INVESTIGATIONS OF THE ROLE OF LIPIDS IN MARINE MAMMAL DIETS, HEALTH AND ECOLOGY

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INVESTIGATIONS OF THE ROLE OF LIPIDS IN MARINE MAMMAL DIETS, HEALTH AND ECOLOGY

A

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ABSTRACT

Lipids are essential to many aspects of marine mammal biology. I investigated the amount, type and flux of lipids under a variety of natural and controlled nutritional and dietary conditions, in order to increase our knowledge of marine mammal diets, health and ecology. First, I examined the influence of biological and environmental variables on the quantity and quality of blubber, and their importance in establishing condition indices in the bowhead whale. Blubber was heterogeneous in composition, varying by both site and depth. Sex, age-class, season and body length were all significant factors in determining lipid content (quality) of blubber. Blubber thickness (quantity) was highly correlated with body length after ~9 m. Blubber lipid content at umbilicus sites and inner depths was most variable and presumably most responsive to nutritional changes. Blubber properties appeared to exceed what was necessary for insulation, further supporting the concept for the need to store energy as a consequence of the large seasonal and annual variability of food availability in the arctic environment. These data establish a baseline for long-term monitoring of bowhead whale health and population condition. Second, I addressed post-mortem changes in blubber composition of a stranded humpback whale. Lipid content decreased due to tissue decomposition by as much as 24%, limiting the ability to accurately assess nutritional status and health. Finally, in response to a growing need for validation of the use of fatty acid profiles as dietary tracers in top marine predators, I investigated the effects of prey switching on fatty acid profiles in plasma and red blood cell membranes (RBCs) of captive harbor seals. In plasma, nine of fifteen fatty acids responded significantly with prey switching, compared to only three plus one ratio in RBCs. Season and total daily lipid intake also affected the level of some plasma fatty acids. Diet was reliably predicted from fatty acid profiles in plasma after two weeks and in RBCs at four months using discriminant function analysis. Plasma and RBC fatty acid profiles provided an integration of dietary history, representing short-term and long-term "dietary windows," respectively.

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BD	– Blowhole dorsal
BL	– Blowhole lateral
BV	– Blowhole ventral
UD	– Umbilicus dorsal
UL	– Umbilicus lateral
UV	– Umbilicus ventral
BH	– Blowhole sites (BV, BL and BV)
UM	– Umbilicus sites (UD UL, UV)
TBL	– Total mean blubber lipid content
SBL	– Site mean blubber lipid content
FA	– Fatty acid
TAG	– Triglyceride
LPL	– Lipoprotein lipase
RBC	– Red blood cell
EFA	– Essential fatty acids
SAT	– Saturated fatty acids
MUFA	– Monounsaturated fatty acids
PUFA	– Polyunsaturated fatty acids

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1 Introduction

Lipids are essential to many aspects of marine mammal biology. Marine mammals utilize lipids in a suite of physiological and morphological adaptations to help solve some of the functional challenges associated with an aquatic lifestyle. For example, lipids in blubber help insulate from the cold ocean, as well as provide energy and metabolic water. Lipids in membranes may allow normal neural and cellular function at high pressure when a marine mammal dives to depth (Williams et al. 2001). Furthermore, life-history strategies of marine mammals are intimately linked with the management of lipids, for they can rapidly assimilate and deposit lipids in the blubber during periods of high prey availability when energy intake is in excess, and subsequently mobilize stored lipids for metabolism and/or reproduction during periods of fasting. This behavior of alternating between fattening and fasting is separated both spatially and temporally, allowing marine mammals to exploit the seasonal productivity of the oceans. Such behavior can be described biochemically through lipid analyses. Specifically, the amount, type and/or flux of lipid can be quantified in either the whole organism or in specific tissues such as blubber, milk or blood. For the researcher, lipids become tracers that can provide insight as to when, how much and where an animal is feeding. When pursued along another avenue, lipid studies can explain adaptations, describing the form and function of a tissue or an organ. Lipid measurements can therefore be a valuable tool to address a wide array of scientific questions including metabolic, nutritional, dietary, ecological, anatomical and management questions. In this thesis, I will utilize lipids as coarse and fine scale tracers to chemically describe or predict changes in marine mammal diets, health and ecology in absence of behavioral observation. To accomplish this goal, first, I will examine the influence of biological and environmental variables on the quantity and quality of blubber and their importance in establishing condition indices in the bowhead whale, *Balaena mysticetus*. Second, I will address the utility and difficulty of assessing the nutritional status and condition of whales based on the compositional analysis of blubber samples from stranded individuals. Lastly, I will examine how

specific lipids, i.e. plasma and red blood cell fatty acids, can be used as dietary indicators in a model marine carnivore, the harbor seal, *Phoca vitulina*.

Lipids as fuel

The advantages of lipid as a long-term source of stored energy are well documented (Hadley 1985). Firstly, lipids are a very compact energy source, yielding greater than two times the metabolizable energy of an equivalent mass of carbohydrate or protein. Secondly, lipid stores are less dense compared with other fuels, because they can be stored in a nearly anhydrous form. This feature provides an additional benefit to marine organisms because it can aid in buoyancy. Thirdly, when lipids are broken down for energy, metabolic water is created as a by-product. For an animal in a marine environment or for one that is fasting, metabolic water can be a significant source of fresh water. Finally, lipid droplets in adipocytes (fat cells) can also expand in volume by at least 10 fold, a volume that would be damaging in other types of cells and allows for the rapid growth of energy stores as fats are deposited. Marine mammals have capitalized on these benefits of lipids as fuel and developed a specialized form of superficial adipose tissue, known as blubber, where most lipids are stored. These lipids are predominantly in the form of triacylglycerols (TAG) in most marine mammals, but significant portions of wax esters have also been found in some toothed whales, i.e., odontocetes (Lockyer 1991). Previous studies have shown that blubber comprises 15-43% of body mass in cetaceans (reviewed by Lockyer 1991). Even larger proportions of blubber can be found in phocid seals, approaching or exceeding 50% of body mass in, for example, recently weaned northern elephant seal pups, *Mirounga angustirostris*, (Rea and Costa 1992). The ability to store energy as lipids in blubber for later use has allowed marine mammals to separate feeding events and energetic events such as reproduction, lactation, molting and migration. Adult southern elephant seals, *Mirounga leonina*, for example, fueled 95% of their ~36 day molting fast with energy derived from lipids that were mobilized primarily from the blubber (Slip et al. 1992).

Determination of blubber energy stores as a measure of body condition

Body condition in mammals is considered to be a general measure of an individual's relative energy stores, and hence, fitness. It can be represented by size (mass or length) to age ratios, mass of fat depots or by numerous other physiological indices (Hanks 1981). Determination of body condition is important for monitoring the health of animal populations, as individual fitness is correlated with reproductive success and survivability and thus ultimately the success of the population (Beck et al. 1993; Boyd 1984; Lockyer 1987; Lockyer 1990). In marine mammals, the lipid-rich blubber layer serves as the primary energy store, in some species representing 90% of the total body lipids. Therefore, the measurement of blubber energy stores is thought to be an accurate representation of overall body condition (Beck et al. 1993).

Body composition studies (percent body fat relative to body mass) of free-ranging pinnipeds, have increased dramatically since the early 1990's due to the development of non-lethal indirect methods such as isotopic dilution (Arnould 1995; Bowen and Iverson 1998; Reilly and Fedak 1990; Speakman 2001), biological impedance (Arnould 1995; Bowen et al. 1998; Gales et al. 1994) and morphometrics indices (Fadely 1996; Gales and Burton 1987; Ryg et al. 1990a; Trumble 2003). In contrast, body composition studies of most cetaceans have declined in recent times due to the lack of availability of fresh carcasses for direct methods and logistical constraints of applying indirect methods on free-ranging fully aquatic mammals. The exception to this has been in the case of strandings (Evans et al. 2003), incidental catch from fisheries (Koopman et al. 2002; Read 1990) and in the few species that continue to have either commercial or subsistence harvests (Ichii et al. 1998; Lockyer 1993; Næss et al. 1998). Because of these constraints, large gaps in our knowledge of current body condition and health exist for many cetacean species, increasing the difficulty of monitoring population health and dynamics.

Functional constraints and consequences of blubber properties

In marine mammals, the potential conflict between the role of blubber as both energy store and insulator has been recognized (Bryden 1968). In addition, other

functions of blubber have now been identified such as providing buoyancy, aiding in streamlining, increasing swimming efficiency and serving as a source of metabolic water (reviewed by Pabst et al. 1999). The differing roles of blubber have resulted in conflicting demands being placed on its composition, thickness and distribution. The resulting properties of blubber must ultimately arise from a balance of these functions. This balance is species specific and depends on life-history strategies, gender, age, size, current nutritional status, diet, availability of prey (seasonal or year-round), environmental temperatures and perhaps other influences that are currently unknown. In light of the multiplicity of function, care must be taken when examining blubber properties (i.e., thickness and lipid content) as indicators of energy stores and, hence, condition.

Evidence of regional specialization in the function of blubber has been found in several species. For example, the primary function of blubber in the throat grooves of balaenopterid whales and the tailstock of small odontocetes is considered to be structural or mechanical (Koopman et al. 2002; Lockyer et al. 1984; Lockyer et al. 1985). The throat grooves allow expansion of the throat for engulfing prey, whereas the tailstock is important in locomotion. In these two types of blubber, the composition is dominated by large quantities of collagen and thickness does not appear to vary with condition; features that are common to structural adipose tissue of all vertebrates (Pond 1998). The relative stability of these tissues ensures that function is not compromised. Consequently, these regions would not be appropriate for condition studies.

Constraints on regional blubber thickness due to locomotor or streamlining effects have also been suggested. For example, dorso-ventral thickening was observed in the caudal peduncle of fast-swimming porpoise and dolphins, while laterally the blubber remained much thinner, even as total blubber energy stores and therefore, body condition, increased (Koopman 1998; Pabst et al. 1999). The collagen matrix in blubber can also function as a spring, which reduces the cost of transport as cetaceans beat their tail flukes to propel themselves. This function may limit the extent that blubber thickness can increase without impeding motion or decreasing efficiency (Pabst 1996). In harp seals,

Phoca groenlandica, the greatest absolute value and relative change in blubber thickness occurred at sites posterior to the maximum girth, allowing changes with condition to occur with minimal effects on drag or streamlining (Beck and Smith 1995). In contrast, blubber remained thinner and was less apt to change behind the fore-flippers, allowing the flippers to be tucked in for streamlining.

Efforts have also been made to try to understand whether the large variation in seasonal blubber energy stores exhibited by many marine mammals has associated energetic costs due to alterations in buoyancy and insulation. An animal in "good" condition with large blubber stores would also be more buoyant. The effect of additional buoyancy on locomotor costs as seals dive to depth was recently explored by Beck et al. (2000) and Webb et al. (1998). Both found that buoyancy affected descent rates, allowing less buoyant (less fat) seals to descend faster. Webb et al. (1998) argued that the benefits of energy storage and insulation due to increased blubber mass were likely a trade-off with the additional energy required to overcome the effects of buoyancy.

Changes in condition, measured by either a change in percent lipid or thickness of blubber, affect the insulative properties of blubber and the thermoregulatory costs to the individual. Several studies provided evidence that the distribution of blubber was important in maintaining thermal balance (Rosen and Renouf 1997; Ryg et al. 1988; Slip et al. 1992). During seasonal mass loss, the rate of blubber mass loss, ratio of blubber thickness to body radius ratio, and distribution of blubber remained fairly constant along the body of southern elephant seals (Slip et al. 1992). In contrast, harbor and ringed seal (*Phoca hispida*) blubber losses were greater in the over-insulated hind regions, but the ratio of blubber thickness to body radius had less variation or was nearly constant along the body in harbor and ringed seals, respectively (Rosen and Renouf 1997; Ryg et al. 1988). Both strategies exhibited by phocids were regarded as mechanisms to maximize available blubber for insulation. These studies, however, did not address changes in blubber lipid content that may have occurred as lipids were mobilized for energy. If changes did occur, the effect on thermoregulatory costs may have been significant, as the

insulative quality of blubber varies directly with lipid content (Kvadsheim et al. 1996; Worthy and Edwards 1990).

Finally, care must be taken to understand the influence of biological factors on blubber composition, thickness and distribution. Differences have been found between sexes (Beck et al. 2003; Ryg et al. 1990b), age-classes (Aguilar and Borrell 1990; Read 1990), and reproductive states (Arnould 1995; Lockyer 1986; Read 1990; Víkingsson 1995). Given the above variables, the definition of "good condition" may differ in absolute value both within and between species.

Lipid biochemistry, deposition and mobilization

The focus of this chapter thus far has concentrated on the factors influencing the *amount* of lipid in marine mammals and how, by tracing that amount, it is possible to understand properties of health and condition, foraging ecology and aquatic adaptation. Much of what I have discussed essentially focuses on *when* an animal is feeding. But, to determine upon *what* an animal is feeding using fatty acid profiles, it is important to understand the nomenclature and fundamental biochemistry of lipid acquisition and metabolism. As stated previously, triacylglycerols (TAG) are the main storage lipids in blubber. Each TAG molecule is made up of three fatty acids linked with an ester bond to glycerol. Fatty acids in storage TAG typically contain carbon chains of 14-24 carbons in total attached to a carboxyl molecule, and are designated by carbon chain length:number of double bonds, and location (n-*x*) of the double bond nearest the terminal methyl group. For example, the fatty acid 16:1n-7 contains sixteen carbon atoms and one double bond located at the seventh carbon from the end methyl group. Fatty acids can be grouped into classes designated according to the number of double bonds. Saturated (SAT) contain no double bonds, monounsaturated (MUFA) contain a single double bond and polyunsaturated (PUFA) contain two or more double bonds.

The pathway of dietary lipids in prey items to their deposition in the blubber layer involves several metabolic steps. Lipids from prey are digested in the stomach through mechanical churning and gastric lipase. Lipases are enzymes that break the ester bonds

linking fatty acids to glycerol and are important throughout many stages of lipid digestion. Once in the intestine the lipids are further broken down by bile salts synthesized in the liver and multiple lipases, especially pancreatic lipases. In the breakdown of TAG, the outer two fatty acids are released, but the middle one remains intact, forming a monoacyclglycerol. In pinnipeds, as with other carnivores and monogastric animals, the fatty acid structures themselves are not modified, but are absorbed through the intestinal cell walls in approximate proportions in which they are present in the digested food (Pond 1998). In contrast, multichamber stomached vertebrates such as ruminants have micro-organisms in the gut that can desaturate and shorten the chain length or otherwise modify fatty acids. Short chain fatty acids (< 12 carbons) generally are oxidized immediately for energy and are not stored in adipose TAG. Cetaceans differ from pinnipeds in that they have a multichambered stomach, but it differs from that in ruminants, and therefore it is not known to what extent microbial action in the gut may affect fatty acid composition. Absorbed fatty acids and monoacylglycerols are repackaged into TAG and assembled into chylomicrons for circulation in the blood. Following a meal the adipocytes increase production of the enzyme lipoprotein lipase (LPL), which attaches to nearby blood vessel endothelium. As circulating chylomicrons pass by, LPL cleaves fatty acids from the TAG, releasing the fatty acids for uptake into the adipocytes where they are repackaged as TAG for storage (Vance and Vance 1990). Because of this pathway, fatty acids from the diet with $\geq$14 carbons should be deposited in the blubber in a predictable and direct manner.

Our knowledge, however, of preferential deposition or mobilization of fatty acids in blubber and adipose tissue in general is incomplete. If preference does occur, this could have a profound impact on diet interpretation from fatty acid profiles. *In vitro* studies in rats and humans suggested that mobilization of fatty acids from adipose tissue was based on molecular structure. For a given chain length, relative mobilization increased with increasing unsaturation and for a given unsaturation increased with decreasing chain length (Raclot et al. 1997; Raclot and Groscolas 1993). However, *in vivo* studies on rats fasted for 1-2 days did not show significant changes in adipose

composition, suggesting that mobilization was not selective under these conditions (Chen and Cunnane 1992). During longer fasting durations (10 days) in rats, selective mobilization of fatty acids was greatly reduced as adipose TAG were depleted (Raclot and Groscolas 1995). Furthermore, although a 4.5-fold difference was found in the fractional mobilization range in 7 day fasted rats, mobilization for ten out of seventeen major fatty acids did not differ from total fractional mobilization (Raclot and Groscolas 1995). Thus, for a majority of the fatty acids, mobilization was non-selective.

Although seals make great candidates to study specific fatty acid mobilization patterns in naturally fasting carnivores, few studies exist. In both hooded seals, *Cystophora cristata*, and grey seals, *Halichoerus grypus*, 20:5n-3 was preferentially mobilized into milk from maternal blubber stores (Iverson 1993; Iverson et al. 1995), consistent with high mobilization for this fatty acid found by Raclot (2003). But if deposition is similarly governed by molecular structure, 20:5n-3 should also be one of the least readily deposited. In contradiction, deposition of milk fatty acids by hooded seal pups was uniform, including 20:5n-3 and at the end of a 4 day lactation period, pup blubber lipids did not differ from milk lipids (Iverson et al. 1995). Similarly, in provisioned king penguin chicks, *Aptenodytes patagonicus*, also marine predators, adipose tissue TAG fatty acids were essentially identical to that of the diet at emancipation (Thil et al. 2003). It appears that in rapidly fattening marine vertebrates, the efficiency of fatty acid deposition has a greater impact on actual deposition than selective deposition based on molecular structure. It is evident that more studies on the potential for selective deposition in marine mammals are warranted that include not only time periods of rapid fattening, but also at maintenance levels and amongst different age-classes.

Lipids as dietary indicators in the marine environment

The potential utilization of fatty acids as chemical tracers of diet has long been recognized in the marine environment. In early studies on the blubber oil of blue whales, *Balaenoptera musculus*, Tveraaen (1935) hypothesized that fatty acids could be directly

deposited into the blubber without modification by the whale and that the specific fatty acids present in the blubber were likely due to the fatty acids of ingested zooplankton and phytoplankton. Furthermore, populations of fin whales (*Balaenoptera physalus*) that foraged in different oceans could be distinguished chemically by their blubber lipids, and this again was attributed to differences in dietary fatty acid intake (Ackman and Eaton 1966). While the fundamental theories of fatty acids as dietary tracers were recognized, many early studies of marine mammal fatty acids were based on single or few individuals and only descriptive in nature with little emphasis on any broader ecological context or lacking in sample size for statistical comparisons (Ackman et al. 1965; Ackman et al. 1971; Jangaard et al. 1963; Stull et al. 1967; West et al. 1979a; West et al. 1979b).

The analysis of fatty acids for physiological and ecological studies has now advanced to become a powerful research tool. The effectiveness of fatty acids as dietary markers and their use in feeding ecology is based on several properties of marine lipids and their consumers. A wide array of fatty acids exist (>70), many of which can be traced to specific species or habitats. This diversity is due to the large number of carbon sources available and subsequent lipid synthesis pathways of marine phytoplankton (Sargent et al. 1988; Sargent 1989). But more importantly, many of the fatty acids of marine origin arise only or mostly from the diet, making them particularly useful as dietary indicators (Iverson 1993). These include essential fatty acids (ESA) 18:2n-6 and 18:3n-3, which mammals must get from the diet because they lack the enzymes to produce double bonds at the n-3 and n-6 position. Also included are long-chain n-3 and n-6 PUFA, such as 20:4n-6 and 22:6n-3. Because PUFA are readily available in the marine environment, there is generally no need for biosynthesis. That is not to say that *de novo* biosynthesis does not occur in marine mammals, for it has been documented in juvenile harp seals under conditions of substantial fat mass loss (Kirsch et al. 2000), but in the case of either adequate fat in the diet or during fasting when large amounts of fatty acids are mobilized from endogenous stores, these abilities are suppressed (Vance and Vance 1990). Finally, as mentioned previously, the transfer of dietary fatty acids from prey to consumer is conservative, that is, dietary fatty acids with carbon chain length $\geq$14 can be directly

deposited into tissues of the consumer without prior modification (Iverson et al. 1995; Pond 1998).

The number of studies utilizing fatty acids as dietary indicators in wild populations of marine mammals has increased substantially since the early 1990's. This is due to the development of "fatty acid signature analysis," an approach that examines the pattern of many fatty acids in combination using multivariate statistical techniques (Iverson 1993; Iverson et al. 1997a; Iverson et al. 1997b; Smith et al. 1997). Differences in fatty acid signatures observed in the blubber, milk or blood of marine mammals have been attributed to differences in foraging associated with trophic level, gender, populations, geographical location and season (Adams 1999; Bradshaw et al. 2003; Iverson et al. 1997b; Olsen and Grahl-Nielsen 2003; Walton et al. 2000; Walton and Pomeroy 2003). While many of these studies could detect differences in diet between known groups using fatty acid signatures, fewer studies have attributed differences to specific dietary prey (Bradshaw et al. 2003; Dahl et al. 2000; Hooker et al. 2001; Iverson et al. 1997b).

To enhance our ability of using fatty acid analysis in a quantitative fashion for dietary studies of wild populations, two more components are necessary. First, large databases of fatty acid profiles of the potential prey base need to be established (Budge et al. 2003; Iverson et al. 2002). Second, because fatty acid profiles present an integration of dietary history, controlled dietary studies with captive animals are necessary to determine rates at which consumer tissues reflect those of the diet, how they might vary given the metabolic state and nutritional status of the animal, and whether biosynthesis or preferential deposition of specific fatty acids occurs. While several controlled dietary studies have been performed at lower trophic levels, from primary consumers such as zooplankton (Fraser et al. 1989; Graeve et al. 1994; Lee et al. 1971), to secondary consumers such as fish (Dos Santos et al. 1993; Kennish et al. 1992; Kirsch et al. 1998; Tidwell et al. 1992), far fewer have been completed for top-level predators such as seals (Grahl-Nielsen and Mjaavatten 1991; Kirsch et al. 2000).

Scope of study

The objective of this dissertation is to further our understanding of lipid utilization in marine mammals through biochemical assessment of the composition of blubber and of fatty acid profiles in blood components under a variety of nutritive and dietary conditions. In chapter 2, "*Natural variation of the chemical composition and thickness of blubber in the bowhead whale, Balaena mysticetes: implications for body condition assessment*," I address the biological and environmental influences on the quality and quantity of blubber in the bowhead whale. This chapter shows how the length of the whale, a proxy for age, is important for determining the thickness and composition of blubber. I address whether other factors such as sex, age-class, and season also impact the blubber properties observed. Regional and vertical heterogeneity of blubber is explored at the level of specific depths, sites and extrapolation to the blubber layer as a whole. Results are used to determine if specific sites or depths are sensitive indicators of nutritional status and condition. This project is the first detailed examination of the natural variation of the composition of bowhead whale blubber and serves as a baseline on which to base further health and condition studies in this species. Preliminary analysis from this work was published previously in a report (Willetto et al. 2002).

Chapter 3, "*Postmortem blubber composition changes from a stranded humpback whale, Megaptera novaengliae: implications for health assessment,*" describes the compositional changes that occur in blubber after a whale has died and stranded. This study addresses whether the use of blubber samples in varying stages of degradation offer any utility in the assessment of nutritive status and condition.

In chapter 4, "*The effects of prey switching on plasma and red blood cell fatty acids in the harbor seal (Phoca vitulina),*" I present the results of a long-term controlled feeding study in captive harbor seals. I examine individual fatty acids and fatty acid profiles in prey fish and, using serial blood samples, their incorporation into both plasma and red blood cell membranes (RBCs) of harbor seals. Using multivariate techniques this allows for the prediction of diet based on fatty acid profiles. Furthermore, the use of multiple tissues is explored in estimating the "dietary window" its profile represents.

Finally, I address the impact of season and total lipid intake on individual fatty acid levels. This study provides an important validation of fatty acid profiles as dietary tracers for use in wild populations of marine mammals.

Chapter 5, "*Lipids as biochemical markers in marine mammal physiological and ecological research: current status and future projects*," is a summary of the utility of lipid-based biomarkers in marine mammal research. I discuss our current knowledge in the field and suggest future directions for increasing our understanding of the feeding ecology of marine mammals.

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2 Natural variation of the chemical composition and thickness of blubber in the bowhead whale, *Balaena mysticetus*: implications for body condition assessment

INTRODUCTION

One of the more obvious external anatomical features of marine mammals is the blubber layer encasing the core. In large cetaceans, blubber can encompass 15-43% of the total body mass and ranges widely in both thickness and lipid content (Aguilar and Borrell 1990; Aguilar and Borrell 1991; Lockyer 1976; Lockyer 1991). The level of energy reserves in cetaceans, much of which is stored as lipid in the blubber, is considered to be an important indicator of body condition or nutritional status (Hanks 1981; Lockyer 1987; Slijper 1954). Many marine mammals undergo seasonal changes in body condition associated with variation in food availability and with the energetic demands of migration and reproduction. Because lipid provides most of the energy reserve for these species, reliable methods for estimating the total lipid content of a marine mammal are critical to models of condition. However, blubber thickness and lipid content are controlled by a suite of biochemical, physiological and anatomical constraints which complicate the assumption that total lipid content equals total available energy.

The blubber layer is a dynamic tissue, the quantity and quality (lipid richness) of which is influenced by the many functions of blubber, including providing insulation, adjusting buoyancy, aiding in streamlining and locomotion, serving as a source of metabolic water and as the main energy reserve (reviewed by Pabst et al. 1999). Furthermore, factors such as size, age and reproductive status, and environmental factors such as temperature and food availability may also influence the quantity and quality of blubber. Because of the many intrinsic and extrinsic factors that could influence blubber and therefore body condition, thorough knowledge of intra- and interspecific variation of blubber lipid stores is necessary to accurately describe condition (Slip et al. 1992). In addition, long-term monitoring of body condition can reveal changes in environmental conditions and are therefore important in understanding population and ecosystem health,

and ultimately, in making management decisions (Arnould 1995; Beck et al. 1993; Fadely 1996; Kirkpatrick 1980; Lockyer 1990).

Blubber responses to changing energetic demands vary among species and may be detected by changes in blubber thickness, lipid content, or both. Blubber thickness has been used as a proxy for body condition in a variety of marine mammals, such as harp seal (Beck et al. 1993), harbor seal (Pitcher 1986; Rosen and Renouf 1997), walrus (Quakenbush et al. 1999), harbor porpoise (Koopman et al. 2002), fin and sei whales (Lockyer et al. 1985), and minke whales (Næss et al. 1998), etc. However, it was not considered a good marker for condition in several other species, such as southern right whale (Tormosov et al. 1998) or gray whale (Rice and Wolman 1971). Variation in lipid mobilization among body sites, as well as the multifunctionality of blubber dictates that selection of measurement sites be established on a species-specific basis. To be an effective condition index, Lockyer et al. (1985) suggested choosing the site from which measurements provided the maximum variability.

Because lipid content does not necessarily vary linearly with blubber thickness (Klem 1935; Slijper 1962), the lipid content of blubber must be measured in addition to thickness to estimate total body lipids and seasonal energy gain or loss. Blubber in whales is composed of a highly structured collagen matrix and adipocytes (fat cells) that expand and contract with varying energetic demands as lipids are deposited and mobilized, respectively. Unlike phocids, in which blubber lipid content is relatively uniform throughout the body (Beck et al. 1993), most mysticete (baleen whale) blubber is heterogeneous in composition across both site and depth (Ackman et al. 1975a; Ackman et al. 1975b; Heyerdahl 1932; Slijper 1962). Aguilar and Borrell (1990) found that the lipid content of the inner layers of fin whale blubber were most likely representative of nutritional status, whereas outer layers were more stable, and thus, less sensitive to nutritional changes. In fin whales known to fatten seasonally, blubber lipid content increased substantially over the feeding season and varied annually, presumably depending on prey availability (Lockyer 1986).

The bowhead whale (*Balaena mysticetus*) and balaenid (right) whales in general, were hunted commercially almost to extinction because of their very large blubber stores, which caused them to float when killed; making them the "right" whales to catch. Despite this potential access to blubber samples for scientific study, no large-scale description of the quantity and quality of bowhead blubber with respect to condition has been carried out. However, since the 1970's blubber thickness measurements have been recorded from subsistence harvested whales (George et al. 2002a). The current work represents the first detailed examination of the natural variation in the quality of bowhead whale blubber and the relationship to blubber quantity (thickness).

