooten and Lad 1991)	644	(Slooten and Dawson 1994)
Cephalorhynchus heavisidii	312.78	n=5	not stated, assumed included	(Best and Abernathy 1994)	68950	n=1	(Best and Abernathy 1994)	64400	n=1	(Best and Abernathy 1994)	724	(Best and Abernathy 1994)
Lagenorhynchus cruciger	4700	n=1	not included, added 17.5%	(Brownell Jr and Donahue 1999)	94000	n=1	(Brownell Jr and Donahue 1999)	88200	n=1	(Brownell Jr and Donahue 1999)	1129.2	(Goodall et al. 1997)
Lagenorhynchus obliquidens	1300.73	n=1	not included, added 17.5%	(Harrison et al. 1972)	79250	n=4	(Harrison et al. 1972)	74875	n=8	(Harrison et al. 1972)	1148	(Marino et al. 2006)
Lagenorhynchus obscurus	2950	n=125	included	(van Waerebeek and Read 1994)	75000	n=500	(van Waerebeek and Read 1994)	72100	n=522	(van Waerebeek and Read 1994)	-	-
Lissodelphis borealis	1300	n=23	not stated, assumed included	(Ferrero and Walker 1993)	141000	-	(Trites and Pauly 1998)	68300	-	(Trites and Pauly 1998)		
Delphinus delphis	2004.67	n=11	not included, added 17.5%	(Harrison et al. 1972)	80250	n=2	(Harrison et al. 1972)	57800	n=10	(Harrison et al. 1972)	815	(Marino et al. 2006)
Stenella coeruleoalba	333.7	n=33	not included, added 17.5%	(Hirose and Nishiwaki 1971)	140830*	n=16	(Miyazaki 1977; Perrin et al.	131630*	n=29	(Miyazaki 1977; Perrin et al.	938.5*	(Marino et al. 2004)

							1994b)			1994b)		
Stenella frontalis	1210	n=4	not stated, assumed included	(Perrin et al. 1994a)	100250	n=7	(Perrin et al. 1994a)	114520	n=4	(Perrin et al. 1994a)	726	(Perrin 2002)
Tursiops truncatus	1323.05	n=1	not included, added 17.5%	(Harrison et al. 1972)	20300		(Trites and Pauly 1998)	17200	-	(Trites and Pauly 1998)	1824.4	(Marino et al. 2006)
Stenella attenuata	996.8	n=181	included	(Hohn et al. 1985)	75140	n=1280	(Perrin and Hohn 1994)	59770	n=3890	(Perrin and Hohn 1994)	726	(Perrin 2001)
Sousa plumbea	650	n=1	not stated, assumed included	(Ross et al. 1994)	142247	n=29	(Ross et al. 1994)	123164	n=10	(Ross et al. 1994)	688	(Jefferson and Kaczmarski 2001; Miyazaki and Perrin 1994)
Lagenodelphis hosei	3826	n=1	included	(Amano et al. 1996)	95400	-	(Trites and Pauly 1998)	95400	-	(Trites and Pauly 1998)	-	-
Stenella longirostris	1393.55	n=7	not included, added 17.5%	(Mead et al. 1980)	65880	n=5	(Mead et al. 1980)	60810	n=6	(Mead et al. 1980)	660	(Marino et al. 2006)
Sotalia fluviatilis	1735.1	n=14	not stated, assumed included	(Da Silva and Best 1994)	42750	n=4	(Da Silva and Best 1994)	45000	n=5	(Da Silva and Best 1994)	688	(Marino et al. 2006)
Sotalia guanensis	2270	n=14	included	(Weber Rosas and Monteiro-Filho 2002)	90800	n=14	(Weber Rosas and Monteiro-Filho 2002)	83000	n=4	(Weber Rosas and Monteiro-Filho 2002)	-	-
Steno bredanensis	1667.22	n=9	not stated, assumed included	(West 2002)	128300	n=16	(West 2002)	112400	n=19	(West 2002)	1541.9	(Miyazaki and Perrin 1994)
Lagenorhynchus acutus	287.5	n=4	included	(Reeves et al. 1999)	110000	n=11	(Reeves et al. 1999)	78000	n=31	(Reeves et al. 1999)	1105	(Reeves et al. 1999)
Feresa attenuata	771.98	n=1	not included, added 17.5%	(Harrison et al. 1972)	122500	n=1	(Forrester et al. 1980)	113600	n=1	(Forrester et al. 1980)	1033	(Ross and Leatherwood 1994)
Globicephala macrorhynchus	7050	n=50	not included, added 17.5%	(Marsh and Kasuya 1984)	819000	-	(Trites and Pauly 1998)	467000	-	(Trites and Pauly 1998)	2890	(Marino et al. 2006)

Globicephala melas	8441.67	n=12	not stated, assumed included	(Sergeant 1962)	104000	n=236	(Bloch et al. 1993)	893000	n=370	(Bloch et al. 1993)	2861.7	(Marino et al. 2004)
Peponcephala electra	1779.5	n=2	not stated, assumed included	(Bryden et al. 1977)	100000	n=1	(Bryden et al. 1977)	89000	n=2	(Bryden et al. 1977)	-	-
Grampus griseus	3005	n=4	not stated, assumed included	(Chen et al. 2011)	296250	n=4	(Kruse et al. 1999)	262500	n=2	(Kruse et al. 1999)	2387	(Marino et al. 2006)
Pseudorca crassidens	8200	n=1	not stated, assumed included	(Odell and McClune 1999)	902000	n=1	(Odell and McClune 1999)	566000	n=2	(Odell and McClune 1999)	3512	(Lefebvre et al. 2006)
Orcinus orca	27730	n=1	not included, added 17.5%	(Harrison et al. 1972)	2587000		(Trites and Pauly 1998)	1974000		(Trites and Pauly 1998)	5059	(Marino et al. 2006)

Table 7.1Testes mass, male and female body mass, and brain mass of 40 odontocete species. Testes masses listed are the masses used in analyses, following the addition of the weight of the epidydimides where noted.* denotes body masses calculated from length weight equations.

7.1 References

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