The Bering-Chukchi-Beaufort Sea (BCBS) stock of bowhead whales is of special management concern because of its endangered species status and its cultural and nutritional importance to many native peoples. Also, as the only true pagophilic (ice-loving), year-round resident mysticete of polar waters, it may potentially serve as an indicator species for the health of the arctic ecosystem in light of changing climatic conditions. Five stocks of bowhead whales exist throughout the arctic, but for the purpose of this paper, reference to bowhead whales denotes solely the BCBS stock. Bowheads migrate seasonally between the northern Bering Sea in winter and the Eastern Beaufort Sea in summer (Moore and Reeves 1993). Knowledge of bowhead feeding habits has been limited to visual observations in mostly summer and fall, collection of stomach contents from harvested whales, and by chemical interpretations (stable isotopes, fatty acids, and contaminant analysis) of various tissues, but these studies have not always been in agreement on the amount or contribution of potential feeding areas to the annual energy budget of bowhead whales (Hoekstra et al. 2002a; Lee 2000; Lowry 1993; Lowry and Frost 1984; Richardson and Thomson 2002; Schell et al. 1989; Sheffield and Lowry 2002). Energy reserves and fecundity have been shown to vary with annual environmental conditions and seasonal food availability in balaenopterids (Ichii et al. 1998; Lockyer 1986; Lockyer 1987; Lockyer 1990). If bowhead feeding is also seasonal, we should be able to detect changes in blubber energy reserves and hence, condition.

However, in order to link changes in condition with changes in seasonal feeding, indicator sites must first be established.

The objectives in this current study are to: 1.) Establish the pattern of natural variation and stratification in the chemical and energetic composition of bowhead whale blubber; 2.) Determine the influence of select biological (e.g., age, size, gender, reproductive status) and environmental (e.g. season, year) variables on blubber thickness and composition; and 3.) Determine if specific sites or layers of blubber are sensitive indicators of the nutritional status of bowhead whales, such that body condition indices can be developed and utilized in long-term monitoring of bowhead health and population status.

METHODS

Sampling regime

Blubber samples were taken from bowhead whales (n=48) obtained from Alaskan Eskimo (Inuit) hunters at Barrow and Kaktovik, Alaska, during fall (n=29) and spring (n=19) subsistence harvests in 1998-2001. Samples were collected within 12 hours of the whale's death by personnel from the North Slope Borough Department of Wildlife Management and Alaska Department of Fish and Game. A suite of biological data (body length, blubber thickness, sex, external scars or parasites, etc.) was recorded for each whale sampled (Table 2.1). Full thickness blubber cores (approximately 5 x 10 cm) were taken at six sites: three sites (dorsal, lateral, ventral) along the girth one meter posterior to the blowholes and three sites (dorsal, lateral, ventral) at the umbilicus girth. Abbreviations of site names are shown in Fig. 2.1, which are used throughout this chapter. Not all blubber core sites were collected from each whale due to inaccessibility of landmarks for sampling or when the flensing process was already underway. Complete sample sets (all six sites) were obtained for 32 whales, whereas 16 whales had only 1-5 sites sampled. Blubber thickness, both with and without the epidermis, was measured on each core with a meter stick to ±0.1 cm after it had been removed from the whale. The blubber core was then further subdivided into five equal depths based on the blubber thickness measurement without epidermis. The five depth samples were labeled in

increasing order from 1-5 starting with the depth just below the epidermis and continuing to the depth just above the muscle (See Fig. 2.1). Samples were kept frozen in individual airtight plastic bags initially at -20° C at the field site and at -80 °C once transferred to UAF, until analysis.

Animal Classification

Whales were classified into age-class categories as mature (>13 m; n=13), or immature (7-12.9 m; n=35) according to body length and upon inspection of the reproductive organs (George et al. 1999). For example, a 12.6 m whale that was pregnant would be placed in the mature category, despite a body length less than 13 m. The immature age-class was further subdivided into subadult (>9-12.9 m; n=21) or juvenile (7-9 m; n=14) when appropriate. Mature females were classified as pregnant (n=5), lactating (n=1) or mature (n=2); however, due to small sample sizes, these categories were pooled unless otherwise noted. Additionally, the blubber lipid content of whale 00B4, a non-pregnant mature female was an outlier statistically and omitted from all statistical procedures. Data from this whale are given as a case study instead.

Composition Analysis

Lipid content was determined gravimetrically in duplicate 0.5 g (wet wt.) samples weighed to ±0.1 mg after 24 hour extraction of lipids by continual reflux in a Soxhlet extraction apparatus with a 2:1 chloroform-methanol solution (Fadely 1996; Lockyer et al. 1985). Samples were then dried in an oven at 70°C until a constant mass was achieved. Calculation of the final percent lipid required the determination of percent water from paired samples. Lipid content was expressed as a percentage of the wet weight of the tissue.

Approximately 0.5 g samples weighed to ±0.1 mg were frozen at -80°C and then lyophilized to a constant mass under vacuum (VirTis Freeze Dryer Model 5463). Water content was determined gravimetrically in duplicate and expressed as a percentage of the wet weight of the tissue.

Ash content was determined gravimetrically in a subset of duplicate samples (n=108) after ignition at 550°C in a muffle furnace for 24 hours. Ash content (average 0.02%) was determined to be insignificant and not continued for the remainder of the samples.

Energy density was determined for a subset of samples (n= 7 whales) with a micro-adiabatic bomb calorimeter (Parr Co.) using approximately 0.3 g (wet wt.) duplicate samples. Because of oil leaching from the blubber sample, each sample was placed in a gelatin capsule (Parr Co.) of known energy density to ensure complete ignition. Capsule energy density was then subtracted from the final determination. Energy density values obtained through bomb calorimetry were compared to calorific conversion of proximate analyses, using 9.45 $kcal \cdot g^{-1}$ for lipid and 5.65 $kcal \cdot g^{-1}$ for protein (Brody 1968) to ensure quality control.

Statistical analysis

As a first approach to understanding the relationships between blubber lipid content and various biological variables, total mean blubber lipid content for whales with complete sample sets (n=32) was regressed against body length. Total mean blubber lipid content (hereafter *TBL*) was defined as the average of all 30 subsamples (six sites x five depths per site) of a given whale and is expressed as a percentage of the wet mass of blubber. Relationships between *TBL* and body length were tested for differences between sex, season or age categories by simple linear regression.

The mean lipid content of all five depths was considered to represent the lipid content of the whole blubber core at a given site, hereafter referred to as "site mean blubber lipid content," or *SBL*. Each of the six sites was initially explored individually by a multiple regression model (Statistica, Visual GLM) for sources of variation due to age-class, sex, season and all two-way interactions. Similarly, sources of variation within the five depths were initially explored by a multivariate multiple regression model. The data were modeled with depths 1-5 (% lipid) as the dependent variables and age-class, sex, season, site and all two-way interaction as factors. Higher order interactions were not

included due to lack of degrees of freedom. An interaction between age-class and length prevented use of analysis of covariance for between age-class analyses, but was suitable for within age-class analyses. Although biological dependence between the six sites on a given whale was expected, inspection of the residuals suggested statistical independence. Lipid content percentages were arcsine-square root transformed prior to statistical analyses in order to better approximate normality (Sokal and Rohlf 1998). For *post hoc* multiple comparisons, a Tukey test for unequal n was used to identify significant factors in determining *SBL* or blubber lipid content at individual depths. Significance was set at $P<0.05$. Data are presented as mean ± SE unless otherwise stated.

Juvenile and subadult whales were pooled into a general "immature" category in order to have sufficient sample size to make seasonal comparisons. Too few mature whales were sampled to make similar seasonal assessments. For immature whales *SBL* was significantly negatively correlated with body length (Pearson's correlation; range –0.499 to –0.792, $P<0.01$ for all 6 sites). Analysis of covariance (ANCOVA) was therefore used with body length as the covariate. Least squares means were calculated to examine seasonal differences. Homogeneity of slopes was tested between seasons at each site prior to ANCOVA analysis and when rejected a separate slopes ANCOVA model was utilized. Multiple contrasts of least squares means for separate slopes data were performed at 0.5 m increments to detect at what length a seasonal difference occurred. Seasonal comparisons were also made for each depth using the same statistical procedure.

The degree of lipid stratification, or the gradation in lipid content from the outer to the inner layer of the blubber core, was examined between age-classes and body sites. The stratification index, termed Δ lipid content, was calculated by subtracting the % lipid of the outer-middle layer (mean of depths 2 & 3) from the % lipid of the inner layer (depth 5). Positive Δ lipid content values indicated that the blubber core increased in lipid content from the outer to inner layers, representing a high level of lipid deposition, while negative Δ lipid content values indicated that lipids were being mobilized. A homogenous blubber core was defined as having a Δ lipid content value between –5 and 5 %.

RESULTS

Relationship of blubber thickness and body length

The thickness of bowhead whale blubber ranged from 11-42 cm. Blubber thickness was highly variable in juvenile whales (< 9 m in length), but increased linearly with body length in subadult and mature bowhead whales (Fig 2.2a). Blubber thickness was highly correlated with body length after whales reached 9 m (Pearson's correlation 0.7423), thus body length was used as a covariate when testing for seasonal differences. No difference was found between spring and fall when mean thickness for each whale was tested or when examining the thickness at each body site individually ($P > 0.05$; all cases). Blubber thickness was uniformly distributed among the six body sites for juvenile and mature whales (Table 2.2). However some variation in site blubber thickness was observed in subadult whales. Site BD was significantly thicker than sites BL or BV and site UV was significantly thicker than site BL.

Relationship of blubber thickness and blubber lipid content

Linear regression revealed that blubber thickness was negatively but weakly related to blubber lipid content (r^2=0.036, P=0.009; Fig. 2.2b), that is, as blubber thickness increased, the lipid content slightly decreased. Given the relationship between blubber thickness and body length, there would likely be age-class differences, so this was also explored with linear regression and further categorized by body site location to try and understand sources of variation. Again relationships between blubber thickness and lipid content were weak but highly variable among age-classes and sites resulting in positive, negative and no relationships (Fig. 2.3). Blubber lipid content was least related to blubber thickness in subadult whales.

Relationship of total mean blubber lipid content and body length

Simple linear regression of whales with complete sample sets revealed a significant negative relationship between *TBL* (%) and body length (m) (Fig. 2.4a), represented by the equation:

All whales (n=32)
TBL (%) = -1.24L + 95.36 $r^2 = 0.422$, $P < 0.001$ (Eqn. 1)

Where *TBL* (%) is the total mean blubber lipid content as defined in the methods and L indicates the body length of the whale.

Relationships between *TBL* and body length were further refined by separating the data by age-class and sex, revealing no relationship for mature females, but stronger relationships for immature whales:

Mature female whales (n=5)
TBL (%) = -0.11L + 81.87 $r^2 = 0.002$, $P = 0.948$ (Eqn. 2)

Immature female whales (n=15)
TBL (%) = -2.31L + 104.37 $r^2 = 0.679$, $P < 0.001$ (Eqn. 3)

Immature male whales (n=11)
TBL (%) = -1.94L + 103.15 $r^2 = 0.496$, $P = 0.016$ (Eqn. 4)

Although the age-class categories used in this project allowed comparisons to many other bowhead whale studies that define groups based on length cohorts and sexual maturity (see methods), it appeared evident from Fig. 2.4a that the relationship between *TBL* and body length changed at approximately 11 m. That is, the *TBL* had a negative linear relationship with body length to about 11 m (eqn. 6), after which no relationship existed for females (eqn. 5). Males also followed this pattern to about 11 m (eqn. 7), after which the slope remained negative, but the sample size was too small (n=3) to produce a significant relationship ($P = 0.178$).

Female whales >11 m (n=6)
TBL (%) = -0.24L + 83.70 $r^2 = 0.018$, $P = 0.802$ (Eqn. 5)

Immature female whales ≤ 11 m (n=14)
TBL (%) = -3.00L + 110.32 $r^2 = 0.791$, $P < 0.001$ (Eqn. 6)

Immature male whales ≤ 11 (n=9)
TBL (%) = -3.37L + 116.49 $r^2 = 0.767$, $P < 0.001$ (Eqn. 7)

In order to test for seasonal variation of *TBL*, a larger sample size than the 32 completely sampled whales was required. Using the data from completely sampled whales, *TBL* was regressed against each site mean lipid content (*SBL*), yielding the regression equations in Table 2.3, and allowing a calculated TBL to be estimated from a single site mean. Because whale 99B14 was an outlier statistically and potentially compromised due to multiple line injuries, regression equations are given both with and with out the inclusion of this whale. Although all regressions were highly significant, sites BV and UL had the highest r^2 values, regardless of whether 99B14 was included or not, and were therefore selected as the best predictors of *TBL*. *TBL* was calculated for whales with incomplete sample sets, first by using the equations for UL, then by using BV if UL had not been sampled. The regression equations including whale 99B14 were chosen for this analysis in order to include the largest range of *SBL* values possible. Using these methods *TBL* could be estimated for nearly all of the whales sampled (45 of 48).

By including both calculated and measured *TBL*, a larger proportion of the variation could be explained by the *TBL*–body length relationship (Eqn. 8 and Fig. 2.4b.) Additionally, the variation in *TBL* explained by body length improved when mature females were excluded (eqn. 9).

All whales (n=45)
TBL (%) = -1.30L + 95.93 $r^2 = 0.519$, $P < 0.001$ (Eqn. 8)

All whales except mature females (n=36)
TBL (%) = -1.94L + 101.79 $r^2 = 0.690$, $P < 0.001$ (Eqn. 9)

Significant seasonal differences in slope were found for both males and immature females (Fig. 2.5; eqn. 10–13; $P < 0.001$); however, too few mature female whales were

sampled to make seasonal comparisons. All slopes were negative, except for spring mature females, which did not differ significantly from zero (eqn. 14).

FALL

All Males (n=13)
TBL (%) = -1.51L + 99.09 $r^2 = 0.746$, $P < 0.001$ (Eqn. 10)

Immature Females (n=14)
TBL (%) = -1.81L + 100.35 $r^2 = 0.788$, $P < 0.001$ (Eqn. 11)

SPRING

All Males (n=4)
TBL (%) = -3.23L + 114.93 $r^2 = 0.921$, $P = 0.040$ (Eqn. 12)

Immature Females (n=5)
TBL (%) = -3.67L + 115.30 $r^2 = 0.919$, $P = 0.010$ (Eqn. 13)

Mature Females (n=7)
TBL (%) = -0.86L + 93.24 $r^2 = 0.368$, $P = 0.148$ (Eqn. 14)

The regression equations for spring and fall samples intersected at whale lengths of 8-9 m, which is not surprising, given that many whales of this length were classified as *ingutuk* by the Inuit (Table 2.1). Ingutuk are believed to be recently weaned, and probably near the limit of maximum fatness for this species. However, as whale length increased, the difference between spring and fall lipid contents also increased. For example, for an 11 m and 12 m female whale, the *TBL* increased by 5.6 % and 7.4 %, respectively, from spring to fall.

Comparison between age-classes of blubber lipid content at different body site locations

No significant differences were found for season, sex*season or age-class*season in determining SBL. However, sex, age-class and the age-class*sex interaction were significant for four of the six body site locations examined in determining *SBL*. Multiple comparisons of the age-class*sex interaction revealed that sexes did not differ in lipid content for the juvenile and subadult groups. However, males and females differed significantly in the mature group. Sex data were therefore pooled for the immature groups

to form four reproductive categories; juvenile, subadult, mature females and mature males. While only one site (BL) did not have significant differences between reproductive categories (Table 2.4), five sites (BD, BV, UD, UL, UV) were highly significant (all $P<0.001$). *Post hoc* comparisons at the BD site indicated that juvenile SBL was significantly higher than all other categories. The latter four sites revealed that mature female SBL did not differ from subadult SBL; however, all other reproductive classes differed significantly from each other. Similar to the trend seen for *TBL*, smaller whales generally had higher *SBL* values than longer whales at each site. The largest difference between categories occurred at the UV site (21.8% difference), whereas the BD site had the least difference (9.1%).

Comparison between age-classes of blubber lipid content at different depths

At all 5 depths sex, age-class, site and the age-class*sex interaction, but not other two-way interactions, were significant in determining lipid content. Similar to results for SBL, lipid content at all five depths varied between sexes only in the mature age-class, thus categories were pooled to form the four reproductive categories as above. Subadults did not differ from mature females at 3 depths (1,2,5), but all other comparisons between reproductive categories were significantly different (Fig. 2.6). Mean lipid content was greatest at all depths in juveniles and then decreased by age-class in the following order:

Depths 1,2,5	juvenile>subadult=adult female>adult male
Depths 3,4	juvenile>subadult>adult female>adult male

The smallest mean difference (~8-9%) between reproductive categories for a given depth occurred in the outer-middle layers, depths 2-3, whereas depth 1 and 4 were intermediate (~14-16%), and mean differences were greatest (~30%) at the innermost layer, depth 5 (Fig. 2.6). The lipid content of a given depth did not appear to be homogeneous across body site locations (Fig. 2.7), however, while the effect of site alone was significant, statistical power was not sufficient to detect a significant site*reproductive category interaction ($P = 0.171$). In general, lipid content at a given

depth was similar for all blowhole sites, but at umbilicus sites, dorsal sites equaled lateral sites, and both were significantly different from ventral sites.

Not considering the depth next to the epidermis (depth 1), the range of means among all the depths was smallest in juveniles and greatest in mature males, suggesting greater and lesser homogeneity of the blubber column, respectively (Fig. 2.7). This was explored in more detail by developing a stratification index as will be discussed below.

Lipid content variation within the immature age-class: the effect of season and year

Blubber lipid contents in immature whales showed seasonal differences but were dependent on body site location. When analyzed on a mean site basis, all three umbilicus sites, but only one blowhole site showed significant seasonal differences, suggesting regional variation in lipid mobilization and deposition (Fig. 2.8). Although the relationship between *SBL* and body length was negative regardless of season, the slopes of the regression lines differed significantly between seasons in three (BV, UD, UL) of the six sites (Fig. 2.8). The regression lines at these three sites intersected between 8.5–9 m. Consequently, utilizing a separate slopes model, significant differences between seasons were not found in short (i.e. young) whales, but beginning at 10–10.5 m in length, *SBL* in fall landed whales was significantly higher than spring landed whales. At sites where slopes were not significantly different between seasons (BD, BL, UV) only the UV site was found to be significant for season, again with fall landed whales having higher lipid contents than spring landed whales.

Seasonal and site differences at the depth level were also found in immature whales (Fig 2.9). Homogeneity of regression slopes was rejected, thus a separate slopes MANCOVA was utilized to examine differences. Multivariate results indicated that season was a significant factor, however, although fall depth means appeared higher than most spring depth means when computed at the mean covariate (body length), univariate results revealed significant seasonal differences only at depth 5, the innermost layer. Site was also significant at depths 4 and 5, but the site*season interaction only at depth 5. Depths 1-3 did not vary significantly by site, depth or their interaction.

In spring, lipid content of depth 5 at BD, BL and UD sites did not differ from the corresponding sites in fall (Fig 2.9b). In contrast, lipid content of depth 5 at BV, UL and UV sites were significantly lower in spring compared to fall sites. Seasonal differences at depth 5 were greatest at the UV site, for example, at a covariate body length of 10 m, fall lipid content means were 72.7 ± 1.8 % (n=24) compared to spring at 58.6 ± 3.9 % (n=6).

The effect of year on *TBL* was examined using a one-way ANCOVA with body length as the covariate. Because spring samples tended to have lower lipid contents but were not represented in all years, this analysis was limited to fall whales. No significant difference was found between years ($P = 0.440$). Least squares *TBL* means at a covariate length of 9.61 m were 83.3 ± 0.6 %, 83.7 ± 0.6 %, and 84.3 ± 0.5 %, for 1998 (n=7), 1999 (n=9) and 2000 (n=10), respectively (data not shown).

The effect of body length, sex and sampling position on blubber lipid content at each depth

Although it was recognized that body length, and hence age, played a major role in determining blubber lipid content, statistical constraints prevented the use of body length as a covariate for between age-class comparisons of depths. Scatterplots of blubber lipid content at each depth vs. body length were therefore plotted, examining each sex separately and further subdividing by sampling position (dorsal, lateral, ventral) to try to understand regional patterns of blubber lipid content (Fig. 2.10-2.13).

Sex differences were evident at depth 1, the layer closest to the epidermis (Fig. 2.10). In males, lipid content decreased linearly with body length at each of the three sampling positions. Lateral sites decreased more rapidly with body length, than did dorsal and ventral sites, for which slopes did not differ. Female lipid content at depth 1 could not be explained by linear relationships, and as a result, LOWESS lines were fit to the data. LOWESS is a robust locally weighted least squares smoother. For the immature female whales, lipid content decreased linearly with length similar to that of male whales. However, after female whales reached approximately 10 m, lipid content of depth 1 did not vary with length, regardless of sampling position.

The outer middle layers, depth 2 and 3, did not differ significantly from each other in within whale variability tests and were therefore pooled. Lipid content was linearly related to length in both sexes, although the slope was more negative in males (Fig. 2.11). Ventral sites in females were the exception to this pattern, in which lipid content did not vary with body length after approximately 10 m.

Patterns between the sexes at depth 4 were similar to that of depth 2 and 3. The lipid content varied negatively for both with length, but decreased more rapidly in males (Fig. 2.12).

Gender and body length differences in lipid content were most striking in the innermost layer, depth 5. An inverse relationship between lipid content and body length was observed for whales up to 10-11 m similar to other depths, after which the pattern between the sexes and regions diverged. In female whales longer than ~10 m, lipid content tended to increase dorsally, level out ventrally, and was quite varied laterally with increasing body length (Fig. 2.13). In contrast, lipid content for males longer than about 11 m did not vary dorsally but continued to decline at lateral and ventral sites with increasing body length.

Degree of blubber lipid stratification

Juvenile whale blubber was the least stratified of all age-class groups (Fig. 2.14). In fact, 72.5 % of all juvenile blubber cores examined were classified as homogeneous (shaded bars). In contrast, only 20.0%, 28.3%, and 41.0% of mature male, subadult, and mature female whale blubber cores, respectively, were classified as homogeneous. Of all the homogeneous cores, 39.4 % occurred at dorsal sites, 30.8 % at lateral sites and 29.8 % at ventral sites. Only three cases exhibited Δ lipid content values ≥ 5 %, one juvenile and 2 mature females, indicating a reverse stratification pattern. The greatest degree of stratification occurred in mature males. Blubber cores exhibiting a large degree of stratification (Δ lipid content arbitrarily chosen at ≤ -30) occurred in nearly half of all mature male whale blubber cores examined, but only in 10.3%, 7.1% and 1.5% of blubber cores examined for mature female, subadult and juvenile whales, respectively.

When examined by body site location, a large degree of stratification occurred most frequently in ventral sites (11 of 79 cores) and lateral sites (7 of 71 cores) and least often at dorsal sites (2 of 86 cores), accounting for 55%, 35% and 10% of the highly stratified cores, respectively. These highly stratified cores were observed more frequently in spring (18 of 81 cores) compared to fall (2 of 155 cores), but may be due in part to the greater frequency of longer (i.e. older) whales sampled in spring.

Comparison of blubber lipid stratification in pregnant and a non-pregnant mature female

A 15.4m non-pregnant adult female (ID: 00B4) was sampled that had very depleted blubber lipid stores (overall mean lipid content 56.9 %, range 8.1–85.1%) compared to all other whales examined. The lipid contents at all depths of the three umbilicus blubber samples obtained for this whale were compared to the means at all depths of the same sites in pregnant females (n=4) to examine stratification and potential lipid mobilization patterns (Fig. 2.15). Stratification of the blubber core lipids was greater in whale 00B4 at all sites examined and represented the only observation of considerable mobilization of lipids from the middle layer (depth 3). The greatest depletion of blubber lipids was observed at the umbilicus ventral site, for example, the inner layer (depth 5) was approximately ten-fold lower in lipid content than the mean for pregnant whales.

DISCUSSION

The intrinsic and extrinsic factors affecting blubber energy stores in balaenopterid whales such as fin and sei, have been reasonably well characterized due to the availability of samples from commercial whaling (Aguilar and Borrell 1990; Lockyer 1986; Lockyer 1987; Lockyer et al. 1984). Since the end of large-scale commercial whaling, access to fresh, uncompromised samples from any baleen whale for scientific study of blubber characteristics in relation to condition has been limited. Because of the Alaska Native hunter-scientist collaboration, the results of this study provide an opportunity for the first detailed examination of possible contributing factors linked to the natural variation of the thickness and composition of bowhead whale blubber.

Blubber thickness

The thickness of bowhead blubber ranged from approximately 11-42 cm, and averaged 20.4 cm. While this range is similar to previously reported ranges for bowhead whales, it is much larger than ranges reported for other baleen whales such as fin, sei or blue (Lockyer 1976), but similar in thickness to Atlantic right whales (12-23 cm; Moore et al. 2001). The positive linear relationship of blubber thickness with body length observed in this study (Fig. 2.2a) is also common among other baleen whales (Ash 1956; Matthews 1937; Slijper 1962). The large degree of uniformity of blubber thickness amongst sites within an age-class suggests that thickness may be largely size related.

The relationship between lipid content and blubber thickness is not a simple one. One could predict that as adipocytes expand with lipid, blubber thickness would increase creating a positive relationship. This positive relationship only occurred with consistency in adult bowhead whales that had larger ranging lipid contents (Fig. 2.3). For comparison, in harbor porpoise (*Phoecena phoecena*), as mean lipid content increased from 70-85 %, blubber thickness also increased, but thereafter the relationship weakened. Blubber thickness continued to increase while changes in lipid content were relatively small (Lockyer 1995). It is conceivable that the current set of bowhead data may fall in this upper range of lipid contents, similar to where the lipid content-thickness relationship weakened in harbor porpoise. The site mean lipid content ranges in bowhead whales were small and lacked the lower values that might be necessary in order to observe a large decrease in thickness. Also, because the collagen matrix in blubber can retain its structural integrity and the lipids and water within the matrix can exchange for one another, thickness may be less responsive to changes in condition. Another note to consider is that blubber thickness is inherently difficult to measure both on the whale, due to slumping and off the whale because of retraction of the elastin fibers, which could blur subtle changes in thickness.

Relationship of blubber lipid content and length

The lipid content of bowhead blubber is heterogeneous in nature, varying both by site and depth in an individual whale. Many factors contribute to the pattern of lipid content, but one pattern that is observed repeatedly is the negative relationship between lipid content and body length, a proxy for age. Before effects of other factors, such as sample site and depth, sex, reproductive status or season were tested, it was apparent that body length explained a significant portion of the variation associated with blubber lipid content (Fig. 2.4). Regression analysis indicated that 42.2% of the variation in *TBL*, which provides a general picture of the lipid content of the blubber layer as a whole, is a result of body length. Furthermore, slightly more than half of the variation in *TBL* could be explained by body length when sample size was increased with the addition of estimated *TBL* values. The strong *TBL*-body length relationship suggests that individual variation was relatively low in bowheads. This may be due in part to the fact that samples from late lactating females, which have shown the greatest variation in lipid content in other cetaceans examined (Lockyer 1987), were lacking from this study. Although the slope of the *TBL*-body length relationship varied, depending on other factors examined such as season, the slope always remained negative. In order to accurately assess condition or energy status in the bowhead whale with blubber lipid content measurements, the results of this study indicate that a corresponding length measurement is also necessary. For example, even though the absolute value of TBL in a juvenile male whale (87 %) may differ greatly from an adult male whale (74%), both values may indicate the same "condition" when examined on a length basis. Compared to other cetaceans, a negative relationship between length and lipid content was also found for male fin whales (Aguilar and Borrell 1990), but no relationship was found in large odontocetes such as the sperm whale (Evans et al. 2003). No other studies of large baleen whales have, to our knowledge, reported the relationship between blubber lipid content and body length.

Several factors may explain the negative relationship between blubber lipid content and length, including development of the blubber matrix, life history strategies,

and energetic and thermoregulatory concerns. The blubber matrix is composed of collagen and elastin fibers. The fibers appear to grow in size and width as the whale grows in length (T. Mau, pers. obs.), making protein a larger proportion of the blubber composition as the whale grows. This necessitates that the proportion of other components comprising the blubber, i.e., lipid or water, decrease. It is a common feature of all vertebrates that body tissues become more fibrous with age. However the majority of whales represented in this study, although "aging" were not yet old enough to reproduce. Hunters have also acknowledged that blubber from "old" whales is tougher, i.e., more fibrous. However, the fact that pregnant females can have the thickest blubber of all groups, thick collagen fibers, and yet also have lipid contents >90%, suggests that "fibrosis" of the blubber with age does not limit the expansion of individual adipocytes. Indeed, adipocyte size was found to increase with increasing lipid content of the blubber in bottlenose dolphins (*Tursiops truncates*) and was greatest in pregnant females (Struntz et al. 2004). It is likely that pregnant bowhead females also deposit large stores of lipid that result in increased adipocyte size and that non-reproductive mature whales do not require the equivalent energy storage in the form of blubber as do pregnant females.

Large lipid stores (30-60% of body mass) are typical in recently weaned marine mammals. This allows the young to survive long periods without food, resulting from patchy and unpredictable nature of marine food resources, inexperience in foraging and physiological limitations to diving. Indeed, the highest lipid content values of blubber observed in this study (>95%) were from juvenile whales believed to have been recently weaned. In addition to the energy that blubber lipids provide, a higher lipid content increases the insulative capacity of the blubber (Worthy and Edwards 1990). Because younger whales have larger surface-to-volume ratios, and hence, greater heat loss to the environment compared with mature whales, they benefit from the insulatory effects that the higher lipid content values provide. Conversely, the largest of whales may have the opposite problem and need to dump heat (Hokkanen 1990). The lower lipid content values found in large mature males may be a response to this problem.

Comparison of blubber lipid content among age-classes

Both regional and vertical heterogeneity of blubber lipid content was observed when compared across age-classes. The blubber of juvenile whales contained more lipid than all other age-classes at every site except the BL site. This appears to be in contrast to studies of fin whales (Aguilar and Borrell 1990; Lockyer 1987), in which pregnant females had higher lipid levels than immature whales. However, several differences between the studies may account for the discrepancy. First, the fin whale studies did not make the distinction between juvenile and subadult whales, and further may have been restricted in taking of the smaller whales, and thus the age-classes may not be comparable. However, bowhead subadult whales did not differ from mature females, which is similar to the results for fin whales. Furthermore, only one whale in this dataset had a full-term fetus, thus it is possible that had the other females lived, the lipid content of their blubber would have continued to increase until commencement of lactation.

The greatest difference in the mean blubber lipid contents among age-classes occurred at the UV site, followed by UL and BV (Table 2.4). In cetaceans, the best indicator sites of nutritional status were found to possess the greatest variability (Lockyer et al. 1985). However, prior studies generally established indicator sites based on variation in blubber thickness, and then measured blubber lipid content. If we apply Lockyer's criteria to lipid content data, then UV, UL and BV are likely the best indicator sites of nutritional status in bowhead whales. All other sites, even when significant, varied less by age-class than did the six sites within the mature male age-class (Table 2.4). The sample size of adult whales in different reproductive conditions is admittedly small in this dataset; the expansion of which would provide additional information as to the variation in condition at different sampling sites. Furthermore, although some whales were noted as "thin," severely emaciated whales were not observed. Thus, differences in condition were probably more subtle in this dataset. Under conditions of starvation in harbor porpoise, Koopman et al. (2002) observed significant changes in blubber thickness and adipocyte morphology in body sites between the nuchal crest and the anus. All six sampling sites chosen for this study (at the girth 1 m posterior to the blowholes and at the

umbilicus girth) are within the same range of sites that Koopman described as important for lipid deposition and mobilization. It is likely that all six sites are important contributors to total energy stores in the bowhead whale, but that UV, UL and BV are the most responsive to nutritional changes. However, it is unlikely that any of these sites serve primarily a structural role, as has been suggested for blubber of the throat grooves in fin and sei whales (Lockyer et al. 1984; Lockyer et al. 1985) or of the tailstock region in small cetaceans (Koopman et al. 2002).

When compared on a depth basis, the greatest difference in lipid content between age-classes occurred in the innermost layer, depth 5. Relatively large differences also occurred in depth 4. This is in agreement with observations of numerous authors who have concluded that the inner blubber layers of cetaceans appear to be more active in fat mobilization and deposition based on greater variability of lipid content, or in earlier literature, because of a larger range of iodine values indicative of dietary fatty acid input (Ackman et al. 1975a; Aguilar and Borrell 1990; Lockyer et al. 1984; Tveraaen 1935). Further evidence is given by Koopman et al. (1996, 2002) who found significant changes in fatty acid composition and adipocyte morphology in the inner layers of starved vs. normal harbor porpoise, but not in outer layers. The inner layer's physical proximity to active muscles also likely contributes to its suspected higher activity, in that it can supply the muscles with fatty acids for oxidation, which releases energy. The inner layer should also be nearer that of the core body temperature, which would allow for two important processes. First, the inner blubber lipids would be more like an oil compared to cooler outer blubber lipids (Castellini 2000) and thus more readily manipulated biochemically. Second, lipoprotein lipase and hormone sensitive lipase, the enzymes responsible for lipid deposition and mobilization in the adipocytes, respectively, should be more active at the higher temperatures found near the core.

Further understanding of the function of different blubber layers in the bowhead whale can be made by comparing them with layers of blubber from other cetaceans. However, the comparison is complicated by the differences in sampling procedure between studies. Owing to the great thickness in the blubber of bowhead whales, the

blubber core was divided into five equal depths, rather than the more typical divisions of two or three depths. This may have led to different definition of layers. For example, in this study significant differences between age-classes for depth 1 (layer nearest the epidermis) were found in contrast to studies in other baleen whales where outer layers did not differ between age-classes (Aguilar and Borrell 1990). Depth 1 in bowhead whales appeared to be the most structured of all the layers, with many collagen fibers in close proximity of each other and a tough papillary dermal layer connecting the blubber to the epidermis. It appears that this layer becomes more fibrous with "age," but eventually levels off in females (Fig. 2.10). Bowhead whales are known to break through sea-ice as great as 0.5 m in depth in order to create breathing holes, and it is possible that the increased structural capacity of the outer blubber may aid this ability. Intraspecific interaction amongst males has been documented in the breeding season, which may be why "tougher" outer layers in males of greater body length are observed. Lower lipid levels and the highly structural nature by gross visual inspection suggest that depth 1 serves mainly a structural role. Adipocyte volumes were found to not change in the outer layer of harbor porpoise blubber with nutritional status, leading Koopman et al. (2002) to hypothesize that the outer layer was more important for structural or mechanical roles such as streamlining or locomotion rather than an energetic role. Current studies of collagen fiber density (C. Rosa, UAF; study in progress) and future studies investigating adipocyte volume, under differing nutritive conditions such as during lactation or across seasons, should help clarify the role of the outer blubber layer in bowhead whales.

The outer-middle layer (depth 2 and 3) in bowhead whales may be more similar to the external stable layer in fin whales described by Aguilar and Borrel (1990). Both are characterized by high lipid levels, approximately 75-80% in fins and 82-90% in bowheads. However, differences between age-classes were not significant in fin whales yet were evident in bowheads. These differences were relatively small, varying by only a few percentage points between the juvenile, subadult and mature female age-classes. Individual group variation was very tight about the mean, allowing for statistical difference, but a difference that may not be biologically significant. Of all the layers

examined, depth 2 and 3 remained the most stable across age-classes (Fig. 2.6). It is likely that these layers serve as a long-term energy storage. These layers appear less structured than other layers and exhibited low protein contents (mean 2.8 - 4.7%, Mau, unpublished data), which may allow for greater expansion of the adipocytes, resulting in the higher lipid contents. This was similar to the low structural fiber density and high lipid contents in the equivalent depths of bottlenose dolphins reported by Struntz (2003).

The effect of season and year on lipid content within the immature age-class

Seasonal comparisons of lipid content within an age-class were possible for immature whales only, due to limitations of sample size. Significant seasonal differences of SBL were highly dependent on body length. It is interesting that although the relationship of lipid content and body length remained negative in each season, the slopes of these relationships were significantly different at three of the six sites. This suggests both regional variation in blubber lipid utilization and that the energy budget of immature bowhead whales appears to be changing with body length and presumably with age. Weaning is generally believed to occur in spring, but it may occur after the spring harvest. Thus it is possible that the smaller whales have a sufficient maternal input that seasonal differences cannot be detected even if seasonal feeding did occur. One must consider, however, that young whales of a given length may not be of the same exact age because of variation in length at birth and in birth date, which spans from March and August (Koski et al. 1993). Such differences in body length may create enough scatter to mask any seasonal differences. If juveniles are then omitted from the site means model, the slope differences are even greater, suggesting greater seasonal differences in the longer subadult whales (data not shown). However, greater sample sizes are needed for whales of 11-13 m in body length to confirm these results.

Of the four sites in which seasonal differences occurred, three were at the umbilicus girth. If the increase in lipid content observed in fall can be attributed to seasonal summer fattening, this would be consistent with results of previous studies of other baleen whales in which posterior blubber sites, especially at the dorsal position,

were found to be the most important sites of energy storage (Lockyer et al. 1985; Víkingsson 1990; Víkingsson 1995).

Although it appeared that fall lipid contents were higher than spring lipid contents for depths 2-5, only depth 5, the innermost layer was significant. Depth 5 represents only $1/5^{th}$ of the blubber thickness, a relatively small proportion, thus seasonal differences would appear to be fairly small in this age-group. However, the average of all the depth differences were sufficient to cause significantly higher fall lipid contents in both site and whole blubber and must not be overlooked. The results suggest that feeding opportunities and subsequent deposition of lipids in the blubber were greater in summer (Beaufort Sea) than in winter (Bering Sea), or that if feeding was rather uniform throughout the year, a greater proportion of lipids ingested were oxidized to meet immediate metabolic needs than were deposited in the blubber in winter compared to summer.

It is possible that the seasonal differences in blubber energy stores are conservative estimates of seasonal feeding given by the limits of the time of year in which whales can be sampled. Some bowhead whales appear to feed intensely on their initial westward migration from the western Beaufort Sea as evidenced from stomach contents of whales landed in September in Kaktovik, AK and in October in Barrow, AK (Sheffield and Lowry 2002). Some evidence suggests that bowhead whales feed in the late fall in the Western Chukchi Sea (off Chukotka) until the advancing ice-cover pushes them south into the Bering Sea, sometimes as late as early December. The contribution of 1.5-2 months of feeding after the fall harvest to the annual energy stores of the bowhead whale could be considerable, and evidence from stable isotope studies of baleen plates support this view (Schell et al. 1989). Furthermore, Lockyer (1987) suggested that seasonal fat storage served primarily as a reserve for reproduction, and was supported by evidence from fin whales in which dorsal/lateral blubber weight increased by 27% in pregnant females, but only 10-14% in other classes over the feeding season (Víkingsson 1995). Efforts should be made to obtain samples from additional mature female bowhead whales in order to test if the seasonal acquisition of energy strategy for reproduction is

also observed in this species or if energy is acquired over a longer time period and in all ranges of their distribution.

Although the effect of year could only be tested on immature whales sampled in the fall, the lack of difference on *TBL* between years suggests that the immature whales' access to prey did not vary during the summers within the study period.

Potential link between lipid content and puberty

The link between nutrition, body composition and various reproductive variables has been well established in mammals. Lockyer (1986; 1987; 1990) demonstrated in cetaceans that the level of fat reserves affected rates of ovulation and pregnancy, success of lactation, neonate size and the reproductive interval. Moreover, the size of whale at sexual maturity remained constant, but the age at sexual maturity varied with nutrition. In the bowhead whale, sexual maturity has been estimated at 13.0-13.5 m mean length in females and 12.0-13.0 m in males (Koski et al. 1993). Evidence for the onset of puberty in any cetacean is more difficult to obtain. Undernutrition has been shown to affect growth rates, and hence body weight and condition, which can ultimately inhibit hormone secretion and delay puberty (Foster and Olster 1985). Although we did not measure reproductive hormones in this study, insights to the timing of puberty may be obtained from the blubber fat-body length relationship.

In females, blubber lipid content decreased predictably with length to about 10-11 m, after which lipid content appeared higher than length would predict, as was found in *TBL* (Fig. 2.4b) and individual depths (Fig. 2.10 - 2.13). Although the length at which this occurs is too short to indicate sexual maturation, we may be witnessing puberty. Other lines of evidence suggest a metabolic or hormonal shift at about this length in bowhead whales. For example, Ylitalo et al. (2002) found that concentrations of lipophyllic PCB's and DDT's increased with length to approximately 10 m in females, after which the values declined gradually with length. In males, differences in seminiferous tubule diameter indicating puberty were found in whales as short as 11.5 m (O'Hara et al. 2002). The biotransformation processes associated with how male whales

metabolized PCB's also appeared to be influenced as males sexually matured (Hoekstra et al. 2002b). Finally, bowhead whales between 11-13 m in length are the least frequently landed whale of all size classes. This may be due to the growth spurt associated with puberty. The relationship between lipid stores, reproductive hormones and reproductive tract morphology and histology warrants future research.

Lipid stratification

The degree of lipid stratification within the blubber column, Δ lipid content, can be utilized as a sensitive indicator of the overall nutritive state of the animal. The pattern of lipid stratification within the blubber column varied by age-class, sex, season and site. Juvenile whales exhibited the least amount of stratification. Initially, juvenile blubber lipid stores are derived primarily from maternal input (nursing), which probably accounts for the large proportion of homogeneous blubber cores observed. Although we do not have exact ages for this group, previous studies have shown that baleen length in young whales can be a good estimator of age (Schell et al. 1989). Whales with baleen less than 100 cm long were probably less than two years of age and recently weaned. Almost half, or six of 13 juvenile whales in this study fit this category. However, the remaining whales in the juvenile age-class were likely one to several years beyond weaning (C. Rosa, UAF; age analysis in progress). The low occurrence of highly stratified blubber in this age-class suggests that utilization of blubber lipid reserves from maternal input is relatively slow, or that additional energy acquisition is sufficient. Adult females also exhibited a fairly high rate of homogeneous blubber. Large energy stores are important for pregnant females in order to support gestation and especially lactation, which in bowheads is believed to be 6-12 months in duration.

Stratification appeared to increase with age and was most pronounced in adult males. Aguilar and Borrell (1990) observed homogeneous blubber cores in younger fin whales, similar to this study, but adult males did not differ from the young. Bowhead whales of this study may differ from fins because of the time of year caught, species differences, differences in sampling scheme, and (or) access to prey or rate of lipid

utilization. Although more observations of highly stratified blubber cores were found in spring than in fall, suggesting less food availability and reliance of lipids mobilized from blubber stores in winter/spring, this conclusion must be met with caution. Large biases in the age-class and sex composition between seasons may account for these differences.

Insights into regional lipid mobilization and deposition can be gained by examining the stratification patterns among different body site locations. While homogenous blubber cores were found most frequently at dorsal sites, their overall distribution was fairly uniform over the body. This may indicate the final stages of fattening. In contrast, body site location differences were evident in the highly stratified cores. It appears that lipids are mobilized from ventral sites first, then from lateral sites and finally from dorsal sites. Because the amount of lipid affects the buoyant properties of blubber, this pattern of mobilization may allow for changes in condition without affecting the ability of a whale to naturally float with its dorsal side up.

Insights into regional lipid mobilization; a case study

The comparison of the lipid poor blubber of whale 00B4, a non-pregnant mature whale, to known pregnant whales offers an opportunity to theorize about potential regional differences in lipid mobilization and deposition. Whale 00B4 was landed in late May on her eastward migration to the eastern Beaufort Sea. Although known to be non-pregnant, the period of time since her last weaning of a calf is not known. Preliminary examination of both ovaries indicated no corpus luteum, several small corpora albicantia, and small but relatively few follicles, suggesting little reproductive activity in the recent past and no ovulation for the current year (E. Follman, pers. comm.). Lipids were depleted in all 3 umbilicus sites examined, suggesting some degree of uniform lipid mobilization. However, the largest degree of stratification occurred at the umbilicus ventral site. For comparison, a whale classified as early in lactation, 99B16, also had greater lipid depletion of the inner layers of ventral sites. Thus, even in the early stages of lactation, it appears that the umbilicus ventral site is preferentially targeted for lipid

mobilization. This conclusion is supported by the fact that differences among reproductive categories were greatest at UV site (Table 2.4).

A strong link among nutrition, body fat condition and pregnancy has been established in mammals (Frisch 1990). The lack of activity in the ovaries of 00B4 suggests that lipid stores were sufficiently depleted to inhibit ovulation. In fact, only whale 00B4 showed evidence of significant mobilization of lipids from the middle layer of blubber, depth 3, which differed from depth 2 by ~20 % at the UV site. Unfortunately it is not known if similar patterns occurred at blowhole sites, for these sites were not sampled. However, all other whales sampled had high levels of lipid at depths 2 and 3, reemphasizing their importance in long-term energy storage.

Functional role of blubber and implications of energy storage

"Extreme" fattening in wild animals is more prevalent in terrestrial polar regions and in rapidly changing, unpredictable climates (Pond 1998; Winstanley et al. 1999). Such extensive fattening allows animals to live off energy from stored fat when food is limited or unavailable. This type of seasonal fattening is seen in many cetacean species that migrate toward the poles to feed in summer and return to lower latitudes in winter for reproduction. Antarctic fin whales, for example, feed intensely for only approximately 120 days of the year and thus exhibit large seasonal fluctuations in body fat condition (Brodie 1975). However, some Atlantic fin whales have been found year-round in cold, high-latitude waters near Nova Scotia in apparently poor body condition. However, the observed thin blubber thickness was presumed attributable to year-round food availability, negating the need to store large amounts of fat (Brodie 1975). If food was always available and more importantly, predictable, then it would not be necessary to store large amounts of blubber fat. The data from this study, in contrast, fit neither of the above scenarios. The maintenance of thick blubber layers year round, as well as high lipid contents in $2/5^{ths}$ to $3/5^{ths}$ of the blubber column, corroborate previous suggestions that bowhead whales have evolved to maximize energy stores in anticipation of fasting periods of considerable and unpredictable durations. Burns (1993) speculated that

bowhead whales may be able to endure one full year without feeding or multiple consecutive years with low food availability, a duration that, if true, exceeds that of all other reports of mysticetes that undergo seasonal fasts. The need to maintain energy stores for such extended periods may mask large seasonal differences and awaits further testing.

Because bowhead whales remain associated with sea-ice year round in polar waters that average less than 0°C (Brower et al. 1988), insulation in the form of blubber is important for maintenance of thermal balance. By comparing blubber characteristics of bowhead whales with those of other species that inhabit the arctic, we can intuit that the quantity and quality of bowhead blubber exceeds that which is necessary for living in arctic waters. For example, Ryg et al. (1993) found that sculp thickness (blubber + skin) scaled linearly with body mass in 8 marine mammal species, many of which inhabit or visit arctic waters. When sculp thickness of bowhead whales was added, the data points were clearly well above the average line of the other species (Fig. 2.16). Given that the higher the lipid content of blubber, the better it can insulate, bowhead blubber (77% lipid) appears superior to blubber from a grey whale of similar body length (40% lipid; Sumich 1986), yet they can both be found in the waters near Point Barrow, AK. The blubber properties of bowhead blubber thus appear to exceed what is necessary for insulation, further supporting the concept for the need to store energy as a consequence of the unpredictability of the arctic environment. Hokkanen (1990) predicted that bowhead whales were so well insulated that a 13.0 m whale with 25 cm of blubber could swim in water at the equivalent temperature of liquid O_2, but that a decrease in metabolic rate could bring the whale closer to a thermal equilibrium at ocean temperatures. Perhaps this is yet another strategy by the bowhead whale to decrease total energy expenditure, thereby allowing more of their daily intake to go towards lipid deposition in the blubber.

Condition and population dynamics

The ability to validate blubber thickness and proximate composition measurements against other indices of condition, such as blubber mass and body mass,

were not feasible in this study, due to logistical constraints. However, we can speculate that the blubber thickness and lipid content are indicative of a population that is generally in good condition, by linking the baseline data provided here with population dynamics data (Lockyer 1990). The BCBS stock is the best characterized of all stocks of bowhead whales. Population abundance has been estimated regularly since 1976 with average annual increases of 3.3% per year from 1978-2001 (George et al. 2002b). Survival of adults is estimated to be high (Zeh et al. 2002). Current population estimates of 9,860 whales (95% C.I. 7700-12,600; George et al. 2002b) are in range with minimum estimated pre-exploitation levels of 10,400, but are still well below the maximum estimate of 23,000 whales (Woodby and Botkin 1993). In addition, record calf production (121 calves) was documented in the 2001 census, which was nearly twice that of any previous recorded numbers (George et al. 2002b). Food availability and/or body condition have been linked with fecundity in several marine mammal species (Boyd 1984; Guinet et al. 1998; Lockyer 1986; Pitcher et al. 1998; Víkingsson 1990). Pregnant females in this study were as small as 12.6 m, a length smaller than the previously reported length of sexual maturation (13-13.5m), which may indicate that food availability has been good in recent years, although both current and previous studies were based on small sample sizes.

We have shown some seasonality to body condition in immature whales, but while small sample sizes preclude direct conclusions regarding potential seasonal, annual or reproductive fluctuations in body condition for the adult age-classes, these data serve as a baseline by which to compare future samples. Perturbations of the Arctic and Bering Sea ecosystems, whether anthropogenic or natural, may impact bowhead health. As global climate change impacts the arctic ecosystem, temperature increases would likely cause changes in bowhead whale prey distribution and abundance, because of declines in the extent and concentration of sea ice. It is not known; however, whether this impact would be positive or negative. A loss of sea ice would result in a loss of productivity due to ice algae. On the other hand, open water productivity may increase in "warm" arctic waters due to accelerated growth and shorter generation times. The continual lack of ice

may alter arctic productivity dynamics in unknown ways. The feeding apparatus of bowhead whales is especially adapted for feeding on low abundance and patchy prey (George et al. 1999; Lowry 1993). Thus, perhaps the bigger impact might come from the loss of sea-ice habitat itself, with which the bowhead remains closely associated. Population size, reproduction and sea-ice extent have been linked for other arctic species such as ringed seal and polar bear (Stirling and Lunn 1997). The summer of 2002 was a record low ice year in the arctic in both extent and area (Serreze et al. 2003). We can speculate on a cascade of consequences for the bowhead in light of recent climate modeling that predicts the trend for decreasing arctic ice will continue throughout the 21st century (Serreze et al. 2003). As ice cover decreases, prey distribution and abundance will likely change. Less ice could also increase the risk of predation from orca, for bowheads are relatively slow moving animals but can escape to heavy ice-cover where most predators cannot venture. Finally, less ice may lead to permanent shipping lanes in the arctic and increased human activities for resource extraction, leading to increased disturbance, ship-strikes and loss of critical habitat (Tynan and DeMaster 1997). The bowhead response to such changes is likely to be reflected in the distribution and quantity of energy stores, which in turn can affect survival and life-history parameters. Continued monitoring of bowhead whale blubber energy stores and vital rates are necessary for understanding their response to ecosystem changes such that relevant conservation and management decisions can be implemented.

Recommendations for future sampling activities

Blubber thickness and composition measurements are necessary and important condition indices for monitoring the health of the bowhead population and its response to oscillations in population size and/or environmental conditions. However, economics, time constraints and statistical power necessitate that recommendations for a viable sampling scheme, far reduced from the current 30 samples per whale, be developed based on the currently available, yet incomplete data.

Lockyer et al. 1985 suggested that to maximize the value of condition indices in marine mammals, the measurements (here blubber thickness or lipid content) should be taken at the site of maximum variability. For many marine mammal species indicator sites were generally found at posterior and dorsal regions of the body (Beck and Smith 1995; Perryman and Lynn 2002; Ryg et al. 1988; Víkingsson 1995). In this study, for nearly every parameter examined (i.e., age-class, season, stratification index), greater variability was evident at the umbilicus sites. Preference should therefore be given to the umbilicus sites for future condition assessments.

Also worthy of consideration is the comparability to indicator sites of previous studies on other cetacean species. Blubber from the lateral flank has commonly been used in condition studies for beluga, blue, fin, minke, pilot, sei, southern right, and sperm whales. Posterior dorsal samples were also commonly chosen in blue, fin and sei whale studies. Anterior dorsal samples were the third mostly likely used sampling site in fin, sei, pilot and sperm whale studies. Finally, the likelihood of obtaining a given sample site is important for consistency. Bowhead whales are flensed in a consistent manner; however, landmarks are not always visible, depending on how the whale is drawn up on the beach or blubber may be removed before the arrival of biologists. Both of these problems are a greater limitation for obtaining samples from umbilicus sites. It is suggested that distances from the tip of the rostrum to the umbilicus be measured and expressed as a percentage of body length. Umbilicus sites could then be sampled with sufficient confidence of location in absence of a landmark. Preliminary measurements on 6 whales of a wide range of body lengths were very promising, with the umbilicus girth consistently at 57% $\pm$ 0.2 of body length and showed little variance (C. George, unpublished data).

It is evident from data on depth differences and stratification that sampling of the complete blubber core should continue. These data assist in assessing condition, and if coupled with studies on adipocyte morphology, enzyme activity and fatty acid profiles, would further our understanding of the relationship between blubber composition and specific functions of the layers.

Also not to be overlooked is the potential contribution of muscle lipids to the overall energy budget and condition of the bowhead whale. Posterior dorsal muscle lipids in Antarctic fin and blue whales contained 20% and 37% lipid, respectively (Feltmann et al. 1948). In Atlantic fin and sei whales, which do not obtain the same level of fatness as their Antarctic counterparts, muscle lipid content at comparable sites was much lower at 16.2% and 15.3%, respectively. The authors concluded that muscle represented an important energy depot and should be considered for whole whale energetics (Lockyer et al. 1985). To date muscle lipids in bowhead whales have not been examined, though samples have been archived. George et al. (2002) found that girth to body length ratios in subadult bowhead whales were greater in fall than in spring. If blubber thickness is not changing seasonally, then girth differences must come from changes in the core. These findings suggest that muscle lipids may play a role in seasonal energetics. Organ weights should also be considered for seasonal changes but with the realization that such efforts are more difficult logistically.

In conclusion, the effects of body length, age-class, sex, season and sampling location were important in the determination of blubber lipid content and therefore condition in bowhead whales. Although the results of this study greatly increase our knowledge of bowhead energy stores, several data gaps exist in this data set and future sampling efforts should focus on these needs. Samples from spring landed whales are needed to increase statistical power in making seasonal comparisons. Furthermore, only 13 of the 48 whales of this study were mature. Obviously more samples are needed to examine the effect of reproductive status on blubber characteristics as well as any seasonal effects. Because signs of puberty may be evident as early as 11m, all whales $\geq$ 11m should be sampled and reproductive organs examined.

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Table 2.1. Biological data and blubber samples from bowhead whales harvested from Fall 1998–Fall 2001 in Barrow and Kaktovik, Alaska.

Whale ID[a]	Sex	Age/Repro. Status	Harvest Date	Length (m)	Blubber Samples Analyzed	Notes/observations at time of harvest
98B10	F	MATURE	09.19.98	12.98	BD,BL,BV,UD,UL,UV	85 cm scar on belly
98B12	F	SUBADULT	09.19.98	11.30	BD,BL,BV,UD,UL,UV	2 – 50 cm scars
98B15	F	JUVENILE	09.22.98	8.46	BD,BL,BV,UD,UL,UV	Mud in mouth
98B17	M	JUVENILE	09.24.98	8.64	BD,BL,BV,UD,UL,UV	Ingutuk[b], benthic organisms in stomach
98B19	M	SUBADULT	09.28.98	9.52	BD,BL,BV,UD,UL,UV	Rope wound or ship strike on peduncle?
98B22	F	JUVENILE	10.05.98	8.03	BD,BL,BV,UD,UL,UV	Polar bear scratches; large white patch on flukes
98B23	M	SUBADULT	10.05.98	11.74	BD,BL,BV,UD,UL,UV	Golfball-sized abscess in thorax; old bomb scars on lung; inflamed lymph nodes
98B24	F	SUBADULT	10.07.98	10.29	BD,BL,BV,UD,UL,UV	2 paired puncture wounds; possible polar bear scars
99B1	F	SUBADULT	04.28.99	10.40	BD,BL,BV,UD,UL,UV	Very skinny, thin blubber, narrow girths
99B2	F	JUVENILE	05.02.99	8.15	BD	
99B3	F	JUVENILE	05.02.99	7.80	BD,BV	Fairly thin girths
99B4	F	JUVENILE	05.05.99	7.90	BD,BL,BV,UD,UL,UV	Very round, ingutuk
99B6	F	PREGNANT	05.06.99	12.60	BD,BV	~13 cm fetus
99B7	F	PREGNANT	05.08.99	15.40	BD,BL,BV,UD,UL,UV	~40 cm fetus; white around eye, base flipper
99B8	M	SUBADULT	05.09.99	10.95	BD,BL,BV,UD,UL,UV	
99B9	M	SUBADULT	05.09.99	9.26	UD,UV	
99B10	F	SUBADULT	05.09.99	9.42	BD,BV	Line scars on peduncle, lip, mouth; baleen damaged by entanglement, thin
99B12	F	SUBADULT	05.13.99	9.20	BD,BL,BV,UD,UL,UV	Thin, long baleen for length
99B13	M	MATURE	05.16.99	14.09	BL,BV,UD,UL,UV	Healed entanglement wounds; white on baleen, flipper, around eye
99B14	M	MATURE	05.17.99	14.15	BD,BL,BV,UD,UL,UV	Rope around R flipper, fluke; blubber froze on ice, looked old
99B16	F	LACTATING	05.21.99	14.83	BD,BL,BV,UD,UL,UV	CA on both L & R ovary, tears on vaginal opening, post parturient
99B18	F	PREGNANT	05.23.99	13.00	BD,BL,BV,UD,UL,UV	Full term fetus ~400 cm
99KK1	F	JUVENILE	09.11.99	7.70	BD,BV,UD,UV	Ingutuk, very fat, copious amount of fat around internal organs, omentum
99KK2	M	SUBADULT	09.12.99	12.88	BD,BV,UD,UV	Just becoming mature

Table 2.1 continued. Biological data and blubber samples from bowhead whales harvested from Fall 1998–Fall 2001 in Barrow and Kaktovik, Alaska

99KK3	M	JUVENILE	09.16.99	8.33	BD,BV,	Scarring–possible ship strike?
99B19	F	JUVENILE	10.09.99	8.05	BD,BL,BV,UD,UL,UV	Ingutuk
99B20	F	SUBADULT	10.10.99	9.03	BD,BL,BV,UD,UL,UV	
99B21	M	SUBADULT	10.10.99	10.49	BD,BL,BV,UD,UL,	
99B22	F	SUBADULT	10.12.99	9.72	BD,BL,BV,UD,UL,UV	
99B23	M	SUBADULT	10.13.99	10.89	BD,BL,BV,UD,UL,UV	
99B24	M	JUVENILE	10.13.99	8.84	BD,BL,BV,UD,UL,UV	Ingutuk
00B1	M	SUBADULT	04.24.00	9.42	BD,BL,BV,UD,UL,UV	Ingutuk?
00B2	F	MATURE	05.25.00	14.46	BD,BL,BV,UD,UL,UV	Large CA, 3-4 small CA's
00B3	F	PREGNANT	05.25.00	14.58	BD,BL,BV,UD	38.2 cm fetus
00B4	F	MATURE	05.25.00	15.36	UD,UL,UV	Possible killer whale scar on tongue; possible follicle on L ovary[c]
00B5	F	PREGNANT	05.30.00	17.50	BD, UD,UL,	~60 cm fetus; deep, healed puncture wound R side
00KK1	F	SUBADULT	09.02.00	9.40	BD,BL,BV,UD,UL,UV	
00KK2	M	SUBADULT	09.03.00	12.10	BD,BL,BV,UD,UL,UV	Scars on lip
00KK3	F	JUVENILE	09.08.00	8.84	BD,BL,BV,UD,UL,UV	
00B7	M	JUVENILE	09.29.00	8.65	BD,BL,BV,UD,UL,UV	Ingutuk
00B9	F	JUVENILE	09.30.00	7.89	BD,BL,BV,UD,UL,UV	3 puncture wounds; possible line scar
00B11	M	MATURE	10.01.00	13.82	BD	Scar on leading edge L flipper
00B12	M	SUBADULT	10.03.00	10.85	BD,BL,BV,UD,UL,UV	~1m long scar on dorsum
00B13	M	SUBADULT	10.06.00	9.40	BD,BL,BV,UD,UL,UV	
00B14	F	SUBADULT	10.06.00	9.94	BD,UD,UL,UV	
00B15	F	JUVENILE	10.08.00	8.89	BD,BL,BV,UD,UL,UV	
00B16	F	SUBADULT	10.08.00	10.03	BD,BL,BV,UD,UL,UV	
01B27	M	MATURE	10.09.01	15.60	BD,BV,UD	

[a] Whale ID format: The first two digits are the year taken, followed by the village (e.g. B=Barrow, KK=Kaktovik), followed by the number for that whale in the serial sequence of whales landed in that village for that year.

[b] Ingutuk - an Inupiat word referring to a small rotund whale. Believed to have been recently weaned.

[c] After harvest date, cross-sections of ovary for 00B4 revealed very few and small follicles, no CA present, appeared quiescent.

Abbreviations: BD-blowhole dorsal, BL-blowhole lateral, BV-blowhole ventral, UD-umbilicus dorsal, UL-umbilicus lateral, UV-umbilicus ventral, CA-corpus albicans.

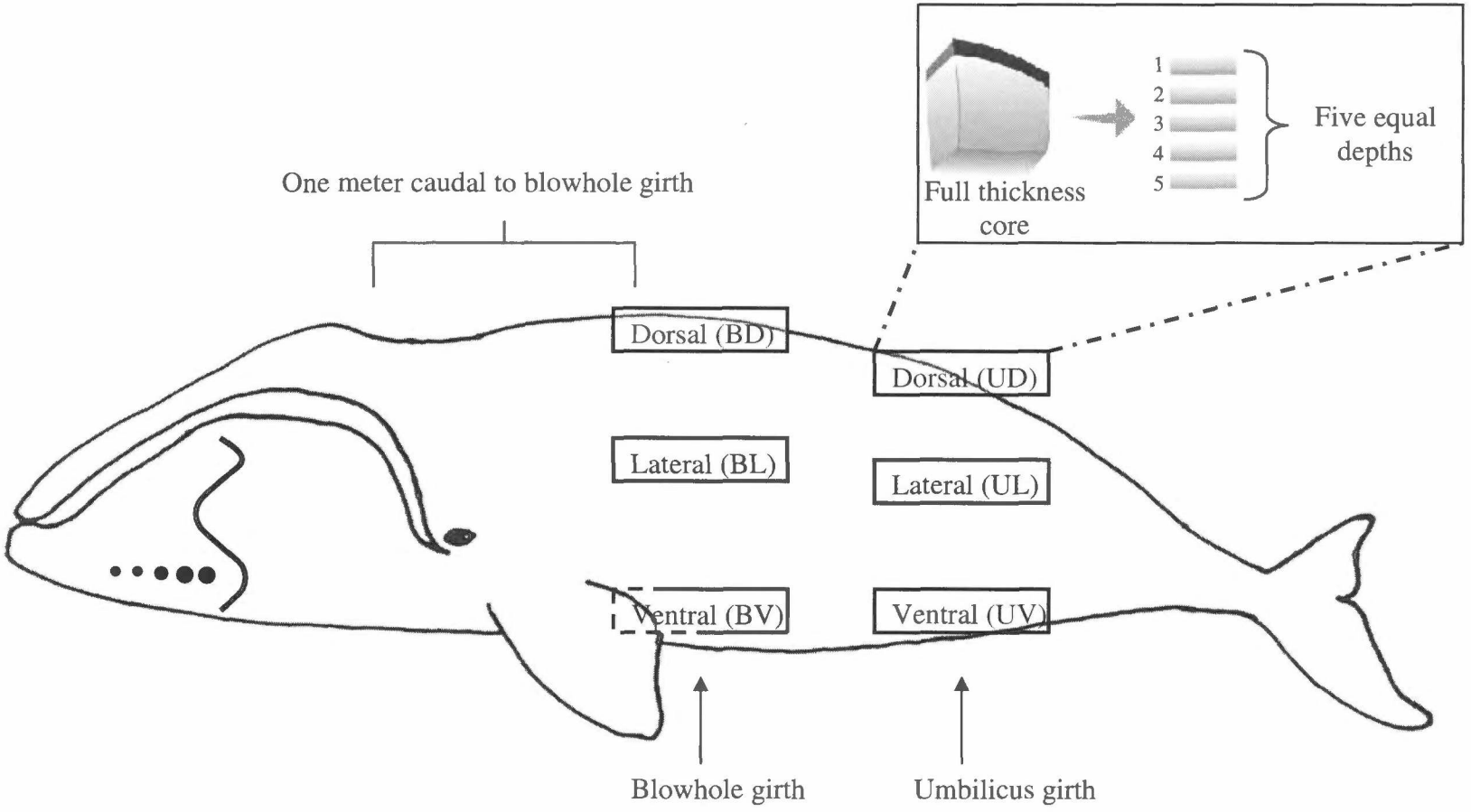

Fig. 2.1. Location of blubber thickness and lipid content measurements on bowhead whales. Measurements were made at three locations (dorsal, lateral, ventral) at two girths (one meter caudal to blowhole girth and umbilicus girth). Full thickness blubber cores were subdivided into 5 equal depths (inset).

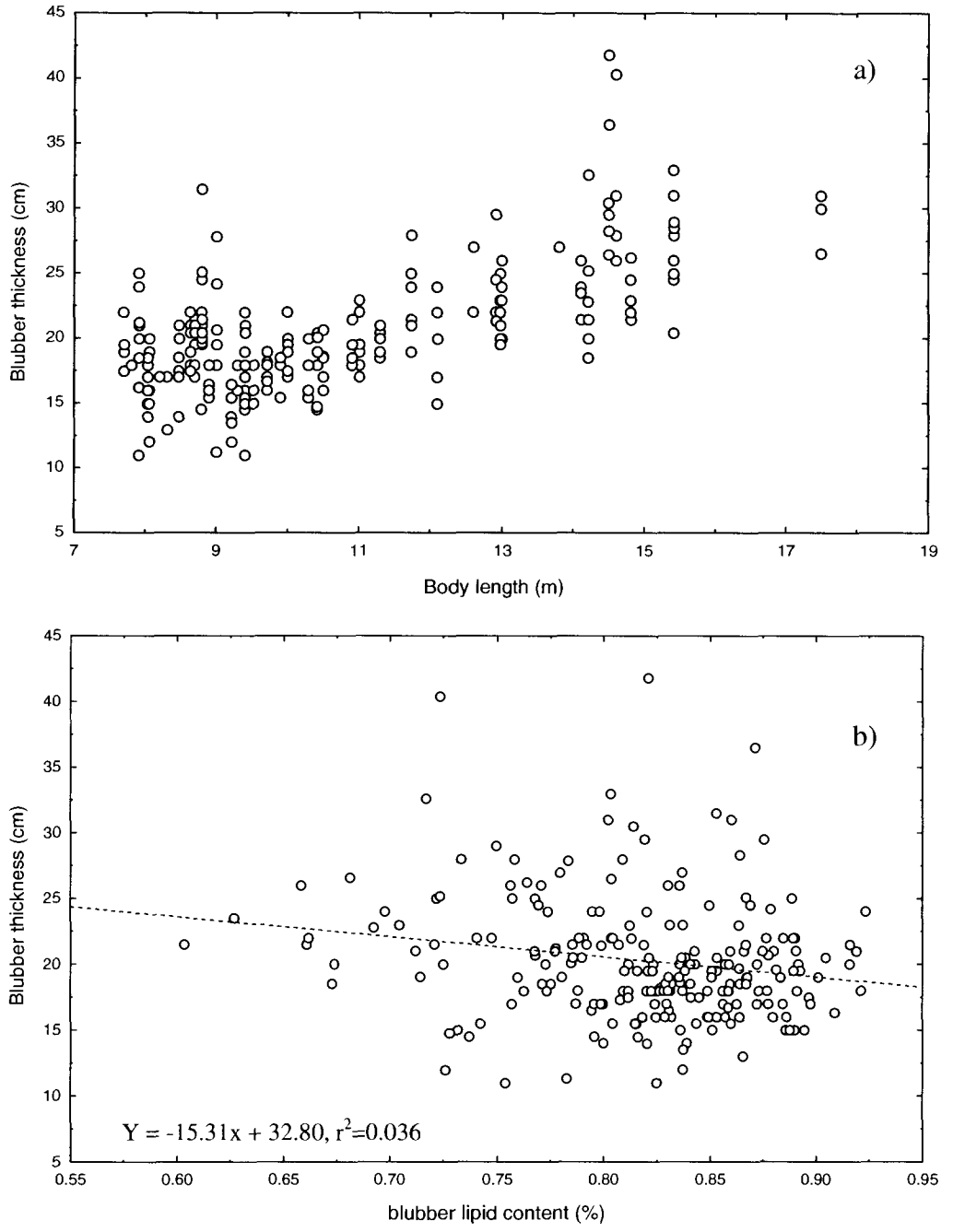

Fig. 2.2 Relationship between (a) blubber thickness and body length and (b) blubber thickness and blubber lipid content in bowhead whales.

Table 2.2 Blubber thickness measurements (cm) at six body site locations in juvenile, subadult and mature bowhead whales harvested from Fall 1998-Fall 2001.

	Juvenile				Subadult				Mature			
Site[1]	Mean ±SE*	Range	s^2	n	Mean ±SE	Range	s^2	n	Mean ±SE	Range	s^2	n
BD	19.3±0.58	16.5-24.5	4.7	14	20.5±0.82[ab]	15.5-29.5	12.7	19	30.2±2.06	23.0-41.8	42.5	10
BL	16.9±1.15	11.0-21.5	13.4	10	17.1±0.83[ac]	11.0-24.0	11.0	16	25.9±1.69	20.0-36.5	25.8	9
BV	17.5±0.75	13.0-22.0	7.2	13	17.6±0.48[b]	14.5-22.0	4.2	18	24.6±1.34	18.5-30.0	16.1	9
UD	19.9±1.13	15.0-25.1	14.0	11	18.6±0.52	15.0-22.0	2.1	19	26.1±1.18	22.0-31.0	12.6	9
UL	18.8±0.82	15.0-22.0	6.7	10	17.7±0.56	13.5-24.0	5.7	18	23.0±1.16	19.5-28.3	9.5	7
UV	20.4±1.25	16.0-31.5	17.2	11	20.5±0.91[c]	12.0-27.9	14.2	17	24.5±1.42	21.0-30.5	14.2	7

[1]Abbreviations as in Table 2.1.

*Within an age-class, means followed by the same letter were significantly different; no letters within an age-class indicate no significant differences amongst sites

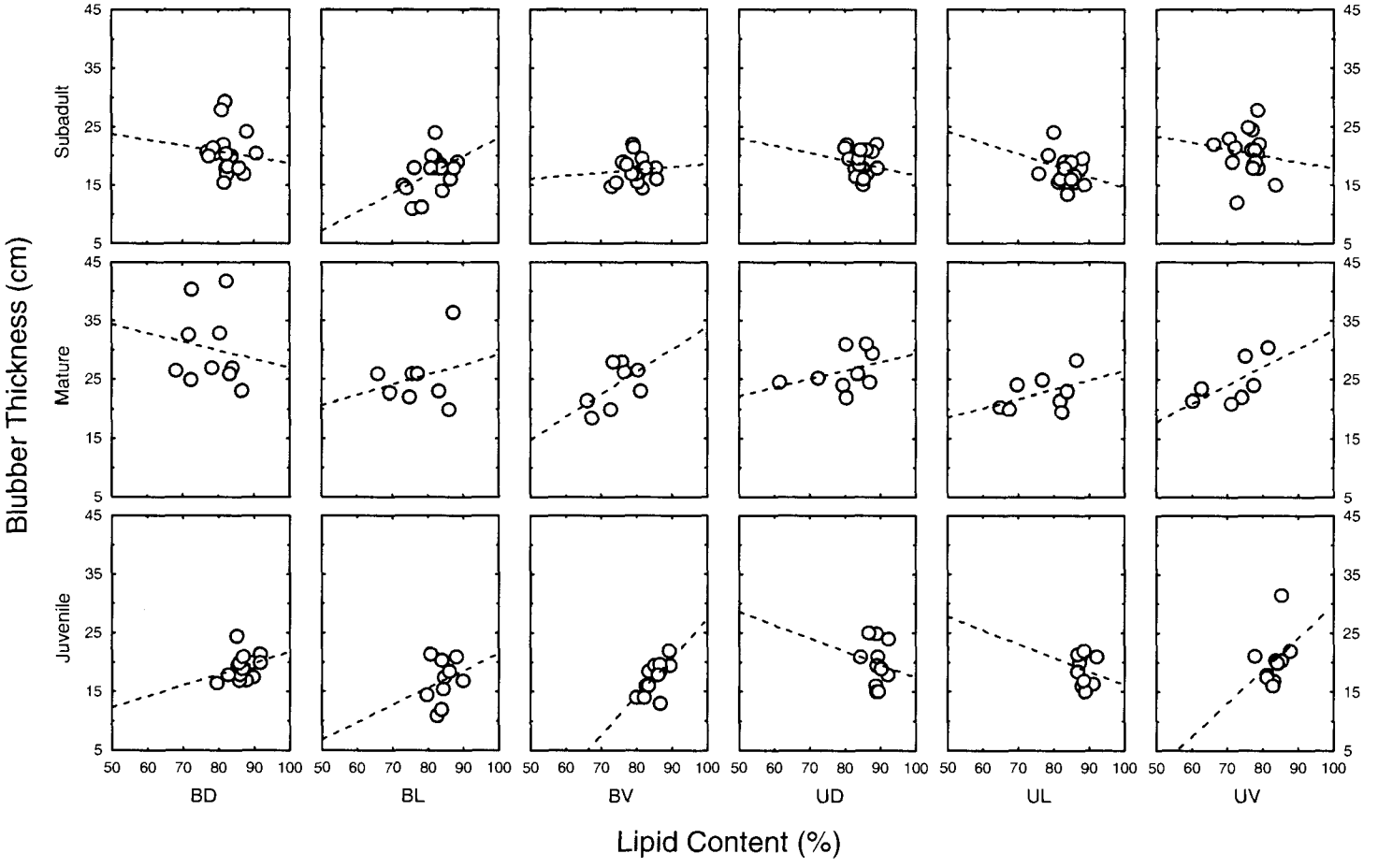

Fig. 2.3. Comparison of the linear relationship between blubber thickness (cm) and blubber lipid content (%) for subadult (top), mature (middle) and juvenile (bottom) bowhead whales at six body site locations. Abbreviations same as in Table 2.1, n as in Table 2.2.

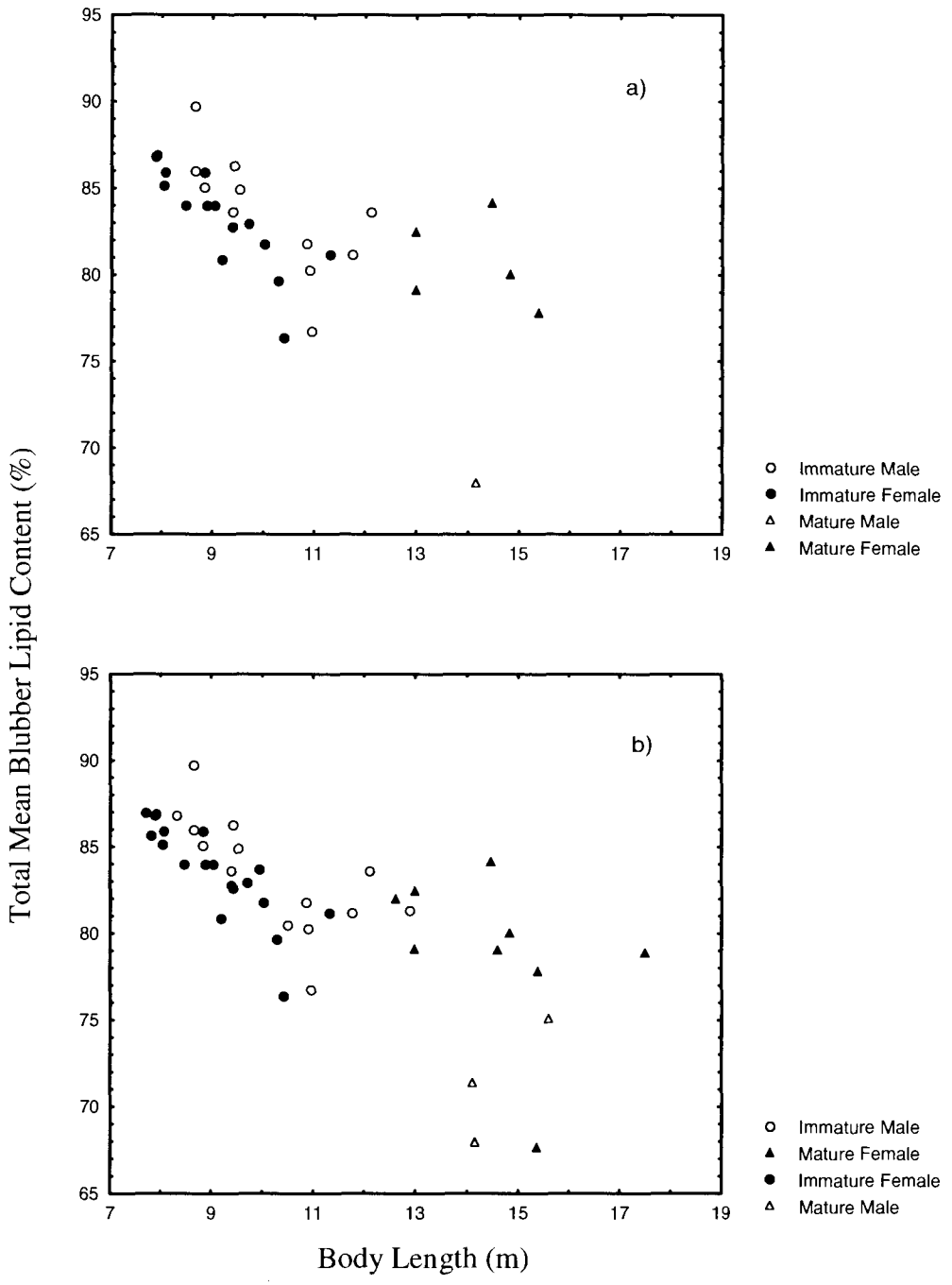

Fig 2.4. Relationship between total mean blubber lipid content (*TBL*) and body length in the bowhead whale. a) Whales with complete sample sets (n=32) Each symbol represents the mean of all 30 blubber subsamples of a given whale. b) Whales from panel a, as well as whales with *TBL* estimated from a site mean (n=45). See equations 1-9 in results for regression relationships.

Table 2.3. Regression equations for the prediction of the percentage total mean blubber lipid content (*TBL*) from the percentage site mean blubber lipid content (*SBL*) in bowhead whales.

Site	Regression equation	r^2 value	*P* value
All complete whales (n=32)			
BD	$TBL = 0.694(SBL) + 24.66$	0.654	<0.001
BL	$TBL = 0.573(SBL) + 35.67$	0.495	<0.001
BV	$TBL = 0.722(SBL) + 24.28$	0.797	<0.001
UD	$TBL = 0.887(SBL) + 6.41$	0.742	<0.001
UL	$TBL = 0.759(SBL) + 18.41$	0.845	<0.001
UV	$TBL = 0.572(SBL) + 38.15$	0.652	<0.001
All complete whales except whale 99B14 (n=31)			
BD	$TBL = 0.556(SBL) + 36.46$	0.582	<0.001
BL	$TBL = 0.418(SBL) + 48.61$	0.362	<0.001
BV	$TBL = 0.610(SBL) + 33.49$	0.756	<0.001
UD	$TBL = 0.766(SBL) + 16.87$	0.590	<0.001
UL	$TBL = 0.698(SBL) + 23.67$	0.745	<0.001
UV	$TBL = 0.456(SBL) + 47.31$	0.505	<0.001

Site abbreviations are given in Table 2.1.

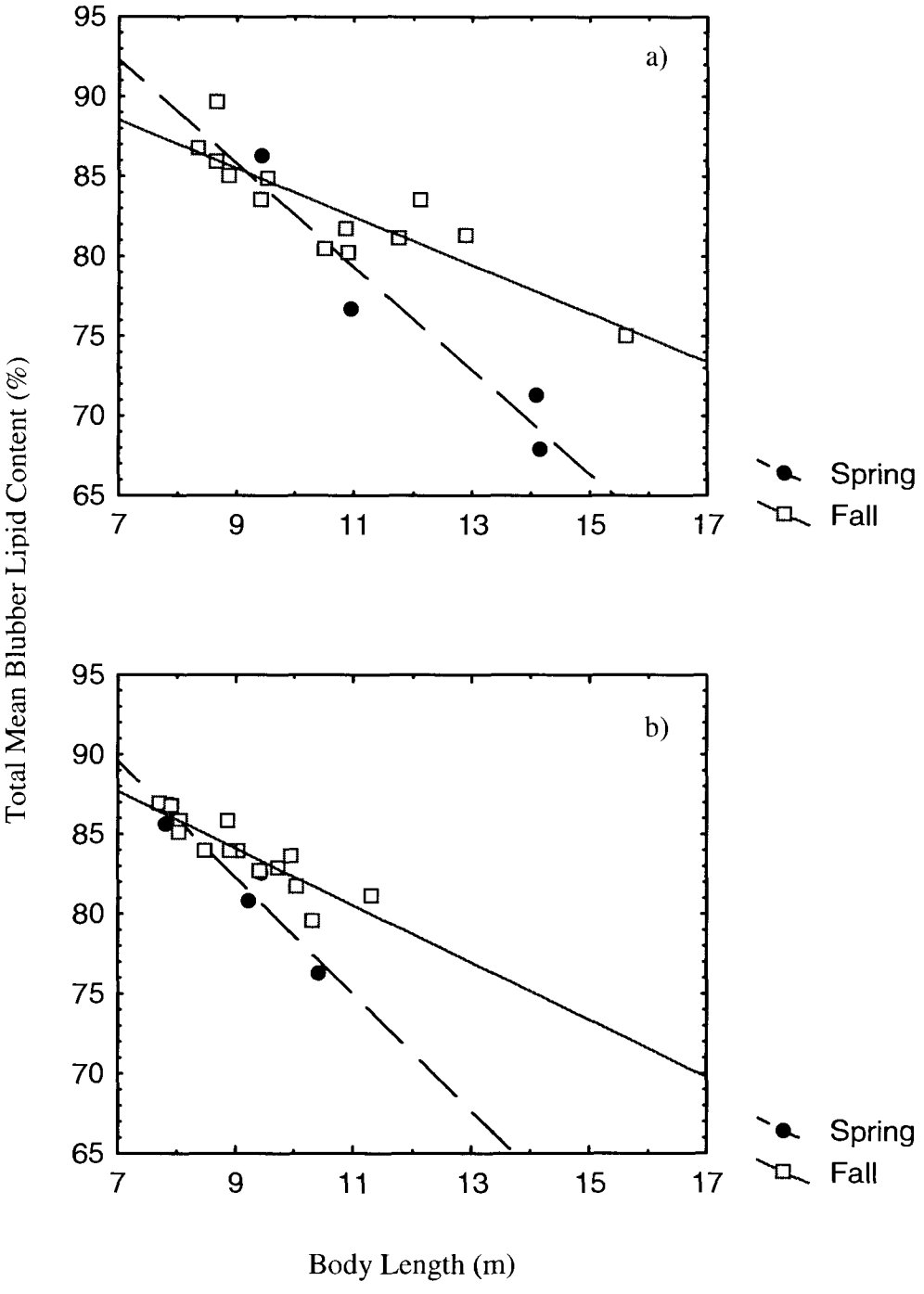

Fig. 2.5. Relationship between total mean blubber lipid content and body length in spring (solid circles) and fall (open squares) for a) all male and b) subadult female bowhead whales. See equations 10-13 in results for n and regression equations.

Table 2.4. Mean ± SE (n) lipid content (%) at each site and the maximum difference between age-class category means at each site for bowhead whales. Within each column, mean site lipid contents of age-class categories followed by the same letter were not significantly different. Sites without letters had no significant differences among categories.

	Blowhole sites			Umbilicus sites		
Age-class	BD	BL	BV	UD	UL	UV
Juvenile	86.6 ± 1.0 (14)[a]	84.3 ±1.5 (10)	85.1 ± 0.8 (13)[b]	89.0 ± 0.8 (11)[b]	88.4 ± 0.8 (10)[b]	83.3 ± 1.1 (11)[b]
Subadult	82.4 ± 0.8 (20)[b]	81.2 ± 1.1 (17)	79.9 ± 0.7 (19)[a]	84.5 ± 0.6 (20)[a]	83.6 ± 0.6 (18)[a]	76.4 ± 0.8 (19)[a]
Mature Female	78.7 ± 1.3 (8)[b]	78.7 ± 1.9 (6)	77.9 ± 1.1 (7)[a]	83.8 ± 1.0 (7)[a]	81.8 ± 1.1 (6)[a]	75.8 ± 1.6 (5)[a]
Mature Male	77.5 ± 2.2 (3)[b]	73.1 ± 3.2 (2)	67.9 ± 1.7 (3)[c]	76.8 ± 1.5 (3)[c]	68.6 ± 1.9 (2)[c]	61.5 ± 2.6 (2)[c]
Maximum difference	9.1	11.2	17.2	12.2	19.8	21.8

Abbreviations for sample sites: BD=blowhole dorsal, BL=blowhole lateral, BV=blowhole ventral, UD=umbilicus dorsal, UL=umbilicus lateral, UV=umbilicus ventral.

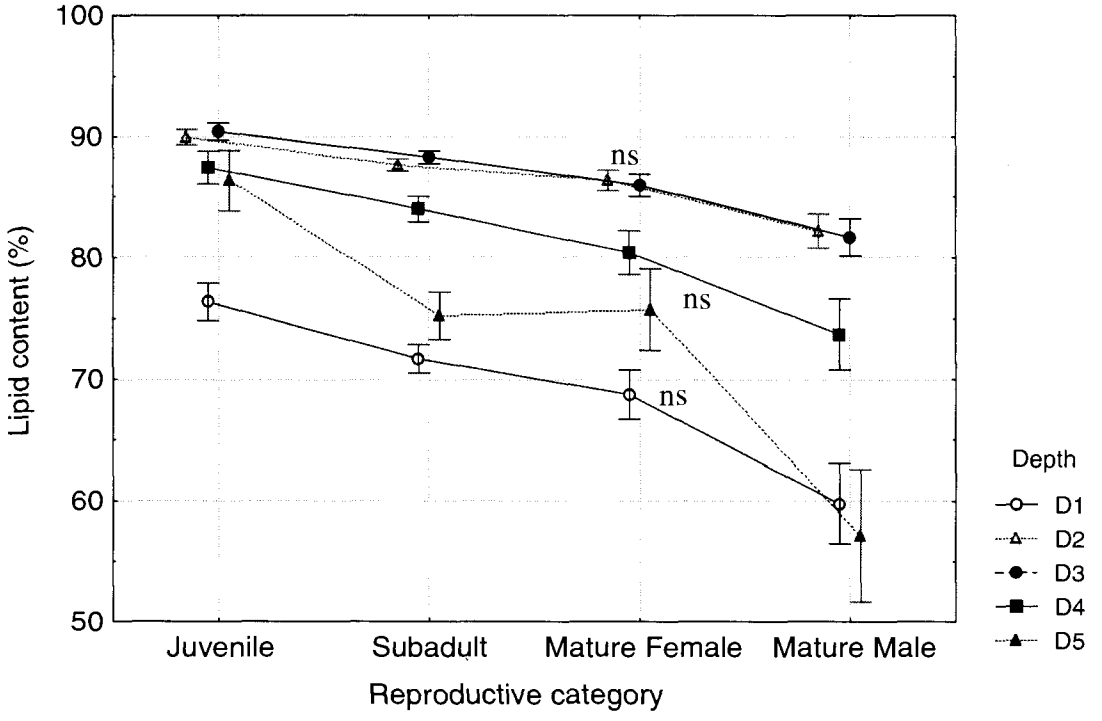

Fig. 2.6. Comparison of lipid content (mean ± SE) at all five depths of the blubber core for juvenile, subadult, mature female and mature male bowhead whales. Depth one is nearest the epidermis, whereas depth 5 is just above the muscle. "ns" indicates no difference between subadult and mature females at that depth. All other comparisons between reproductive categories were significantly different at each depth.

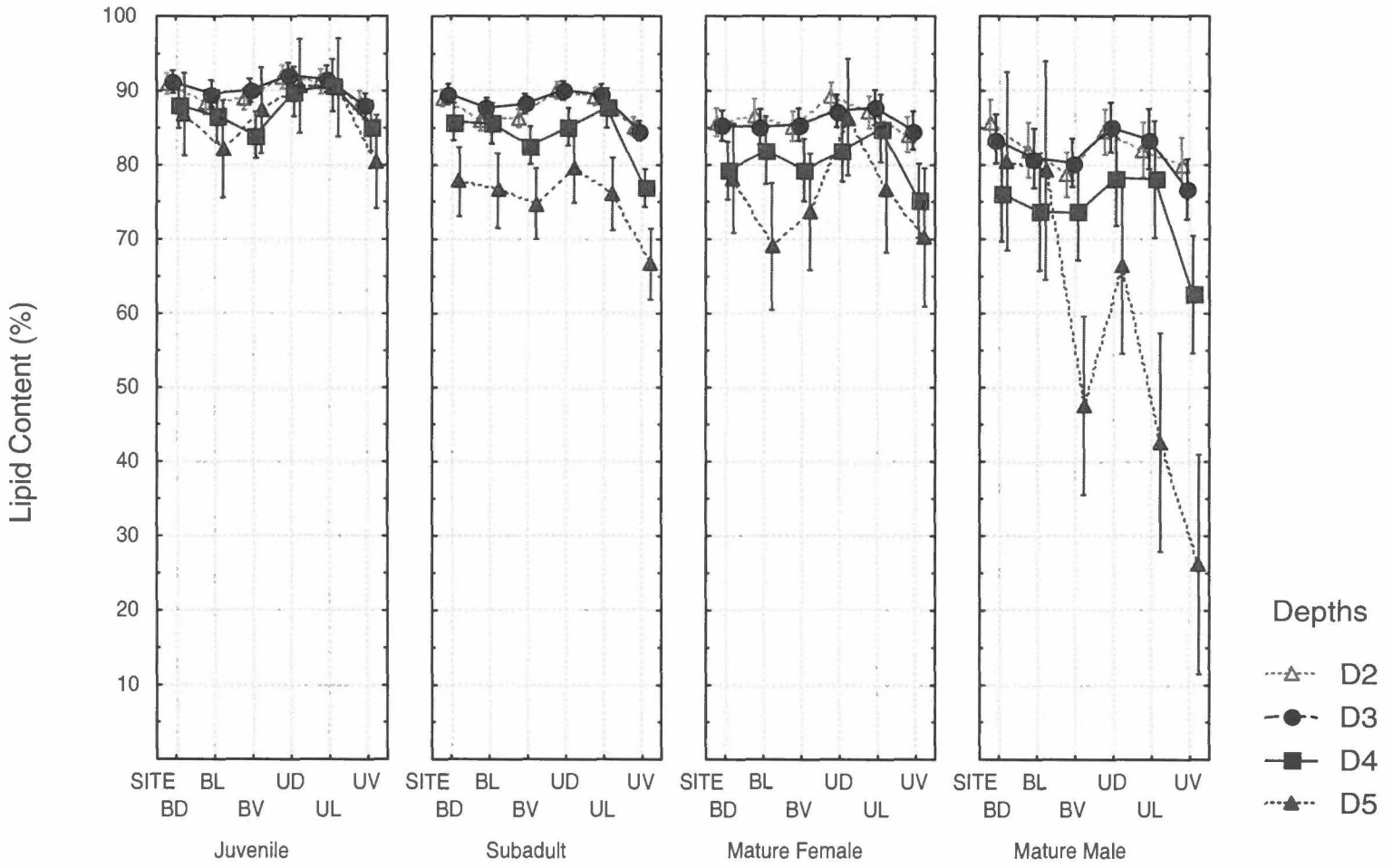

Fig. 2.7. Mean ± SE depth lipid content at six body site locations for juvenile, subadult, mature female and mature male bowhead whales. Depth 1 omitted for graph clarity.

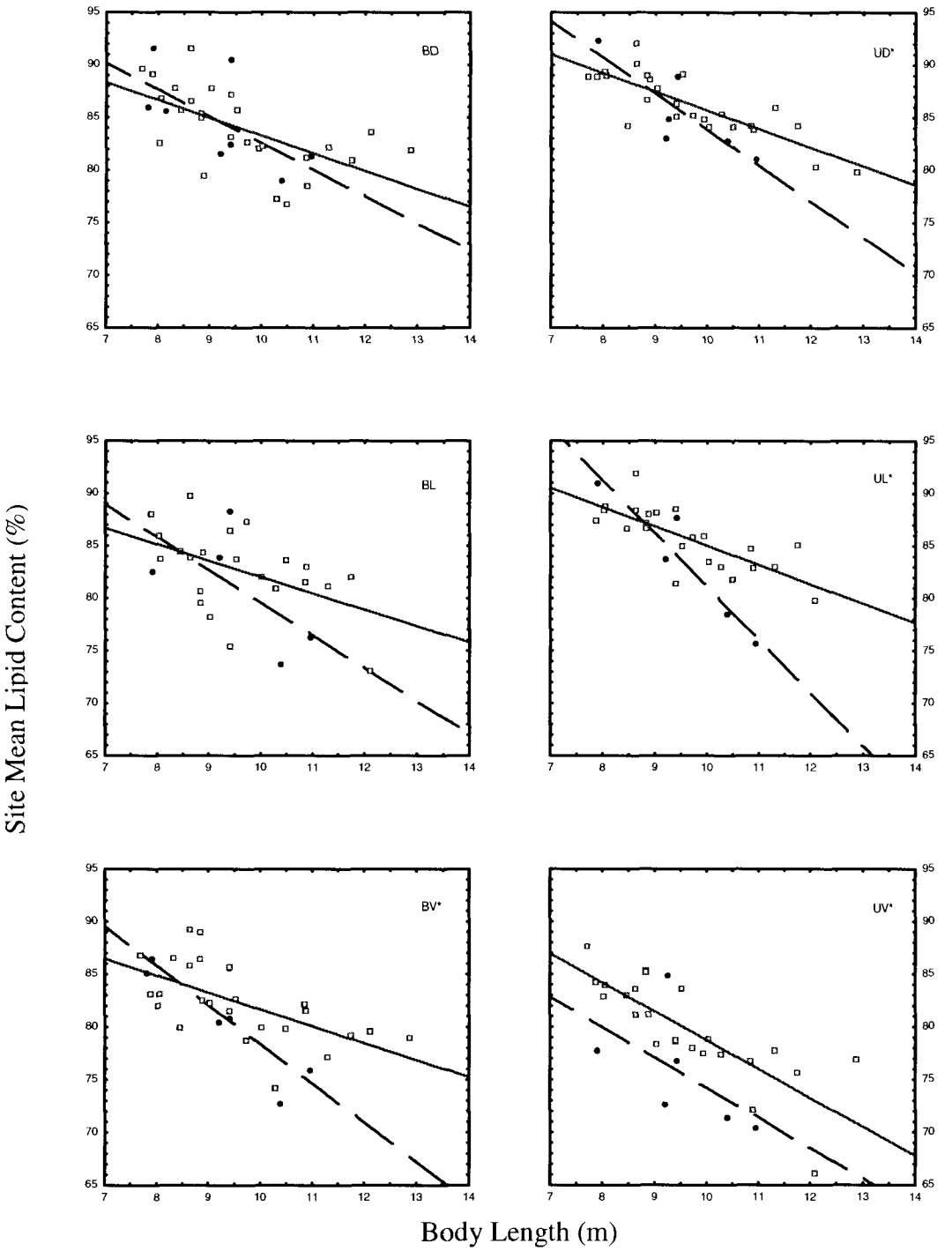

Fig. 2.8. Relationship between site mean lipid content and body length for spring (solid circles) and fall (open squares) landed bowhead whales. Site names with an * indicate a significant seasonal difference. Slope of linear regressions was significantly different between seasons for BV, UD, and UL.

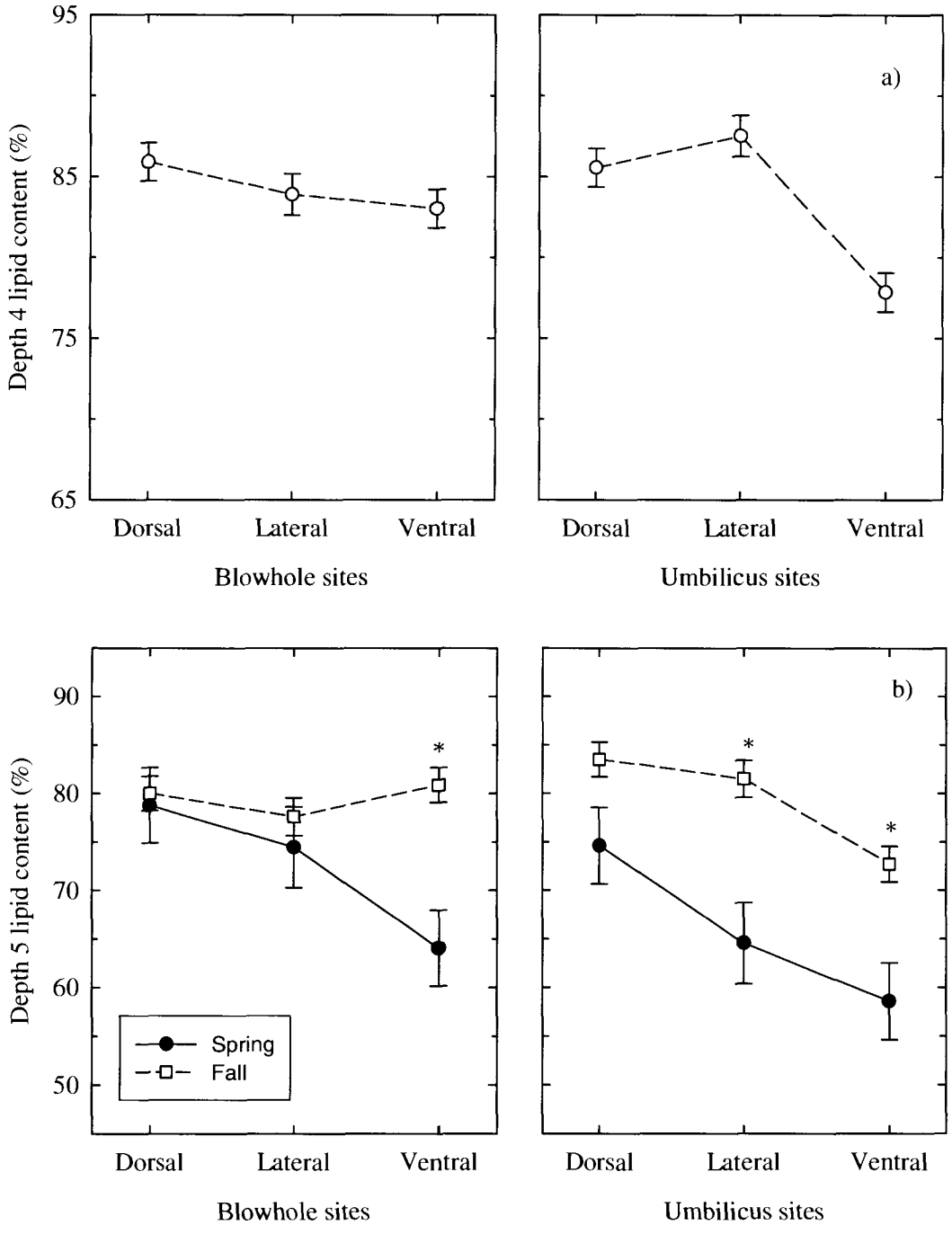

Fig. 2.9. Mean ± SE lipid content computed at a body length of 10 m for six body site locations in immature bowhead whales at a) depth 4, indicating site differences and b) depth 5, indicating site and seasonal differences. Closed circles indicate spring values, whereas open squares indicate fall values. Stars indicate a significant seasonal difference at that site.

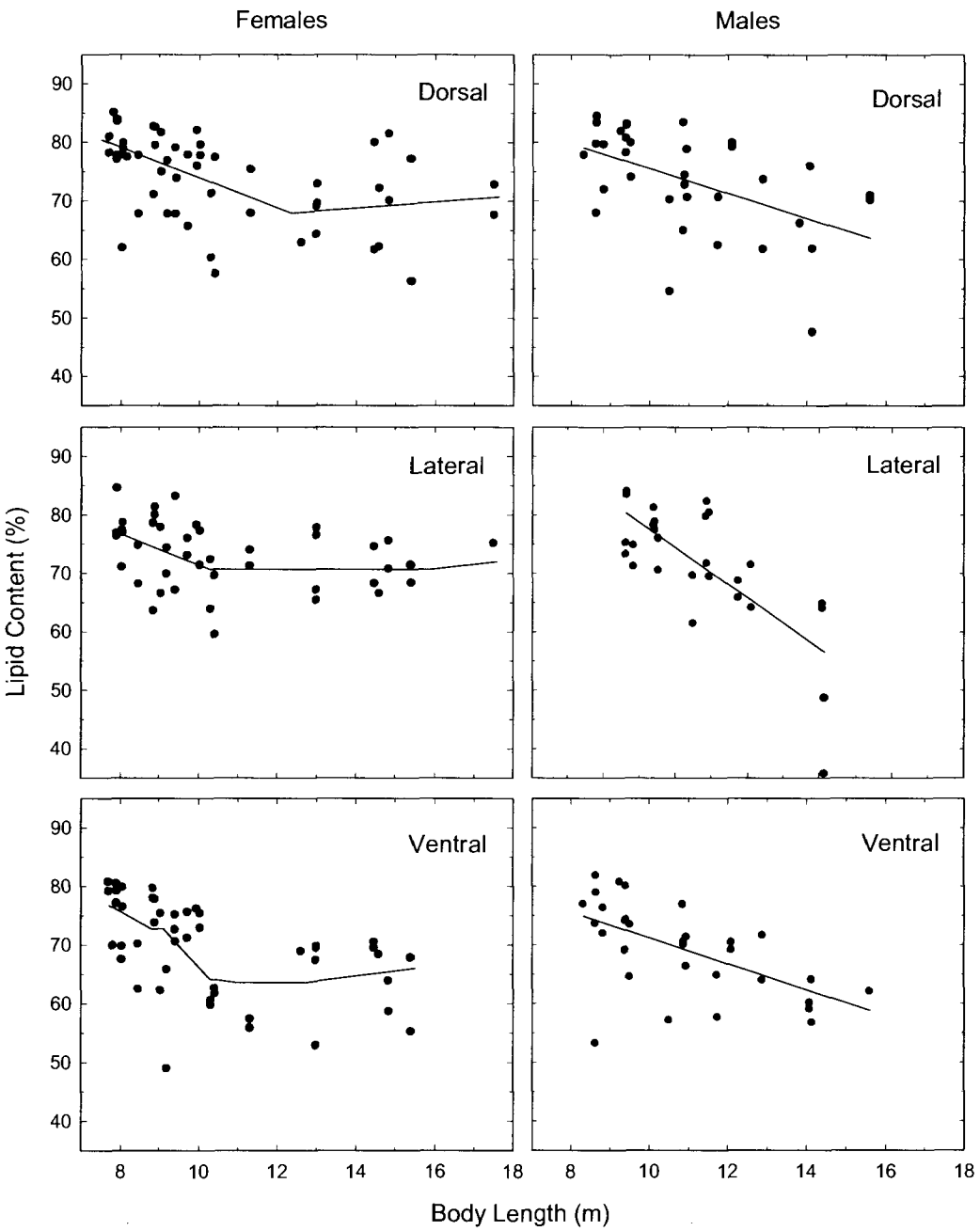

Fig. 2.10. Linear or Lowess relationship of percent lipid content at depth 1 (nearest the epidermis) of the blubber core and body length for females (left) and males (right) at three sampling positions (dorsal, lateral, ventral).

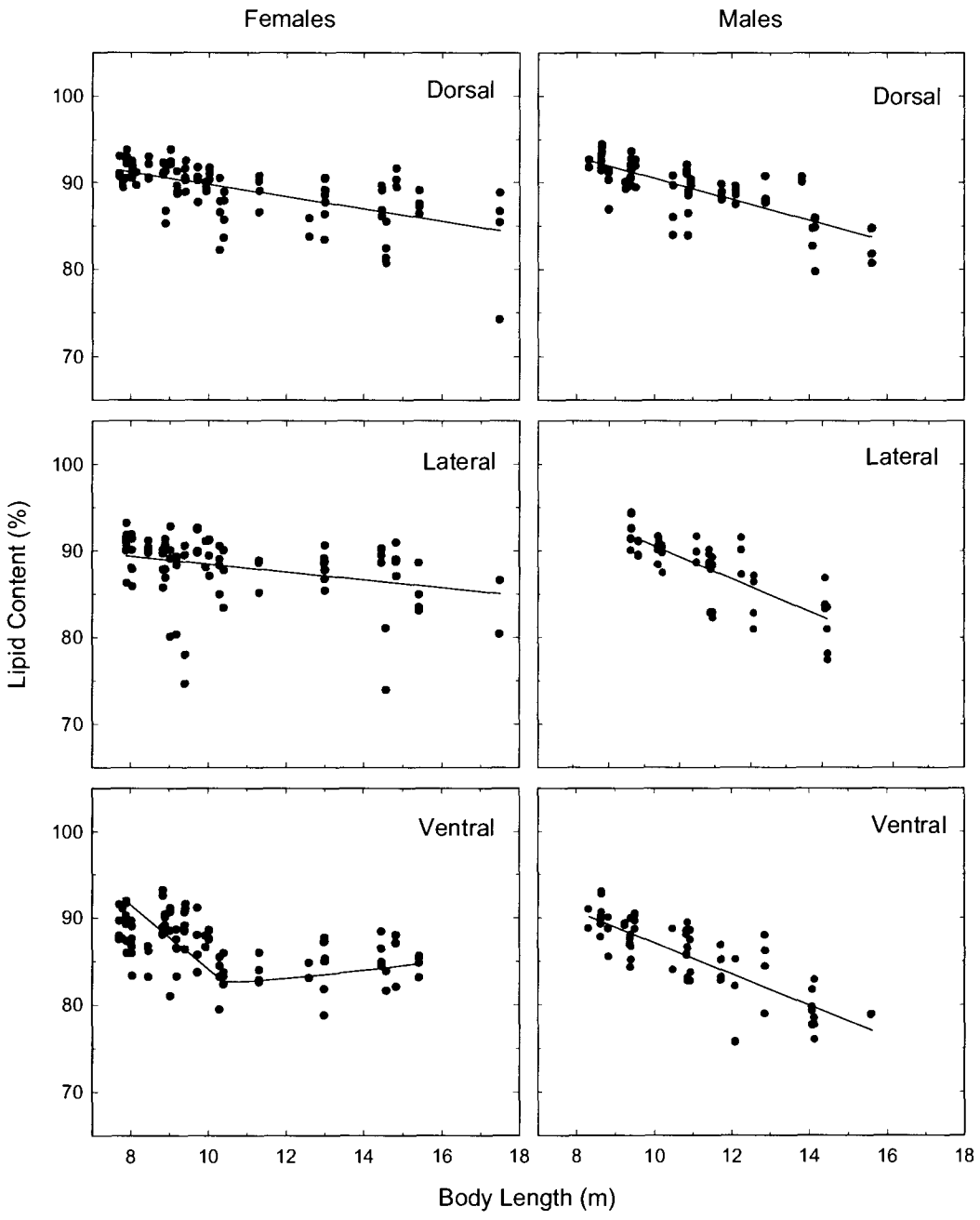

Fig. 2.11. Linear or lowess relationship of percent lipid content at depth 2 and 3 of the blubber core and body length for females (left) and males (right) at three sampling positions (dorsal, lateral, ventral).

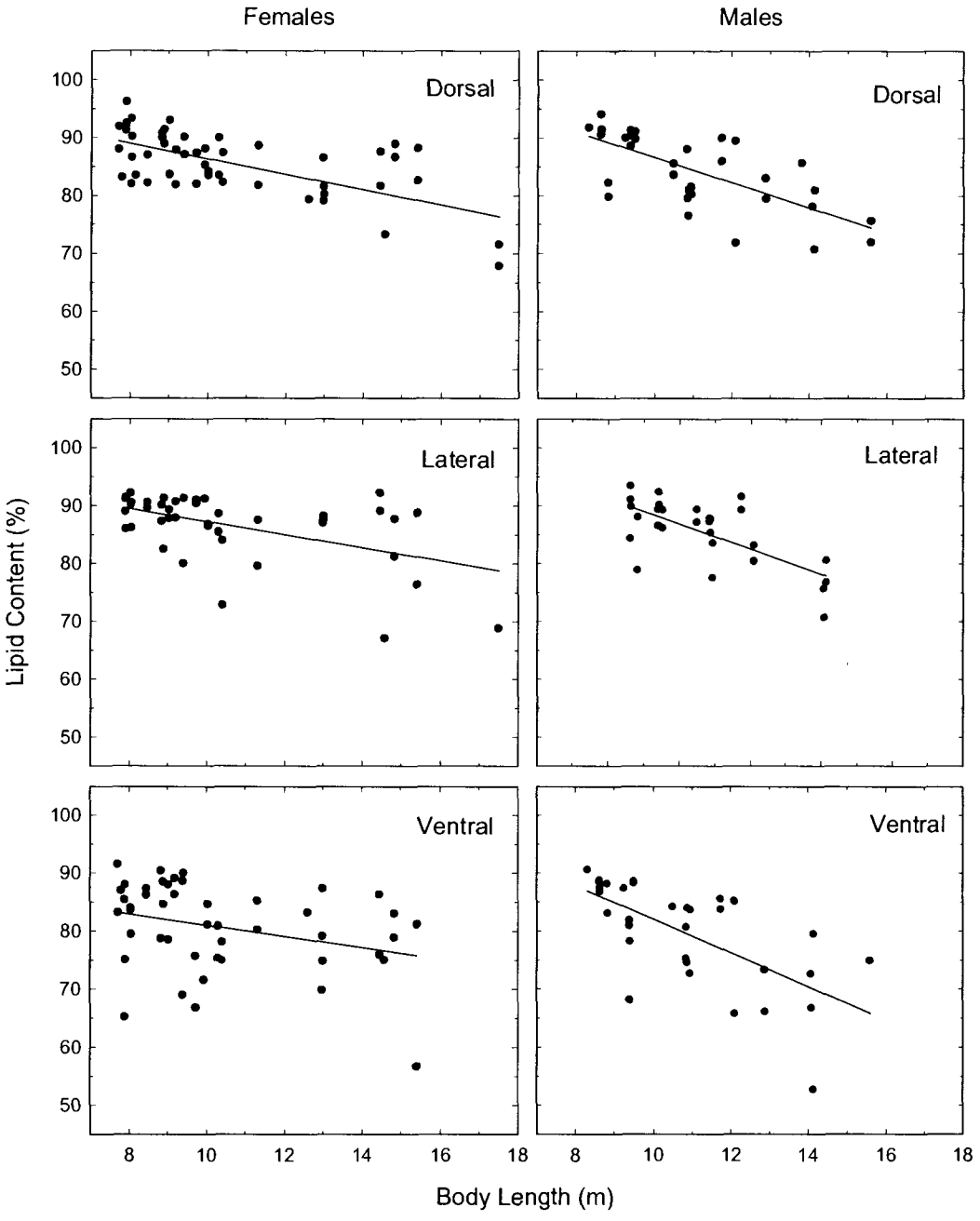

Fig. 2.12. Linear relationship of percent lipid content at depth 4 of the blubber core and body length for females (left) and males (right) at three sampling positions (dorsal, lateral, ventral).

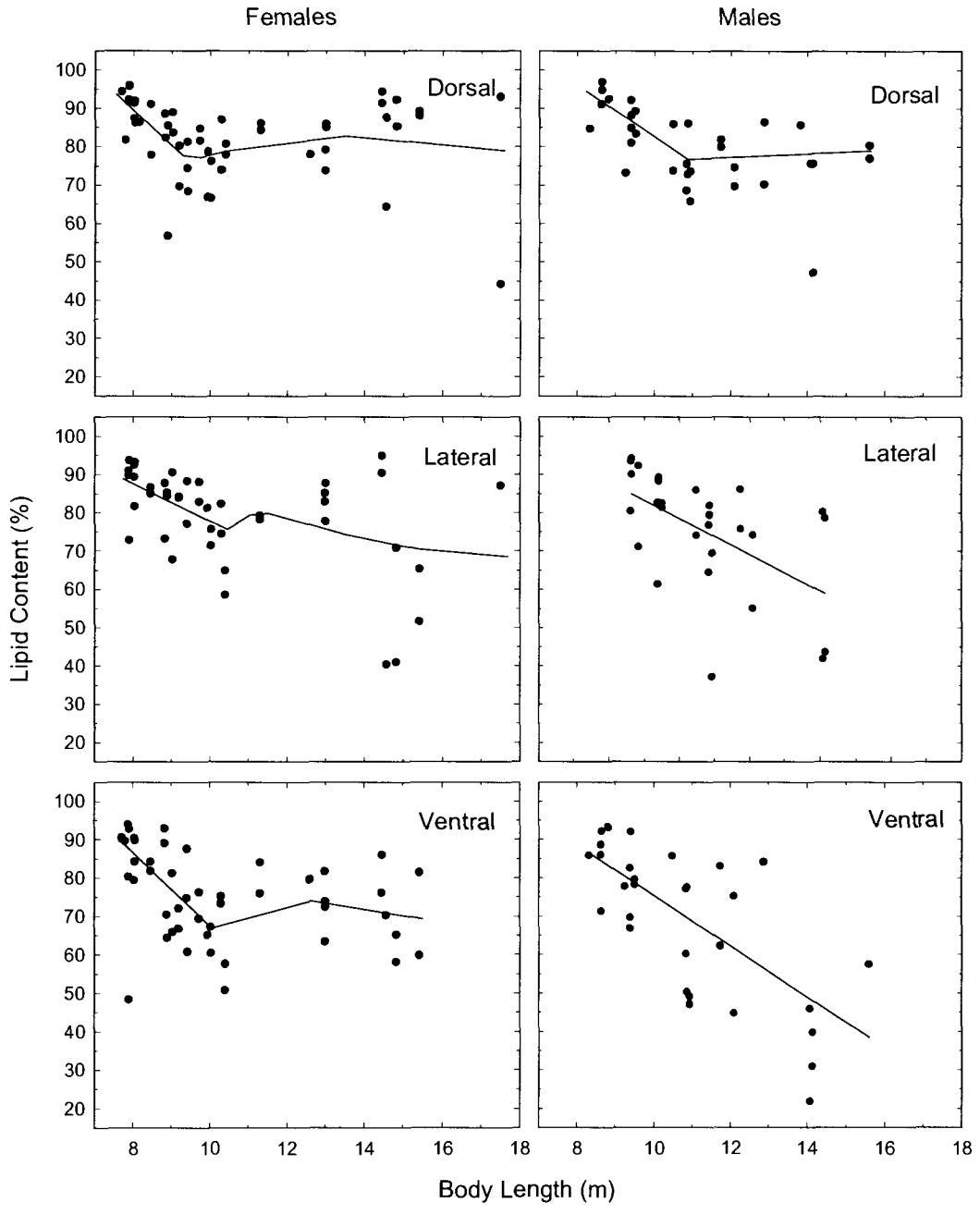

Fig. 2.13. Linear or lowess relationship of percent lipid content at depth 5 (nearest the muscle) of the blubber core and body length for females (left) and males (right) at three sampling positions (dorsal, lateral, ventral).

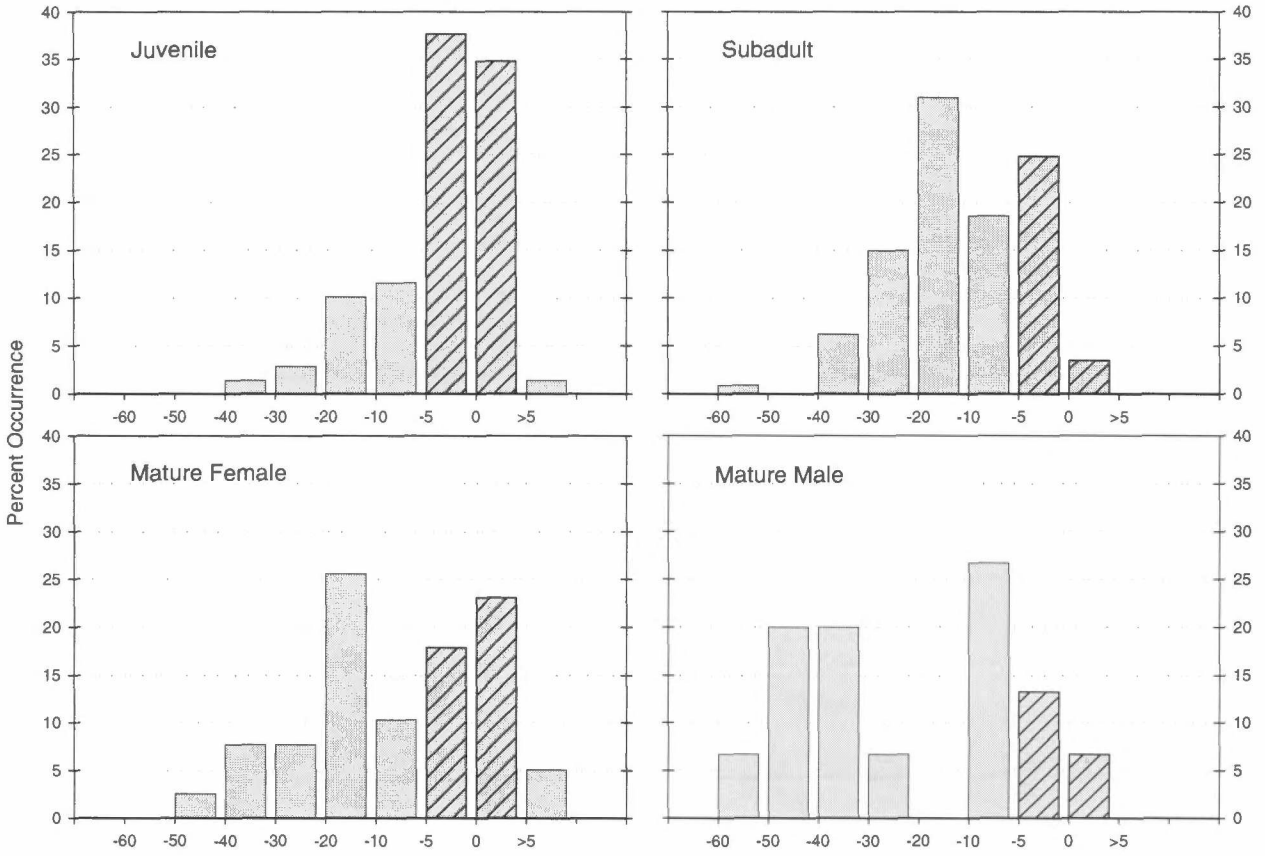

Fig. 2.14. Percent occurrence of sample sites with degree of lipid stratification, or Δ lipid content, for four reproductive categories in the bowhead whale. More negative Δ lipid content values equal a greater degree of stratification. Hatched bars indicate homogeneous blubber cores.

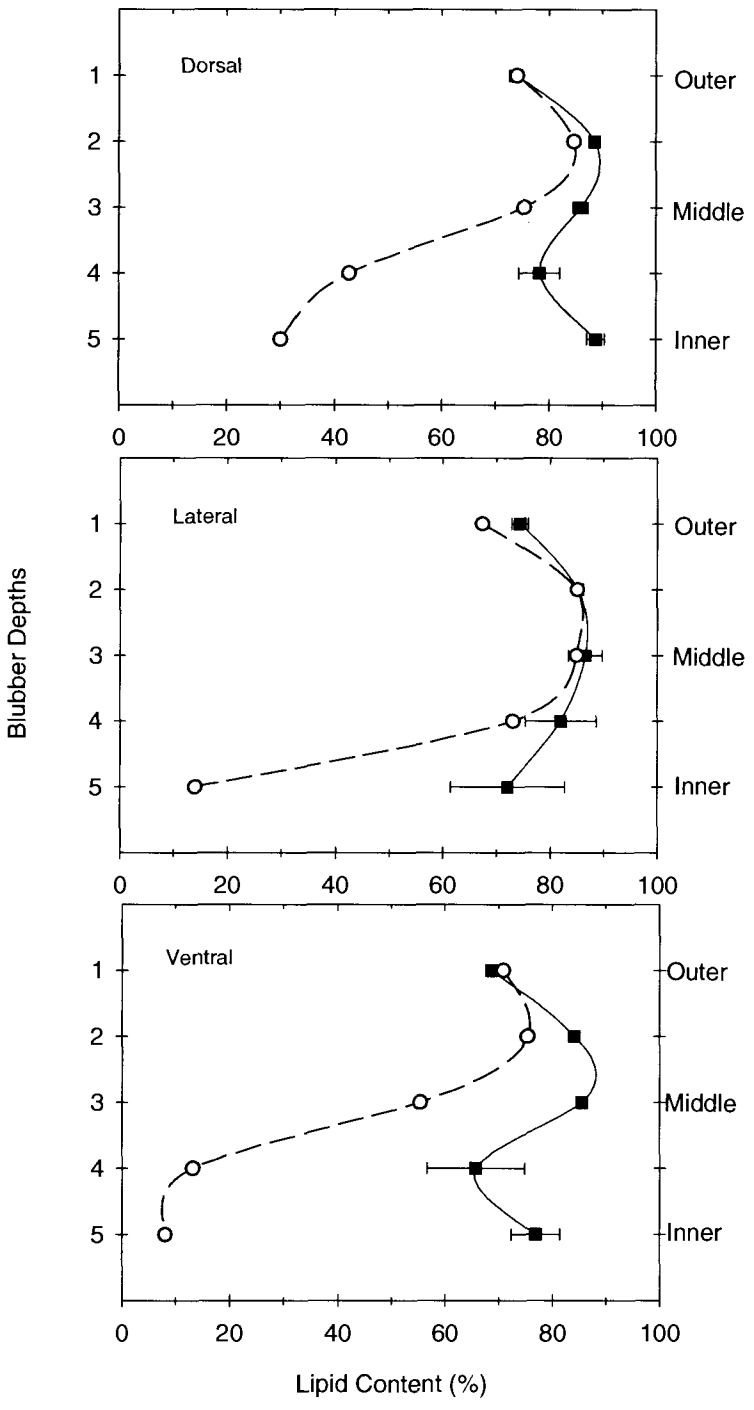

Fig. 2.15. Comparison of mean ± SE percent lipid for each depth at the three umbilicus sites between pregnant bowhead whales (solid squares; n=4) and whale 00B4 (open circles, n=1), a non-pregnant mature female bowhead whale. Points are joined by a cubic spline curve.

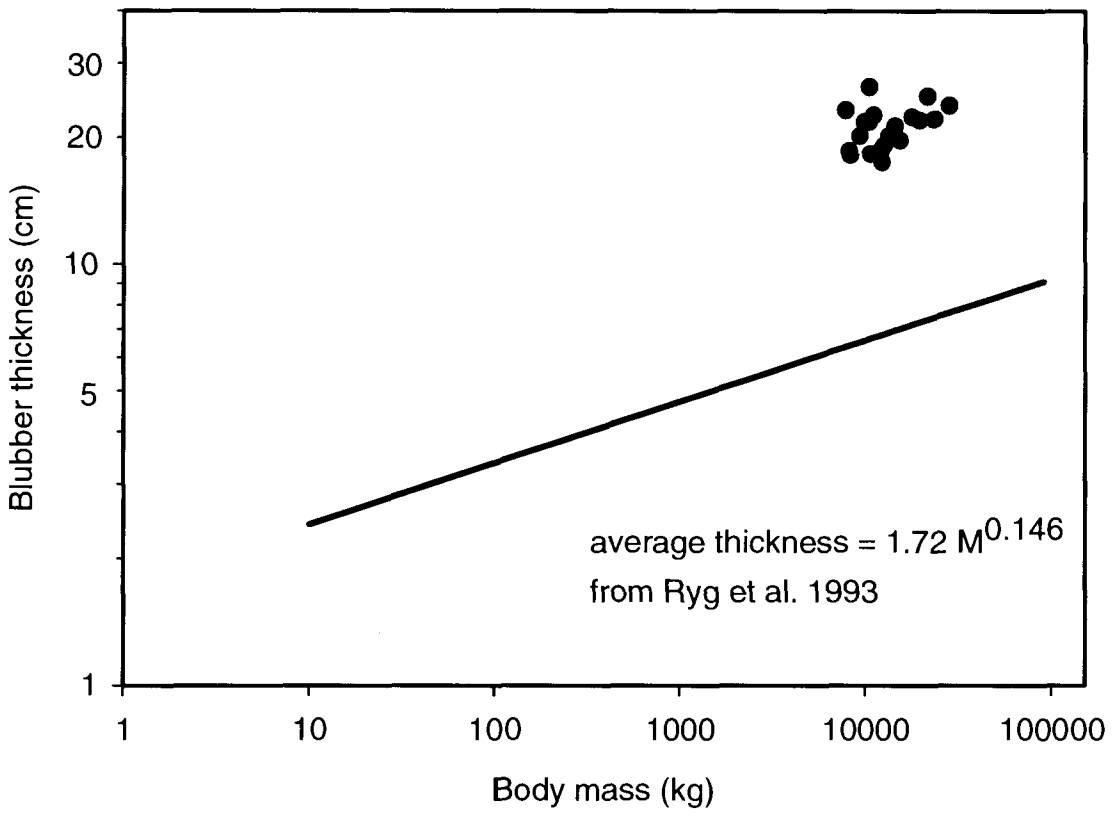

Fig. 2.16. Comparison of Bowhead average sculp thickness (solid circles, this study) with the linear regression of sculp thickness in other polar marine mammals (solid line, n=8 species; from Ryg et al. 1993). Note log-log scale.

3 Postmortem blubber composition changes from a stranded humpback whale, *Megaptera novaengliae*: implications for health assessment

INTRODUCTION

Humpback whales (*Megaptera novaengliae*) are distributed worldwide and most make annual migrations from the tropics to higher latitudes. The Central North Pacific stock migrates annually from winter/spring breeding grounds near the Hawaiian Islands to summer/fall feeding grounds in waters surrounding British Columbia/Southeast Alaska or Prince William Sound/Kodiak Island, Alaska (Baker et al. 1990; Calambodkis et al. 1997; Perry et al. 1990).

Because humpback whale feeding and breeding in the North Pacific Ocean is separated in both time and space, a large variation in the nutritive condition of individuals is likely to occur. Such variation has been shown in their southern hemisphere counterparts by marked seasonal changes in blubber thickness (Matthews 1937). A substantial body of work on several species of baleen whales, mostly from the Atlantic and Antarctic, has demonstrated the usefulness of body fat measurements (i.e., mass, girth, blubber thickness and blubber lipid content) in determining the nutritive status of individuals (Aguilar and Borrell 1990; Heyerdahl 1932; Lockyer 1986; Lockyer 1993; Lockyer et al. 1985; Slijper 1954; Víkingsson 1995). Similar studies on humpback whales of the North Pacific are currently lacking.

In the North Pacific, the humpback whale population was historically estimated at approximately 15,000 individuals, but was reduced by commercial whaling to perhaps as few as 1000 individuals before protection in 1965 (Rice 1978). The current population of humpback whales in the Central North Pacific is estimated at 4005 individuals (Calambodkis et al. 1997) and is designated as 'endangered' under the Endangered Species Act and 'depleted' under the Marine Mammal Protection Act. Knowledge of individual and population nutritive condition is important in understanding the recovery of this species. However, due to the pelagic life of this species and the end of commercial

harvesting, it has not been possible to examine anatomical and biochemical measures of health and condition in the general population.

Biopsies and sampling from opportunistic strandings and entanglements are the only current mechanisms for obtaining blubber samples for health and condition assessment in humpback whales. Although guidelines exist for the collection of condition data from stranded whales (Rowles et al. 2001), useful interpretation of the data is often limited due to (i) the relative state of decomposition and the inability to determine its effect on measurements, (ii) assessments that are anecdotal or subjective, for example, whale was "thin," or (iii) the lack of collection of systematic data on condition to be quantifiably useful (Le Boeuf et al. 2003). Often it is unknown if the state of the animal at the time of necropsy is representative of the state of the animal at the time of death. To address these limitations blubber samples were taken at two time periods following the death of a presumably healthy, pregnant humpback whale. This study had two objectives, (i) measure the proximate composition of blubber at three depths (outer, middle, inner), and (ii) describe postmortem changes in proximate composition over time. The ultimate goal of this case study was to address the utility of samples from stranded whales in various stages of decomposition for making accurate health assessments.

METHODS

A presumably healthy, pregnant, 13.9 m (45.5 ft.) adult female humpback whale was estimated to have died on the afternoon of July 13, 2001 due to blunt trauma to the head from a ship strike in Glacier Bay, Alaska. Damage to the skull was considered sufficient to have caused immediate death (DOI-NPS news release, 2001). The dead whale was first spotted in the water by Glacier Bay National Park (GBNP) staff at 16:20, July 16, 2001. Evidence from examination of the skin, such as a wet appearance and the presence of live cyamids, also known as "whale lice," suggested that the whale had recently surfaced following the submergence that occurred at the time of death. The whale was towed to a beach near Pt. Gustavus, Alaska on July 17 at 14:30, where it remained for serial sampling. GBNP biologists performed a preliminary necropsy the

following morning at 05:00. A full thickness blubber sample was taken from an anterior lateral position caudal to the eye. A second blubber sample from the same area was taken 4 days later on July 22 by a veterinarian who led a second, more comprehensive necropsy. Both samples were deep frozen at -80°C for later analysis. The sample from the first necropsy will be referred to as 'fresh' and the second necropsy as 'decomposed' for the remainder of the paper. Local air temperatures averaged 13.8 ± 1.35°C (56.1 ± 2.3°F), wind speed ranged from 4.6-6.6 knots, and no precipitation occurred throughout the sampling period.

Blubber thickness of both samples was measured in the laboratory with a metric ruler to the nearest 0.1 cm and the general odor and color were recorded (Table 3.1). Each blubber sample was further subdivided into inner, middle and outer samples corresponding to distances of approximately 6.5, 3.5 and 0.5 cm perpendicular to the epidermis-dermis interface, respectively.

Determination of blubber composition

All blubber composition measurements were made in duplicate as per methods described in chapter 2 of this thesis, but are repeated here for clarity. Lipid content was determined gravimetrically in duplicate 0.5 g (wet wt.) samples weighed to ±0.1 mg after 24 hour extraction of lipids by continual reflux in a Soxhlet extraction apparatus with a 2:1 chloroform-methanol solution (Fadely 1996; Lockyer et al. 1985). Samples were then dried in an oven at 70°C until a constant mass was achieved. Calculation of the final percent lipid required the determination of percent water from paired samples. Lipid content was expressed as a percentage of the wet weight of the tissue.

Approximately 0.5 g samples weighed to ±0.1 mg were frozen at -80°C and then lyophilized to a constant mass under vacuum (VirTis Freeze Dryer Model 5463). Water content was determined gravimetrically in duplicate and expressed as a percentage of the wet weight of the tissue.

Protein content was estimated by subtracting the percent lipid and water from 100, and thus also included any carbohydrate content, ash content and any accumulated

measurement error. However, carbohydrates are considered to be negligible in balaenopterid blubber and ash contents are considered minor components, ranging from 0.18-0.35% in fin whales, *Balaenoptera physalus*, and 0.18-0.26% in sei whales, *Balaenoptera borealis*, (Lockyer et al. 1984; Lockyer et al. 1985).

RESULTS

The fresh blubber sample was homogeneous in composition throughout its depth, averaging (mean ± SD; n=3) 70.8 ± 1.5 % lipid, 20.0 ± 0.6 % water and 9.2 ± 1.0 % protein. In contrast, the decomposed sample varied in composition between each depth, and the overall mean for lipid was much lower (50.5 ± 13.8 %), whereas water (34.1 ± 8.0 %) and protein (15.4 ± 5.8 %) were higher compared to the fresh sample. Compositional changes over time were observed at all three depths of the blubber core (Fig. 3.1). The difference in lipid content between fresh and decomposed samples was greatest at the inner layer adjacent to the muscle, which decreased by 24.1%. The least difference between fresh and decomposed samples (12.7%) was observed in the middle layer.

DISCUSSION

The duration of postmortem time prior to tissue sampling was an important factor in the determination of blubber composition and therefore the meaningfulness of the data's application as an indicator of condition. Differences in both the physical description and chemical composition of blubber were readily detectible in the four days between sampling periods as the tissues continued to deteriorate.

Owing to the scarcity of blubber lipid content measurements of humpback whales in the literature, comparison with other healthy whales of similar size and reproductive status was not possible. Blubber of baleen whales has typically been described as stratified, with lower lipid levels found at the inner depths and a gradation from lower to higher lipid content from the inner to outer depths (Ackman et al. 1965; Ackman et al. 1975a; Ackman et al. 1975b; Aguilar and Borrell 1990). Pregnant females have often

been found to vary from this pattern, having either homogeneous blubber cores or reverse stratification patterns (Aguilar and Borrell 1990). The results of the current study agreed with this observation, with lipid contents near 70% at all three depths. Although many baleen whales have relatively high lipid contents (50-80%) during the latter half of the feeding season (Aguilar and Borrell 1990; Klem 1935; Lockyer 1986; Lockyer 1987; Víkingsson 1990), Ackman et al. (1975a) found fairly low lipid contents in a single 9.8 m immature female humpback whale landed in the summer of 1970 off Nova Scotia. Blubber lipid content values in this whale ranged from 10.0-47.3%, much lower than the values observed in this study. Differences between the two studies may have been due to extraction technique, age differences, reproductive status and duration of feeding season. Blubber thickness measured in this study; however, was within the same range as values reported for female humpback whales of the same body length caught off the coast of South Africa (Matthews 1937).

The fresh blubber samples appeared to be in good condition as suggested by the homogeneous and high lipid content, tissue firmness, normal odor and normal coloration. These observations are in agreement with the definition of "freshly dead" in necropsy protocols (Rowles et al. 2001). Although some autolysis is likely to have occurred, given the homogeneous composition and the high lipid content consistent with other pregnant baleen whales, it can be assumed that negligible compositional changes occurred from the estimated time of death to the time of the first sampling. It was therefore assumed that condition as determined by the 'fresh' set of samples was indicative of the condition of the whale at the time of death.

The extent to which lipid content decreased as the whale decomposed was variable with depth. The differences in the decomposed sample were likely caused by differences in degree of leaching and rate of decomposition. Leaching of lipid was probably higher in the outer and inner depths because of pathways through the skin in the former or muscle in the latter. Decomposition was likely to have played a greater role in the inner depth, where the greatest difference between fresh and decomposed samples occurred. The inner depth exhibited the greatest extent of discoloration, ranging from

dark red/brown to green, suggesting that blood from the muscle had leached into the inner depth of blubber. The presence of hemoglobin from blood in muscle tissues is known to catalyze lipid oxidation (Love 1983; Rhee 1988) and may have had a greater impact on the degradation of the inner blubber layer compared with other layers less exposed to hemoglobin. Had the decomposed sample been the only sample available for analysis, the large difference between the inner and middle depths would have probably led to the conclusion that this whale was mobilizing blubber lipid stores for metabolism (Ackman et al. 1975a), when in fact no such evidence was found in the fresh sample. Overall, the changes in composition were sufficient in the decomposed sample to affect interpretation of condition in this whale.

Differences in the total depth of the blubber core between fresh and decomposed samples were also evident. However, it cannot be determined with certainty whether differences were caused by the natural variation in blubber thickness at any given site or from the loss of lipids due to leaching or general autolysis. However, the trend of smaller blubber thickness coupled with lower lipid contents in the decomposed sample may have led to the conclusion of moderate nutritional stress, when in fact this whale was likely not nutritionally compromised at the time of death, based on evidence from the fresh sample.

The results of this study have emphasized the effect of decomposition on the ability to accurately describe condition in stranded whales. The significance is far reaching, because other measures important to health assessment are often lipid dependent. For example, because the distribution, mobilization and metabolism of contaminants such as organochlorines are intimately linked to that of lipids, and for comparison across tissue types, values are often lipid normalized (Aguilar and Borrell 1991). The lower lipid values in decomposed samples would likely result in an artificial elevation of organochlorine levels.

Often the duration of time since death or initial stranding of cetaceans is unknown. A good estimation of the state of decomposition in blubber can be made by measuring the level of lipid classes present (Krahn et al. 2001). In fresh samples, blubber from baleen whales is composed of mainly neutral lipids such as triglycerides. During the

decomposition process triglycerides break down to their fatty acid building blocks. Thus the degree of decomposition can be estimated by either a low percentage of triglycerides or the ratio of free fatty acids to triglycerides. Unfortunately, lipid class measurements such as these require specialized equipment and could not be performed in this study.

In conclusion, the state of decomposition of blubber samples from stranded whales is a significant factor in the determination of lipid content and when moderate to severe, likely to cause erroneous conclusions about whale health. Also, for any health biomarkers that are lipid normalized, decomposed samples are likely to produce results that are significantly elevated compared with results obtained shortly after the whale's death. Samples obtained from stranded whales within 24 hours of stranding, such as in this study, appear to produce good estimates of condition, that is, they reasonably represent condition of the whale at the time of death. Although the duration of time spent floating in the water may also be a contributing factor, the rate of decomposition is temperature dependent and was likely decreased given mean ambient ocean temperatures of 8.7 ± 0.7 °C (NOAA archives). Larger differences in decomposition rate are likely to occur once an animal strands due to both higher ambient temperatures and radiant heat from the sun. Within a relatively short time period (5 days in this study) the utility of blubber samples for health assessment was severely reduced due to lipid loss. Future studies of this type would benefit by a greater frequency of serial sampling in the initial days following the stranding event to aid in the understanding of blubber degradation with time. Local ambient water and air temperatures will play a large role in the rate of decomposition and thus utility of samples from other strandings may vary from those reported here. Evaluation of health or condition from blubber samples of moderate to severe decomposition must be interpreted with caution.

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Table 3.1. Sampling times and characteristics of blubber samples taken from a stranded humpback whale in Alaska.

Sample ID	Date	Blubber thickness[a] (cm)	General Description
Fresh	07/18/03	8.0-10.0	Normal odor, coloration pink, no obvious signs of oxidation, firm
Decomposed	07/22/03	6.0-7.0	Rancid odor, outer depth pink, gradation of green color towards inner depths. Dark red/green color at inner depth

[a] Does not include the epidermis, which was 8.0 mm in both samples

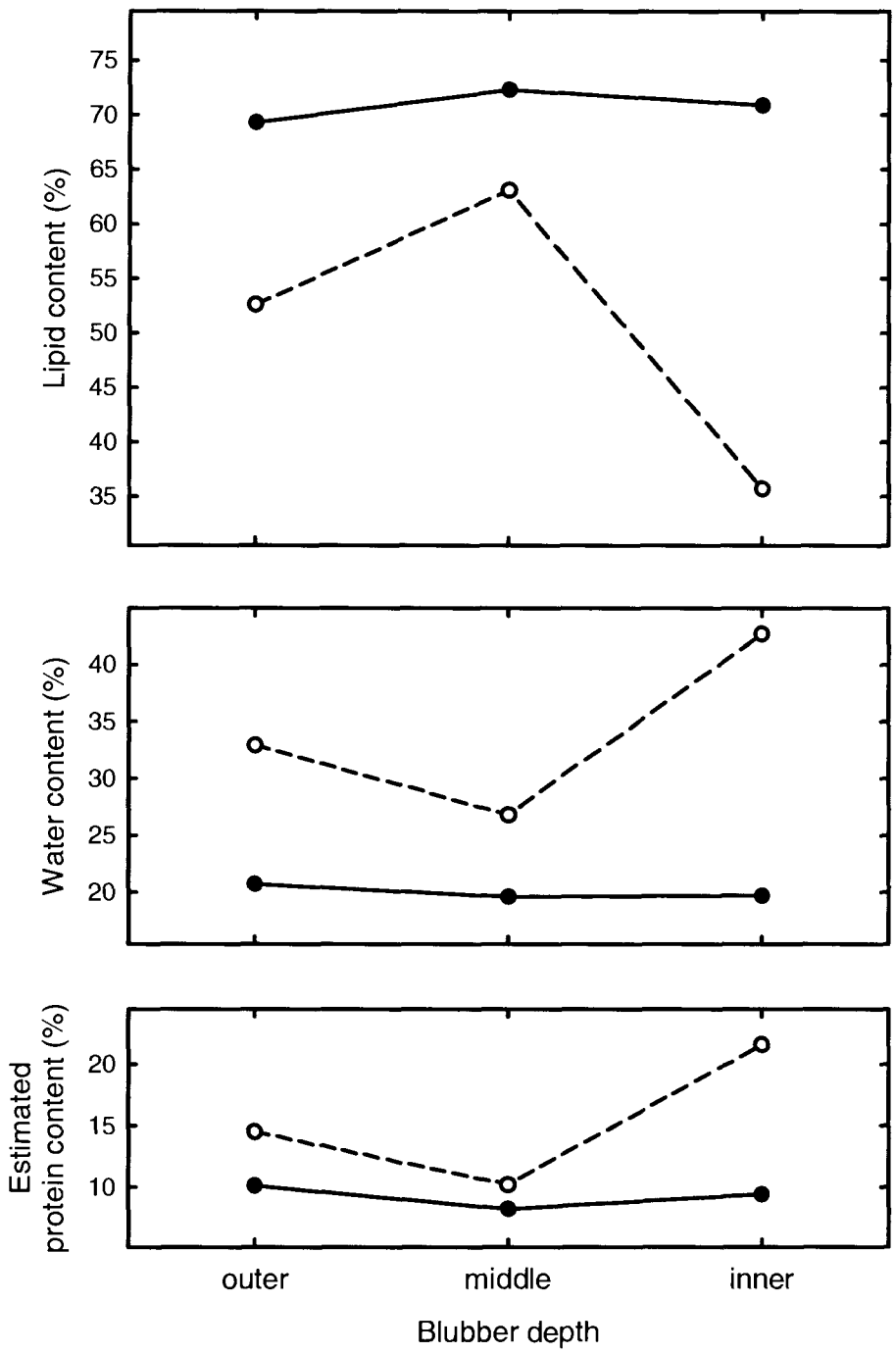

Fig. 3.1. Percentage composition (lipid, water and protein content) at outer, middle and inner depths of humpback whale blubber from fresh (solid circles) and decomposed (open circle) samples. Note differences in scale on the y-axis.

4 The effects of prey switching on plasma and red blood cell fatty acids in the harbor seal, *Phoca vitulina*

INTRODUCTION

The development of dietary biochemical tracer methods such as serology, stable isotopes and fatty acid analysis has greatly enhanced the ability to determine differences in diet and habitat use in a variety of wild populations of marine and terrestrial vertebrates (Hoekstra et al. 2003; Iverson et al. 1997b; Kelly 2000; Pond et al. 1995). The utilization of fatty acid analysis in dietary studies of marine mammals in particular, has greatly increased over the last decade. The effectiveness of such studies is based on the diversity of individual fatty acids (>70) in the marine environment (Ackman and Lamothe 1989) and the conservative nature of the transfer of fatty acids from prey to consumer in marine food webs.

Understanding the diet preference of marine mammals is of particular importance in Alaskan waters, where it has been hypothesized that nutritional stress from a shift in the primary prey base, has contributed to the decline of many marine mammal species, including harbor seals (*Phoca vitulina*) and Steller sea lions (*Eumetopias jubatus*) over the past three decades (Calkins et al. 1998; Pitcher 1990; Small 2000). In addition, knowledge of marine mammal diets is necessary in addressing the interaction of commercially important fish species with the conservation and management of protected or endangered marine mammals.

Stomach samples have been important in providing dietary information from culled animals, or more recently from subsistence harvested or stranded animals in Alaska. However, these studies are restricted in the dietary information they provide, because the contents reveal at most only a recent snapshot of dietary history, that of the last few meals, or no information if the stomach is empty. Fatty acid analysis, on the other hand, provides an integration of dietary information, reflecting a time period that is dependent on the tissue type examined, the total dietary fat intake and the energy balance of the animal. Numerous studies have utilized fatty acid profiles (patterns of multiple

fatty acids) determined from blubber, milk or blood samples to describe feeding ecology in free-ranging marine mammals, especially pinnipeds (Best et al. 2003; Bradshaw et al. 2003; Dahl et al. 2000; Iverson 1993; Iverson et al. 1995; Iverson et al. 1997a; Iverson et al. 1997b; Walton et al. 2000). Few studies have utilized longitudinal sampling (Rea et al. 1997), and validation studies utilizing controlled diets in captive marine mammals have been rare, with only two published papers to date (Grahl-Nielsen and Mjaavatten 1991; Kirsch et al. 2000). Thus, our understanding of incorporation rates of fatty acids into various tissues, whether particular fatty acids are preferentially mobilized or deposited, and the effect of energy or nutritional status on fatty acid incorporation, is only marginally understood in marine mammals.

There are advantages to sampling several tissues simultaneously for fatty acid analysis. Because turnover rates of fatty acids differ among body tissues, differences occur in the time period required for the dietary lipids to be completely reflected in the fatty acid pattern of the tissue. By sampling multiple tissues simultaneously, several "dietary windows," representing short, medium, to long-term dietary histories can be examined. For example, previous studies in humans have shown that the incorporation half-life of certain dietary fatty acids into serum cholesterol esters takes about 5 days, into red blood cell membranes (RBCs) one to two months, and into subcutaneous fat longer than one year (Katan et al. 1997). In seal species that have lipid rich diets and cyclical fattening and fasting, incorporation rates of fatty acids into tissues may be substantially faster. In hooded seals, *Cystophora cristata*, reflection of the diet in the blubber fatty acid profile can be as rapid as 2 to 4 days in a nursing pup consuming a lipid rich (~60% fat) milk diet (Iverson et al. 1995). In contrast, when juvenile harp seals, *Phoca groenlandica*, were fed low-fat (1.7%) pollock, they lost fat mass, and blubber fatty acids shifted more slowly in the direction of the diet, representing an estimate of only 25-50% of prey fatty acids in one month's time (Kirsch et al. 2000). Harbor seals naturally undergo seasonal changes in body fat condition (Renouf and Noseworthy 1991), feed on fish that vary widely in their fat content and fatty acid composition (Iverson et al. 2002) and can easily be trained for repeated scientific sampling. This species can

therefore serve as a good model for elucidating "dietary windows" obtainable through fatty acid analysis of multiple tissues. Dietary trials in a captive situation facilitate improved modeling of the transfer of fatty acids from prey to predator with the use of known diets, intake rates and physiological states of animals.

This study examined the effects of prey switching, from a low-fat pollock diet to a high-fat herring diet, on the fatty acid composition of plasma and RBCs in captive harbor seals. This study had three main objectives within a larger program designed to better understand how specific diets and seasons affected the health and body condition of harbor seals (Castellini et al. 2002; Trumble 2003). The objectives of this study were to (i) examine whether the fatty acid profiles of harbor seal plasma and RBCs were influenced by diet, and whether they reflected the fatty acid profile of prey consumed, (ii) determine the incorporation rate and residence time of fatty acids in these two tissues, and (iii) determine whether other factors which affect metabolic demands such as seal age and season affect fatty acid metabolism and therefore the profiles observed.

MATERIALS AND METHODS

Animal maintenance and feeding trial design

This study was conducted from August 1998 to September 2000 at the Alaska SeaLife Center (ASLC) in Seward, Alaska under terms of the Marine Mammal Protection Act permit # 881-1143 and with approval from the University of Alaska and ASLC Institutional Animal Care and Use Committees. Eight harbor seals (ages 2-23 years, see Table 4.1.) were housed in large outdoor pools (~350,000 l) or indoor pools used for public display with ample haulout spaces and under ambient temperature and light conditions. All seals were trained for frequent handling to obtain blood samples and morphometric measurements at two-week intervals. Each seal was fed individually to a level of satiation where they would still respond to training commands. Thus, the actual mass of fish ingested was determined by the individual seal and varied throughout the experiment. All food consumed by each harbor seal during each feeding trial was weighed in kilograms and presented as daily intake.

The seals were maintained on a mixed but predominantly herring diet for >5 months prior to the start of the feeding trials which began in September 1998. Seals were then divided into three dietary test groups. Group A and B seals received alternating diets of either herring (*Clupea pallasi*) or pollock (*Theragra chalcogramma*), whereas group C seals, i.e., the control group, were maintained on a mixed diet of equal proportions by mass of herring and pollock throughout the study. Six feeding trials were performed in a repeated crossover study design over two years as shown in Table 4.2. Each feeding trial lasted four months and was designed to reflect three metabolically defined times of the year: season one (S1; Sep. 15–Jan. 15), season two (S2; Jan. 15–May 15) and season three (S3; May 15–Sep. 15). This crossover-feeding matrix allowed seals in groups A or B to experience a different diet at similar physiologically relevant times of the year; that is, during the molting, winter or breeding season. Group A, for example, was fed a pollock diet during season one in year one and fed a herring diet during season one in year two. Due to behavioral and palatability adjustments, the seals were phased onto the new diets over the course of several weeks at the beginning of each trial.

As part of the larger program objectives to test the effect of diet, and that of diet "quality" on health and body condition in harbor seals, a high-fat fish, Pacific herring (*Clupea pallasi*) and a low-fat fish, walleye pollock (*Theragra chalcogramma*) were chosen as the two test diets. Fish were purchased in large commercial batches from Cook Inlet Processors (pollock) and Icicle Seafoods (herring) and stored at -20° C in 40 lb. watertight boxes. All fish were thawed whole in a water bath on the day of feeding. Fish quality (based on lipid, protein, water content and energy density) was monitored throughout the feeding trials and remained constant, as reported previously in Castellini et al. (2002). The mean lipid content (mean ± SD wet mass) of herring was substantially higher (16.8 ± 2.2 %, n=104) compared with pollock lipid content (4.9 ± 1.1 %, n=34).

Blood sampling and handling

Blood samples were collected from each seal after an overnight fast at two-week intervals throughout the experiment. Seals were either manually restrained or, in some

cases, trained to position without physical restraint while samples were taken. Blood was drawn from the extradural intravertebral vein using 18G x 3.5 inch spinal needles into 5 ml Vacutainer® blood collection tubes containing the antioxidant ethylene diamine tetra-acetic acid (EDTA) and chilled immediately to 5°C. Whole blood was spun at 5°C and 2950 RPM for 10 minutes in a Sorvall refrigerated benchtop centrifuge (model GPR). Plasma was separated and transferred into cryogenic storage vials, flushed with argon to inhibit oxidation, and stored at -80°C until analysis. The RBCs were isolated by washing and respinning 3 times with ice-cold physiological saline, which removed white cells and other plasma components. The final packed RBCs were re-suspended with saline to an approximate hematocrit of 50% and stored under argon at -80°C until analysis.

Not all blood samples taken were available for fatty acid analysis. In total, 336 samples were available from 7 seals (Tina, group C omitted) for plasma fatty acid analysis, which corresponded to 7-10 samples per seal per four-month feeding trial or an average of once every two weeks. One seal (Pender, group A) was removed from the latter half of trial 5 for a medical condition but returned for trial 6. For RBC samples, only midpoint and endpoint samples were analyzed for 4 of the 6 trials for a total of 48 samples. In addition, higher frequency sampling of RBC fatty acids was possible in two seals, Poco and Travis, at approximately every two-week intervals for at least 3 trials (n=52).

Chemical analysis–plasma and RBCs

Fatty acids in plasma and RBCs were derivatized into their corresponding fatty acid methyl esters by direct transesterification without prior lipid extraction as described by Lepage and Roy (1986) and modified by Rodríguez-Palmero et al. (1997). Either 200μl of plasma or 400 μl of RBCs were added to PTFE-screw-capped pyrex tubes containing 250 μg/ml or 200 μg/ml of internal standard heptadecanoic acid (17:0), respectively. The samples were freeze-dried approximately 24 hours (VirTis Freeze Dryer Model 5463) to remove any water. Direct transesterification was accomplished with 2ml of toluene-methanol, followed by 200 μl of acetyl chloride. The tubes were

tightly capped and heated to 100°C in a dry bath for 1 hour and then allowed to cool to room temperature. The samples were neutralized with 5 ml of a 6% potassium carbonate solution and centrifuged for 10 minutes. The upper toluene phase containing the fatty acid methyl esters was transferred to a PTFE-screw capped autosampler vial and diluted with toluene to 2 ml.

Fatty acid methyl esters from plasma were identified and quantified by temperature-programmed gas-liquid chromatrography using a Varian 3800 Gas Chromatograph equipped with a Varian 2200 Ion Trap Mass Selective Detector and a Varian 8400 autosampler. The instrument was equipped with a 60 m x 0.25 mm i.d. fused-silica capillary column (0.2 μm film thickness; Supelco SP-2330) and linked to a computerized integration system (Saturn Workstation version 5.52, Walnut Creek, CA). The oven temperature was initially set at 125°C, raised by 3°C/min to 240 °C and held for 1.67 min for a total 40 min run time. Helium was used as the carrier gas at a flow rate of 1.0 ml/min. A 1.0 μl sample volume was injected at a split ratio of 1:10 at 250 °C. Nineteen individual fatty acids were identified by comparing retention time and mass spectral data from known standards obtained from Sigma Chemicals. Quantification was accomplished by peak area response comparison with an internal standard (17:0) using a 7-point calibration curve representing the range of fatty acid concentrations typically observed in plasma samples. Calibration curves were run several times weekly and mid-level standards were run after every 12^{th} sample to verify consistency in peak response and quantification. Of the fatty acids quantified, four (15:0, 18:3n-3, 20:0, 22:0) regularly fell below the detection limit in plasma samples and were thus eliminated from subsequent statistical analysis.

Fatty acid methyl esters from RBCs were identified and quantified utilizing a 30 m Supelco SP-2330 column and the same quantification methods as for plasma, but utilizing a Perkin Elmer Sigma 2000 Gas Chromatograph equipped with a flame ionization detector and linked to a computerized integration system (PE Nelson 900 Series) and PE Nelson Turbochrom version 4.1 software system. When necessary, identification of individual fatty acids was accomplished by comparison of retention

times to known standards and to mass spectral data obtained by separate runs on an HP 5890 Gas Chromatograph equipped with a 5070A Mass Selective Detector.

Chemical analysis–fish diets

Whole herring (n=14) and pollock (n=10) were thawed and individually ground to a homogeneous paste in commercial food grinders and processors. Homogenates were kept in air-tight plastic jars at –80 °C until further analysis. Lipids from individual fish were extracted from subsamples of approximately 1.0 g of pollock homogenate or 0.5 g of herring homogenate using a Dionex ASE 200 automated solvent extraction system. The solvent mixture was equivalent to that used in the modified Folch et al. (1957) method (2:1 chloroform/methanol containing 0.005% BHT). The extraction was performed at 100 °C and 2000 lb. pressure under nitrogen. Non-lipids were removed from the extracted filtrate by adding 50 ml of aqueous 0.7 % sodium chloride in a separatory funnel. Once the solution equilibrated, the organic solution containing the lipids was separated and passed through sodium sulfate crystals to remove residual water. The organic fraction was concentrated to 0.5 ml with a Zymark TurboVap II Concentration Workstation at 40 °C. The evaporation was completed by placing tared vials containing the lipid extract into a heating block at 25 °C and then passing nitrogen over the samples. After the evaporation was complete, the samples were weighed and then placed under argon gas and kept frozen at -30 °C until analyzed. Fatty acid methyl esters were prepared from 5.0 mg of lipid extract by saponification in a 5-ml capped test tube by heating the lipid at 80 °C for 30 minutes in 1.0 ml of 0.5 *M* KOH in methanol. After allowing the mixture to cool to room temperature, 2.0 ml of 10 % boron trifluoride in methanol (v/v) was added and the mixture was heated at 100 °C for 10 minutes. The reaction was quenched with 2 ml of de-ionized water after cooling to room temperature. The esterified fatty acids were extracted from the solution with 2.0 ml hexane.

The fatty acid methyl esters were identified by comparing their retention times and mass spectral fragmentation to a set of 20 standards (same as for plasma and RBC analysis), the FAME mixture "Supelco 37" and a FAME reference sample extracted from

NIST SRM 1946. The standards and samples were analyzed using the same instrument, temperature program and GC conditions as for the plasma samples but with internal standard C21:0 and a 60m x 0.25 mm i.d. fused-silica capillary column (0.25 μm film thickness; J&W DB-23).

Individual FA's from plasma, RBCs and fish were expressed as a mass percentage of total fatty acids and were designated according to the short-hand nomenclature of the IUPAC (International Union of Pure and Applied Chemistry) for carbon chain length:number of double bonds, and location (n-x) of the double bond nearest the terminal methyl group. For example, a fatty acid with a carbon chain length of 18, one double bond, and the location of this double bond located 9 carbons from the terminal methyl group would be designated as 18:1n-9.

Data analysis and interpretation

The use of additional standards in the prey analysis allowed for the identification and quantification of a further 46 fatty acids in addition to the same 15 fatty acids identified in plasma and RBC samples. The complete set of 61 fatty acids (see Table 4.3) was utilized to calculate the mean sum of fatty acid classes designated as saturated (SAT, containing no double bonds), monounsaturated (MUFA, containing a single double bond), polyunsaturated (PUFA, containing two or more double bonds), n-3 and n-6 (PUFA's of the omega-3 and omega-6 series) in herring and pollock. However, for consistency, only the 15 fatty acids in prey that were also determined in plasma and RBCs were used, after renormalizing to 100%, in all other statistical analyses (See Table 4.4). The fat content, fatty acid classes and fatty acid composition of diet samples (herring, n=14, or pollock, n=10) were compared by one-way ANOVA after arcsine-square root transformation. Because of the large number of comparisons, the likelihood of obtaining a significant difference could arise by chance alone. Thus, although $P < 0.05$ was considered significant, comparisons with a significance level of $P < 0.01$ were emphasized and used as dietary indicator fatty acids for subsequent comparison of plasma and RBC fatty acids in harbor seals.

The mean mass percentage of individual fatty acids from the last three plasma samples of a feeding trial or endpoint RBC sample of a feeding trial were analyzed using repeated-measures analysis of variance (RM-ANOVA) with diet (herring and pollock) and season (1,2,3) as between-subjects factors. To improve normality and reduce heterogeneity of variances, percentages were arcsine-square root transformed prior to statistical analysis (Sokal and Rohlf 1998). The means of individual fatty acids at each trial end were then plotted to visually indicate dietary and/or seasonal differences. Data points were joined using a cubic spline procedure which connects data points by a series of piecewise cubic polynomials as to form the simplest continuous smoothed curve passing through each point.

Forward-stepwise linear discriminant function analysis (DFA), a multivariate statistical approach, was used to classify seal plasma or RBC samples into dietary treatment groups. This method determines the linear combination of variables, i.e., fatty acids, that best separates the samples into dietary treatment groups. The optimal combination of fatty acids were plotted so that the first function (axis) provided the most overall discrimination between groups and the second function explained the greatest part of the remaining unexplained variation. Four of the fifteen fatty acids (16:0, 16:1n-7, 18:1n-9, 20:4n-6) were removed prior to modeling because of high pairwise correlations with other fatty acids leading to multicollinearity, which limited the ability of the model to reliably assess the relative importance of the predictor variables. Plasma samples from the last month of a given feeding trial were used to develop a model that would best discriminate between herring, pollock and mixed diet groups. The ability of the discriminant model to predict diet group membership was then tested on "new" samples obtained in months one and two of each trial combined, which included the transition period between diets, or month three of each trial, in which the seals had been on a 100% diet for at least 2 weeks.

As with the univariate methods, percentages were arcsine-square root transformed prior to use in the DFA model to improve normality. All tests were performed in

SYSTAT (ver.10) and Statistica (ver.5.5). *P* values <0.05 were considered significant and all data are presented as mean ± SE, unless otherwise noted.

RESULTS

Dietary fish composition

The total lipid content and fatty acid composition of the herring and pollock diets fed to the harbor seals were significantly different. The mean ± SD lipid content of herring was 16.8 ± 2.2 %, whereas pollock was significantly lower in lipid content at 4.9 ± 1.1 % (*P* <0.05, data previously published in Castellini et al. 2002). Levels of total PUFA, n-3 fatty acids, and the n-3/n-6 ratio were significantly higher in the pollock diet, whereas MUFA were higher in herring. SAT and n-6 fatty acids did not differ between diets (Table 4.3). The 15 fatty acids in the diets used for statistical comparison with the plasma and RBC fatty acids represented 89.8% of total fatty acids measured in the fish. A total of 7 of the 15 fatty acids were significantly different between dietary fish (all *P* <0.01, Table 4.4). Of these fatty acids, five (18:0, 18:1n-7, 20:4n-6, 22:6n-3 and 20:5n-3) were higher in pollock, whereas two (14:0, 22:1n-11) were higher in herring. Stable isotope analysis of these same prey samples revealed significant differences in batches of fish within a species (Zhao 2002) and thus it was reasonable to assume that the fatty acid composition might also show differences between batches. Indeed, significant differences were found for five fatty acids between herring batches (H4 vs. H6) and four fatty acids between pollock batches (P2-3 vs. P5, Table 4.4). Because H4 and P2-3 batches were fed to seals for the majority of the feeding trials, these prey were also tested for differences. The same seven fatty acids as found in the overall herring vs. pollock test plus 20:1n-11 were significantly different. Batch H6 and P5 were used in trial 6 only.

Effect of diet on plasma and RBC fatty acid composition

A total of 15 fatty acids were identified in above trace amounts in harbor seals plasma, with 13 fatty acids occurring as greater than 1% of the total fatty acids (Table 4.5). Significant differences in the fatty acid composition of harbor seal plasma due to

diet were evident in 9 fatty acids (RM-ANOVA, n = 7 seals, $P < 0.05$). Of these fatty acids, five (14:0, 16:0, 18:1n-9, 18:2n-6, 20:4n-6) were found in greater amounts in the plasma of seals fed herring, whereas four fatty acids (18:1n-7, 20:1n-9, 22:5n-3, 22:6n-3) were found in greater amounts in the plasma of seals fed pollock. These differences are shown by plotting means of the mass percentage of particular fatty acids in each seal group as they switched back and forth between the two diets over the course of the six feeding trials (See Fig. 4.1). In addition the total percentage of SAT in plasma was significantly higher on the herring diet compared to on the pollock diet, (30% vs. 27.9%), whereas the reverse was true for the percentage of MUFA (28.6% vs. 30.2%). Although the total percentage PUFA in plasma did not vary between diet treatments, all individual PUFA except one, 20:5n-3, varied significantly with diet treatments.

Harbor seal plasma samples from the last month of a given feeding trial were correctly classified into diet treatment groups using discriminant function analysis as shown in Fig. 4.2a. The classification matrix indicated that all 117 plasma samples were correctly classified (Table 4.6a). The fatty acids 18:1n-7, 22:6n-3, 14:0, and 22:5n-3 were the most important contributors to the separation of groups. If the model was confined to only the eight fatty acids that were significant between herring and pollock diets (see Table 4.4), the model still clearly separated groups (Fig 4.2b), but correct classification decreased slightly to 96.6%. Misclassifications were only made between herring and mixed diets (Table 4.6b). When the original model was tested for its ability to predict diet group membership on "new" samples, misclassifications increased. The original model was less able to predict correct diet group membership of plasma samples from seals that had spent less time on a given diet (Table 4.7). Plasma samples from the third month were classified correctly 92.5% of the time, with most of the errors occurring in the herring group that were misclassified as mixed. Correct classification was reduced to 66.2% when samples from the first two months were examined. This was not unexpected, as seals were transitioning onto new diets during this period, with the ratio of new to old diet increasing over the course of several weeks.

There were far fewer differences in fatty acid composition of RBCs compared to plasma from harbor seals fed herring or pollock (Table 4.5). Because only 4 of the 6 feeding trials were used in this comparison, only dietary differences were examined. Only 3 fatty acids (14:0, 18:1n-7, 18:2n-6) plus the ratio 22:5n-3/22:6n-3 were significant between dietary treatments (RM-ANOVA, $P < 0.05$). However, for the fatty acids that were significant in both plasma and RBC, both differed by diet treatment in the same direction, i.e., if values were higher on the herring diet for plasma, that was also true for RBCs. Many of the RBC fatty acids appeared to change in the same direction as did plasma with diet; however, given the small sample sizes, statistical power was reduced to detect significant difference. Although fewer fatty acids were significantly different in RBCs due to diet, diet group membership could still be classified correctly using discriminant analysis (Fig. 4.3 and Table 4.8).

Effect of season on plasma fatty acid composition

Only one plasma fatty acid (20:4n-6) that was significant for diet was simultaneously significant for season (RM-ANOVA, $P < 0.05$). Three other fatty acids (18:0, 16:1n-7 and 20:5n-3) also exhibited significant seasonal differences (Fig. 4.4). Additional seasonal effects were found by examining plots of plasma fatty acid levels over time in "Snapper," an adult male seal that received a continuous mixed diet (Fig 4.5). While nine fatty acids did not vary with season, six fatty acids did (16:0, 18:0, 18:1n-7, 18:2n-6, 20:4n-6 and 20:5n-3). Some seasonal effects occurred outside the time periods tested by the RM-ANOVA, that is, during the beginning or middle of a trial.

Effect of daily dietary lipid intake on plasma fatty acid levels

Seals were allowed to self-regulate the amount of fish in kg eaten per day. Consequently, given the difference in mean lipid content of herring and pollock, the mean total lipid intake (g/day) varied greatly amongst individual seals and between and within diet treatments (Fig 4.6). Both the daily total lipid intake and the mass percentage of 18:1n-7 in fish were important in determining the mass percentage of 18:1n-7 in

harbor seal plasma. Using the seal "Poco" as an example, 18:1n-7 and daily total lipid intake were examined over time (Fig 4.7). The level of 18:1n-7 in plasma increased on pollock diets and decreased on herring diets. However, when total lipid intake was low, the level of 18:1n-7 increased in plasma regardless of diet. The highest correlation between total lipid intake and 18:1n-7 in plasma was found when the mean daily total intake from the week prior to blood sampling was used in the correlation (-0.803). The correlation increased when only pollock values were used (-0.831) and decreased when only herring values were used (-0.698). The reduced correlation on the herring diet reflected the reduction of the effect when lipid intake was high, which for Poco (body mass ranging 57-73 kg), exceeded 300-350 g lipid/day.

Incorporation rate and resident time of fatty acids in plasma and RBCs

A direct quantitative measure of incorporation rate and residence time of specific fatty acids could not be modeled with simple kinetic equations because the seals had to be transitioned onto diets and transition length varied by trial. However, a relative time response could be assessed by comparing time series plots of the relative intake (%) of a given fatty acid with plasma and RBC fatty acid response. The seal "Poco" had the longest continuous record for RBCs and was thus chosen for initial analysis (Fig 4.8). The difference in response time to prey switching among plasma and RBC samples varied for specific fatty acids. Response time for 14:0 was similar and fairly rapid for both tissues. In general, a shift towards the new diet level was detected at two weeks and stabilized between 4-6 weeks. In contrast, while response time for 18:1n-7 was similarly rapid in plasma, RBCs appeared to have an approximately two-month time lag compared with plasma.

Next the transition between trial two and three was examined because it was the shortest in duration of all transitions. Only seal "Travis" had a series of samples from this time period for both plasma and RBCs. Travis was sampled on May 19, transitioned from pollock to herring from May 22 to May 29 and was sampled again on June 1, 15 and so forth (Fig. 4.9). Again apparent response time varied both by fatty acid and by tissue

type. At 3 days (June 1) following the 8 day transition period, 14:0 in RBCs had already reached the new diet level, whereas 18:2n-6 was slower at 24 days (June 15). New diet levels were reached in at least 24 days post-transition in the two plasma fatty acids; however, the 3-day post sample was missing, and prevented a more conclusive assessment.

The final effort was to examine whole fatty acid profile changes instead of individual fatty acid changes. Using the DFA classification matrix, misclassifications were examined in greater detail to determine within what time period the model could correctly predict diet. Looking specifically at samples from the first two months of every trial (Table 4.7a), seals still in transition between diets were routinely misclassified. Most of the samples from seals that had fed on a 25:75 percent ratio of pollock to herring lipid intake for 1-2 weeks prior to sampling were classified as Mixed. This ratio matched exactly the ratio fed to the mixed diet control group seals. When seals ate 100% of the new diet for 3-7 days prior to sampling, samples were always misclassified. However, all but one of the samples were correctly classified as the new diet after two weeks on 100% new diet. Together with the data on individual fatty acids this suggested that even if individual fatty acid incorporation varied in rate, whole profiles could be used to predict diet for the preceding 8-14 days.

DISCUSSION

Understanding the dietary habits of marine mammals is important for both species and ecosystem management, yet data are invariably difficult to obtain because of the pelagic nature of marine mammal foraging. Harbor seals are opportunistic feeders, thus in wild populations the diversity of prey species in the diet can be very complex, contributing to the difficulty of characterizing the general diet. In this controlled study, limiting the dietary input to a two-prey system, herring or pollock, has allowed for the detailed examination of the influence of diet on individual fatty acids and whole fatty acid profiles in blood components. Assessing the fatty acid composition of plasma in

marine mammals in a longitudinal fashion allows for a better understanding of fatty acid metabolism, factors influencing its composition, and its use as a dietary indicator.

Influence of dietary fatty acids on plasma fatty acid profiles

It has been well established through human and animal trials that the fatty acid composition of plasma lipids largely reflects that of the diet, especially during high fat intakes (e.g., Zock et al. 1997). The current study of harbor seals supports this finding in that when examined individually, a large proportion of the plasma fatty acids (9 of 15 fatty acids or > 60 % of total fatty acid mass) exhibited significant differences depending on whether the seals consumed a herring or pollock diet. RBC fatty acids also changed with diet, albeit fewer in number, than in plasma. When fatty acids were examined with a multivariate model using DFA, harbor seal plasma and RBC fatty acid profiles were clearly separated into dietary groups (Fig 4.2 and 4.3.)

Direct incorporation of dietary fatty acids without prior modification into seal tissues is expected in carnivores such as harbor seals. This is because in simple stomached mammals, fatty acids are not altered by gut flora as seen in ruminants (Gurr et al. 2002). Furthermore, fatty acids can be absorbed non-discriminantly through the gut in proportion to that of the digested prey. It has been found in carnivores that the capacity to elongate or desaturate fatty acids is weak, owing to either the lack of enzymes or weak activity (Pond et al. 1995). Also, *de novo* synthesis of fatty acids is relatively low in all tissues except the liver and to a lesser extent, the adipose tissue. Three additional factors 1) diets high in fat and calories, 2) diets such as marine fish that are rich in essential and n-3 fatty acids, and 3) the fasting state, a regular and predictable state for pinnipeds, are all potent inhibitors of *de novo* fatty acid synthesis (Herzberg 1991), suggesting that it should be greatly reduced in harbor seals under most, but perhaps not all, dietary regimes.

In this study both essential fatty acids (EFA) and non-EFA varied in the plasma with respect to diet. EFA are defined on the basis of whether or not they can be synthesized endogenously and if they are needed for normal development and physiological function of mammalian cells throughout the life cycle. The "classic" EFA

are 18:2n-6 (linoleate) and 18:3n-3 (α-linolenate) and can only be obtained from diet, because mammals lack the enzymes to produce double bonds at the n-6 and n-3 position (Gurr et al. 2002). The extended definition EFA include the n-6 and n-3 long chain PUFA series, which cannot be synthesized efficiently, and only in the presence of adequate classic EFA, and thus are obtained almost exclusively through the diet (Arts et al. 2001; Cunnane 2003).

Of the typical fatty acids that can potentially be synthesized *de novo* as well as arise from diet (14:0, 16:0, 16:1n-7, 18:0, 18:1n-9 and 18:1n-7), three fatty acids (14:0, 16:1n-7 and 18:1n-9) appeared to follow dietary input regardless of total fat intake. This contrasts with Kirsch et al. (2000) who reported excess of 16:1n-7 and 18:1n-9 in harp seal blubber when fed low-fat pollock for one month. They suggested that excess amino acids from the high-protein intake from pollock could be used to biosynthesize these fatty acids. In the current study, evidence suggesting biosynthesis was found in the elevation of 18:1n-7 on the pollock diet and (or) when total daily lipid intake was low (Fig. 4.7). Fatty acid 18:1n-7 can be produced from biosynthesized 16:0, which is desaturated to 16:1n-7 and subsequently elongated to 18:1n-7. Hence, both the Kirsch et al. study and the current study suggest evidence of Δ9 desaturase activity in seals along the same pathway.

Several differences were found among the significance tests for plasma and those of the dietary fish (significant results of Table 4.4 and 4.5 summarized in Table 4.9). Study design and statistical limitations, as well as specific fatty acid function and individual variation may be responsible for the discrepancy. Issues surrounding RBCs will have some similarities, but have some additional considerations that will be discussed separately.

Four fatty acids (18:0, 20:1n-11, 22:1n-11, 20:5n-3) were significantly different among dietary fish (H4 vs. P2-3), yet were not significantly different for diet among plasma samples. Two of these fatty acids, 20:1n-11 and 22:1n-11, are frequently cited as "dietary indicators" (Arts et al. 2001; Iverson 1993; Iverson et al. 1997b; Kirsch et al. 2000; Sargent 1989), owing to their specific marine algae origin and usefulness in

identifying consumer-prey relationships. However, large intraspecific variation in these fatty acids can occur due to location, season and year of catch as well as fish age and size (Budge et al. 2003; Iverson et al. 2002). The level of these two fatty acids has been shown to increase three- to four-fold with fish size in adult herring (Iverson et al. 2002). In this study, these two fatty acids had the highest variation within a species and exhibited large batch variation (Table 4.4). Given the small sample size of fish tested and the high intraspecific variability, it is likely that significant differences could arise from chance alone. It is thus feasible that seal plasma did respond to dietary intake of these two fatty acids, but that variability in the fish ingested masked any apparent dietary or seasonal pattern. A higher frequency of fish sampling, such as monthly or at the end of each trial would have improved our ability to relate the plasma response to actual dietary fatty acid intake. The other two fatty acids (18:0 and 20:5n-3) that were significantly different between fish exhibited seasonal differences in plasma, which may have masked dietary differences. It is also possible that the large dietary input of 20:5n-3, especially from pollock, was converted to 22:5n-3, causing no difference in plasma for 20:5n-3, but significantly higher levels of 22:5n-3. A higher synthesis rate of 22:5n-3 from 20:5n-3 in humans was attributed to a large circulating pool of 20:5n-3 obtained from a fish diet compared to a beef diet (Pawlosky et al. 2003). However, it is not known when and if the enzymes responsible for such a conversion are active in seals.

Five fatty acids (16:0, 18:1n-9, 18:2n-6, 20:1n-9 and 22:5-3) were significantly different among plasma samples from different dietary treatments, but were not significantly different amongst fish. The statistical design may have had a role in some of these differences. The repeated measures analysis test (RM-ANOVA) was performed on plasma fatty acids to account for the fact that multiple samples were taken for each seal. However, this test assumed that dietary input was the same for each trial of the same diet species, when in fact it was known that batches of fish differed in fatty acid composition (Table 4.4). For example, 18:1n-9 was fairly constant in the plasma of all seals across trials 1-5 and similar to diet, but when switched onto new batches of fish for trial 6, plasma levels increased on herring and decreased on pollock, again consistent with this

new diet. The effect of this one trial may have largely driven the significant result in plasma. Thus, if this fatty acid was examined considering the batch differences, plasma and diet were actually in agreement.

Finally, the level of one fatty acid, 20:4n-6 (arachidonic acid) was significantly higher in pollock than in herring, but in seal plasma, the opposite was true. Because this fatty acid serves several physiological roles, the levels in plasma may be influenced by mechanisms other than diet. For example, 20:4n-6 is important in blood clotting, and the immune, reproduction and gastrointestinal systems. It is also important as a precursor to eicosanoid and prostaglandin production (Gurr, 2002; Pond, 1998). 20:4n-6 had the largest accumulation in plasma of any fatty acid, with mass % increasing 18 to 33-fold over intake levels. Although dietary intake significantly affected 20:4n-6 levels in plasma, the functional roles of 20:4n-6 appeared to determine its concentration.

Unlike in depot lipids such as blubber that are composed of almost entirely triglycerides, post-absorptive plasma lipids are complexes of lipids called lipoproteins. All lipid classes in lipoproteins (phospholipids, cholesterol esters, triglycerides, non-esterified fatty acids) can be influenced by dietary intake. However, just like with 20:4n-6, the functional role of lipoproteins in transporting lipids in the bloodstream can affect the concentration. If quantitative (as opposed to qualitative) methods are applied to plasma fatty acids in future studies to determine diet, calibration coefficients must be applied in order to compensate for lipoprotein chemistry (Cooper et al. 2001).

Predicting diet group membership with multivariate methods

When fatty acids were examined with a multivariate model using DFA, harbor seal plasma fatty acid profiles were clearly separated into dietary groups (Fig 4.2a) When the model was rerun using only the eight fatty acids significant in fish, the misclassification rate increased slightly, with three herring predicted as mixed and one mixed as herring (Fig. 4.2b). Nevertheless, the restricted model still resulted in a high degree of separation between diet groups. In fact, the separation between herring and pollock was identical between both models and only the separation between mixed diet

and herring declined. Although the mixed diet contained equal proportions of herring and pollock by weight, approximately 75% of the lipids came from herring due to its higher lipid content. The DFA plot revealed a visual confirmation of the closer relationship of mixed diet to herring compared to pollock, and it's ability to distinguish between the two. Separation between mixed diet and herring occurred in the 2nd discriminant score (Y-axis). The contribution of 18:2n-6, 24:1n-9 and 22:5n-3, which were present in the original model but were absent in the restricted model, increased the separation between groups, but the difference was not large. The choice of which fatty acids to include in any DFA is first restricted by model assumptions, that is, only n-1 variables of the smallest group can be used and variables with high pairwise correlation should be removed. Beyond that, choice of fatty acids to include in a model is largely arbitrary. Because upwards of 70 fatty acids can actually be measured, most studies reduce the number of fatty acids to include only major fatty acids (>1 % of total), fatty acids with the largest variance amongst groups and (or) specific fatty acids known to be "indicator" fatty acids of particular prey (Budge et al. 2003; Iverson et al. 2002; Kirsch et al. 1998). In this study, limits to the specific fatty acids used in the two models had little effect on overall diet prediction.

The true test of the appropriateness of the DFA model is to apply it to new samples. In this study "new" samples were samples from seals that had spent less time on a given diet. Arguably, new samples should have come from a different population of seals. However, the "new" samples were appropriate to assess how quickly fatty acid profiles could reliably predict diet. Our model had only three diet group options, 100% pollock, 100% herring or 25:75 pollock to herring (in % lipid, not mass), which resulted in many misclassifications during the transition period. But these misclassifications occurred in a consistent manner that followed the actual lipid ratio of the diet they had received in the previous weeks. It would be interesting to increase the number of mixed diet groups in different lipid proportions such as 90:10, 25:75, 50:50, 75:25, 10:90, to determine how fine a variation could be detected by the model.

Unlike clinical trials where subjects are dosed with very specific levels of fatty acid, this study used wild-caught fish diets that can vary fairly substantially within a species yet still maintain a distinct fatty acid profile (Budge et al. 2003; Iverson et al. 2002). If this technique is to be used in ecological studies at the individual or population level, it needs to be sufficiently robust to deal with potentially large natural variation. This is why multivariate analysis has an important advantage in predicting diet group membership over individual t-tests or ANOVAs. DFA classifies groups based on a cumulative discriminant score. For example, a plasma sample from a herring trial might have a score for 2 fatty acids typical of pollock, but the remaining fatty acids fit the herring profile. The cumulative score would dictate that this sample be classified as herring.

Effect of season on plasma fatty acid composition

Seasonal effects on the mass percentage of specific fatty acids needs to be considered because of the potential impact on the interpretation of dietary effects. Four fatty acids (20:4n-6, 18:0, 16:1n-7 and 20:5n-3) were found to exhibit seasonal differences by RM-ANOVA (Fig. 4.4), using only samples from the last month of each trial. However, peaking of fatty acids sometimes occurred at the beginning or middle of a given trial, which would have gone undetected by the RM-ANOVA method. Additional seasonal effects were identified by examining the plots of plasma fatty acid levels over time in "Snapper," an adult male seal that received a continuous 50/50 mixed diet and had a consistent lipid intake throughout the study compared with group A and B seals (Fig. 4.5a and Fig. 4.6). Because of the higher lipid content in herring, the proportion of daily lipid intake from herring was higher (77.5% ± 0.2, range 71.6-81.7) than pollock (22.5% ± 0.2, range 18.3-28.4). If changes in fatty acid composition were due to dietary differences alone, one would expect no variation throughout the six trials (except due to batch differences). This was true for nine fatty acids with the example of 22:1n-11 shown in Fig. 4.5.b. Yet six fatty acids (16:0, 18:0, 18:1n-7, 18:2n-6, 20:4n-6 and 20:5n-3) exhibited seasonal effects, with the majority of effects occurring in summer (S3, May 15

- September 15) and persisting for at least 4-6 weeks. See Fig. 4.5c-e. Seasonal effects in S3 may be due to stimulated lipolysis induced by hormonal changes associated either with breeding (fighting noted between males in July) or the molt, the peak of which is generally between late July and early September (Daniel et al. 2003), or by other factors which may impact fatty acid metabolism, but which we did not measure.

The seasonal differences in fatty acids detected statistically or visually did not impact the ability to separate harbor seals into dietary groups based on their fatty acid profiles. However, as the use of fatty acid profiles develops from a qualitative to a quantitative method to predict diet composition in wild seals (Cooper et al. 2001), seasonal effects may have a larger impact on interpretation. Whether the seasonal changes seen in plasma are also observed in blubber deserves attention, but may be difficult to address due to limitations placed on the frequency of blubber sampling. It is possible that the plasma samples in this study, which are postabsorptive samples, may be more responsive to greater metabolic processing than the lipids in blubber. Even though fatty acids are continuously recycled between plasma and blubber (adipose), the greatest effect on the fatty acid composition of blubber likely occurs directly following a meal. This is because the fats ingested are typically greater than immediate energy needs and are therefore deposited in the blubber for storage. The percentage stored can be very high, ~90% of dietary fatty acids were deposited in adipose stores during the postprandial period in normal and obese humans (Binnert et al. 1996; Maffeis et al. 1999).

Influence of dietary fatty acids on RBC fatty acid profiles

The fatty acid composition of RBC membranes has been used widely in human clinical trials as an indicator of specific dietary fatty acid input (Dougherty et al. 1987; Farquhar and Ahrens, Jr. 1963; Prisco et al. 1996). Lipids, especially phospholipids and cholesterol, comprise approximately 50% of the mass of RBC membranes (Shohet 1986). Mature RBCs are unable to synthesize fatty acids *de novo*; however, lipids of the membrane bilayer are constantly remodeled by both passive and active exchange pathways with the plasma. This process is most prevalent in the outer leaflet of the

membrane, whereas acylation of lysophospholipids is more common in the inner leaflet (De Gier and Van Deenen 1964; Shohet 1986). In light of this, if plasma fatty acids are representative of the diet, RBC fatty acids should also reflect the diet, albeit in a longer time frame.

In this study although RBC fatty acids generally increased or decreased similar to that of the diet, only 3 fatty acids (14:0, 18:1n-7, 18:2n-6) plus the ratio 22:5n-3/22:6n-3 were significantly different between dietary treatments when analyzed individually. Several factors may explain why dietary differences were less prevalent in RBC fatty acids. First, it may have been more difficult to detect differences because the magnitude of the response in RBCs was smaller than that observed in plasma (Table 4.5, Fig. 4.8-4.9). Second, differences in dietary intake may not have been large enough to elicit a detectible response. In human clinical trials, studies generally involved dosing of single or multiple EFA or PUFA in patients whose normal intakes were generally low or lacking in these fatty acids. The concentration of long-chain PUFA in RBCs was found to be linearly related to daily dosage (Katan et al. 1997). Seals, in contrast, regularly ingest high levels of EFA and PUFA in diets that are typical of the species. It is not known if this would affect incorporation rates. However, differences in the fatty acid classes of RBCs although significant between diets, were very small suggesting that the lipid composition of RBCs is likely under greater homeostatic control than plasma in order to maintain membrane-associated physiologic processes. Because fatty acid composition also affects the fluidity of RBC membranes, a characteristic likely of great importance to diving marine mammals that experience great pressure at depth, some level of homeostatic control of composition is expected. However, RBC fatty acids of both fur seal and elephant seal were found to be more unsaturated as compared to humans (~60% vs. 50%; Fayolle et al. 2000), which was attributable to a marine-based diet. Furthermore, increased fluidity caused by changes in fatty acid composition can be counteracted by changes in the cholesterol content, thereby preserving membrane function despite dietary modification.

Detecting whether RBC fatty acids had reached a steady state after 4 months of dietary treatment was sometimes difficult, but for 14:0 and 18:2n-6, both of these fatty acids appeared at a similar rate as plasma fatty acids and stabilized in 2-6 weeks (Fig. 4.8). This time frame for incorporation is in agreement with human trials ingesting corn oil (high in 18:2n-6) at 40% of total calorie intake (Farquhar and Ahrens, Jr. 1963). Fatty acid 18:1-7 in RBCs was generally slower than in plasma, and for 22:5n-3/22:6n-3 the pattern was unclear. In humans, long-chain PUFA like 20:5n-3 and 22:5n-3 appeared rapidly in RBCs but were slower to reach equilibrium. The half-life of each varied from 28.1 to 38.5 days, respectively (Katan et al. 1997). Because incorporation rates can vary by fatty acid type, some fatty acids may have never equilibrated with respect to diet in the four months of each trial. It is likely that the majority of the fatty acids reflect that of diet in the range of one to several months, but more results are needed. Dosing experiments in seals over a long time period (6-12 months) could help clarify a more precise time range.

When multiple fatty acids were analyzed in combination using discriminant analysis, RBC fatty acids could successfully predict dietary group membership, suggesting that sufficient change in the RBC fatty acid profile had occurred to detect dietary differences (Fig. 4.3). However, the discriminant results for RBCs should be viewed as exploratory and descriptive in nature, because of the small sample size of this analysis and lack of cross-validation.

Multiple fatty acid profiles and "dietary windows": conclusions and future directions

The time period required for dietary fatty acids to be detected in the tissue of the consumer is important for understanding the "dietary window" a fatty acid profile represents. In this study, fatty acid profiles of plasma represented short-term windows of approximately 8-14 days, whereas RBC fatty acid profiles reflected longer-term integration over one to several months. Results from each tissue type could serve to answer different diet history questions. For example, RBC fatty acid profiles would be best suited for representing whole seasons of foraging activity. An example is the comparison between pre-molt and pre-breeding foraging trips of northern elephant seals.

RBC fatty acids could provide an integration of the diet of each whole trip. In addition to representing a longer period than plasma, RBCs would have the added benefit in that they would not be affected by lipemia, a postprandial condition, which would represent only the most recent meals.

The short dietary window provided by plasma samples would be suited to studies in which short-term dietary information is needed, or when multiple sampling of individuals is required. Plasma has the advantage over blubber in that sampling is quick, can often be taken without anesthesia and can be taken with minimal stress or impact on the animal. One plausible application would be studies of young, independently foraging, seal pups. Often young pups feed on alternate prey from adults because they are either physiologically limited in their diving capacity (Burns and Castellini 1996) or they lack experience in catching fast-swimming prey. Plasma fatty acid profiles could potentially distinguish the switch from, for example, sedentary benthic prey to more pelagic prey. It is likely that blubber fatty acid turnover at this stage would be slower because pups would ingest much lower daily lipid intakes than during the nursing period. The effects of maternal input on pup lipid stores may also persist for some time and thus a shift in diet over a short time period during independent foraging may not be detectable in blubber. Only one study to date has examined changes in plasma fatty acids in independently foraging pups (Rea et al. 1997). Although foraging could not be confirmed by changes in individual fatty acids, the authors acknowledged that small sample sizes, short periods of fasting, low prey intake and similar prey and blubber lipids may have made such a determination more difficult to detect. Perhaps if a similar study was performed in a more temperate region that has larger diversity in prey and longer food chains, diet changes could be detected. Also, as shown in the current study, fatty acid profiles rather than individual fatty acids may be better suited for determining dietary differences.

Plasma and RBC fatty acid profiles might also be able to help resolve the question of whether killer whales, *Orcinus orca*, are consuming harbor seals or Steller sea lions around Alaska. We currently do not have data on the whole body fatty acid profile of seals and sea lions, but we do know that stable isotope ratios for these animals overlap

considerably (Hirons et al. 2001). If whole body fatty acid profiles are different, then this type of profiling might be a relevant diagnostic tool that would be preferred over blubber if blood samples could be collected from killer whales. Blubber biopsies would not necessarily work because they access only superficial blubber and most of these shallow lipids are probably structural (Chapter 2, this thesis; Koopman et al. 2002; Struntz et al. 2004) and blubber fatty acids may turn over too slowly to be good indicators of diet. There are no current techniques for obtaining blood samples from wild killer whales. However, work on captive killer whales consuming different fish diets where blood samples could be easily collected would be an important starting point for this type of work.

In conclusion, this is the first long-term study to show the direct effect of prey switching on the plasma and RBC fatty acid profiles in the harbor seal. This study has shown that dietary fatty acids are reflected quickly in these tissues and represent two different "dietary windows." The ability to quantify an estimate of the dietary window was hampered by differences in the length of the transition period between trials and the frequency of a sampling. Future studies should use an abrupt diet switch and sample weekly, which would allow for the application of simple kinetics models and increased resolution in determining the appearance of dietary fatty acids and the dietary window they represent. Nonetheless, general time frames of incorporation could be determined by this study.

Seasonal changes were observed on a constant mixed diet, suggesting that more data are needed to determine what influences fatty acid composition in the plasma. Although not directly tested in this study, the fact that the majority of the seasonal effects occurred in summer/early fall suggests that breeding or molting may have been involved. Hormones associated with molt (thyroid hormones, cortisol), reproduction (estrogen, progesterone and testosterone) and growth (growth hormone) can have dramatic effects on the mobilization of lipids from body stores and fatty acid metabolism (John et al. 1980; John et al. 1987). Monitoring of these hormones is needed to address their impact on the levels of specific fatty acids that are also important for determining diet. This is

especially urgent since most sampling of wild seals is conducted near or at the time of molt and reproduction when the animals are most accessible. Similarly, total lipid intake and energy balance can impact the level of certain fatty acids (this study; Kirsch et al. 2000). Wild phocids are often sampled during periods of rapid fattening and so the impact on diet interpretation may not be an issue. However, in comparison otariids are typically leaner than phocids and rely less heavily on building large lipid stores in blubber for later use. Fatty acid metabolism is likely to differ between these pinniped families and thus, biosynthesis, desaturation and elongation of fatty acids may be more common in otariids and deserves further testing.

How the plasma and RBC fatty acid profiles and the dietary windows they represent compare to blubber fatty acid profiles is unknown. It would be beneficial to have matched serial plasma, RBC and blubber samples over long periods to address this question over a variety of lipid intake levels.

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Table 4.1. Biological information for eight captive harbor seals at the Alaska SeaLife Center participating in a repeated crossover designed feeding trial.

Seal ID	Age*	Age class	Sex	Dietary test group
Poco	23	Adult	F	A
Skeezix	23	Adult	F	A
Cecil	15	Adult	M	A
Sydney	2	Juvenile	F	B
Travis	2	Juvenile	M	B
Pender	2	Juvenile	M	B
Snapper	14	Adult	M	C
Tina	7	Adult	F	C

* Age at the start of feeding trials in September 1998.

Table 4.2. Repeated crossover design of feeding trials for captive harbor seals fed either herring or pollock or an equal herring/pollock (w/w) mixed diet. Group designation as in Table 4.1.

			Harbor seal group on a given diet		
Trial	Season	Time Period[a]	Herring	Pollock	Mixed
1	S1	Sept. 1998-Jan. 1999	B	A	C
2	S2	Jan. 1999-May 1999	A	B	C
3	S3	May 1999-Sept. 1999	B	A	C
4	S1	Sept. 1999-Jan. 2000	A	B	C
5	S2	Jan. 2000-May 2000	B	A	C
6	S3	May 2000-Sept. 2000	A	B	C

[a] Each trial began and ended on approximately the 15th of the specified months

Table 4.3. Fatty acid composition (mass %) of prey species fed to harbor seals during controlled dietary studies. Values are means ± SE of all 61 fatty acids and isomers identified and sums of fatty acid classes.

Fatty acid	Herring n=14	Pollock n=10
SAT		
C12:0	0.04 ± 0.00	0.01 ± 0.00
C13:0	0.02 ± 0.00	0.01 ± 0.00
Iso14	0.02 ± 0.00	0.00 ± 0.00
C14:0	7.56 ± 0.24	4.61 ± 0.29
Iso15	0.17 ± 0.01	0.11 ± 0.01
Anti15	0.05 ± 0.00	0.02 ± 0.00
C15:0	0.30 ± 0.01	0.24 ± 0.01
Iso 16	0.05 ± 0.00	0.06 ± 0.00
C16:0	14.41 ± 0.48	13.67 ± 0.57
7Me16:0	0.22 ± 0.01	0.23 ± 0.02
Iso 17	0.09 ± 0.00	0.09 ± 0.01
C17:0	0.41 ± 0.16	1.86 ± 0.19
C18:0	1.56 ± 0.16	2.97 ± 0.19
C20:0	0.30 ± 0.02	0.12 ± 0.02
MUFA		
C14:1n-9	0.15 ± 0.01	0.09 ± 0.02
C14:1n-5	0.08 ± 0.00	0.08 ± 0.00
C15:1n-6	0.00 ± 0.00	0.00 ± 0.00
C16:1n-11	0.33 ± 0.01	0.20 ± 0.01
C16:1n-9	0.10 ± 0.00	0.12 ± 0.01
C16:1n-7	6.16 ± 0.33	7.19 ± 0.39
C16:1n-5	0.11 ± 0.01	0.08 ± 0.01
C16:1 iso	0.04 ± 0.00	0.03 ± 0.00
C17:1	0.16 ± 0.01	0.10 ± 0.01
C18:1n-13	0.34 ± 0.13	1.14 ± 0.16
C18:1n-9	13.62 ± 0.86	11.38 ± 1.02
C18:1n-7	2.82 ± 0.31	5.82 ± 0.36
C18:1n-5	0.59 ± 0.02	0.24 ± 0.03
C20:1n-11	9.21 ± 1.13	8.14 ± 1.34
C20:1n-9	2.78 ± 0.27	2.62 ± 0.32
C20:1n-7	0.29 ± 0.02	0.23 ± 0.02
C20:1n-5	0.14 ± 0.01	0.08 ± 0.01
C22:1n-11	10.92 ± 1.01	6.54 ± 1.20
C22:1n-9	0.78 ± 0.14	0.49 ± 0.16
C22:1n-7	0.20 ± 0.02	0.17 ± 0.02
C24:1n-9	0.75 ± 0.09	0.49 ± 0.10

Table 4.3 continued

	Herring n = 14	Pollock n = 10
PUFA		
C16:2n-4	0.21 ± 0.01	0.13 ± 0.01
C16:2n-6	0.07 ± 0.00	0.05 ± 0.00
C16:3n-6	0.43 ± 0.02	0.44 ± 0.02
C16:3n-4	0.29 ± 0.02	0.22 ± 0.02
C16:4n-1	0.41 ± 0.03	0.23 ± 0.03
C18:2d5,11	0.05 ± 0.01	0.02 ± 0.01
C18:2n-7	0.02 ± 0.00	0.02 ± 0.00
C18:2n-6	0.78 ± 0.04	0.70 ± 0.04
C18:2n-4	0.19 ± 0.01	0.18 ± 0.01
C18:3n-6	0.08 ± 0.00	0.11 ± 0.01
C18:3n-4	0.11 ± 0.01	0.06 ± 0.01
C18:3n-3	0.56 ± 0.05	0.37 ± 0.06
C18:3n-1	0.01 ± 0.00	0.00 ± 0.00
C18:4n-3	1.49 ± 0.08	1.04 ± 0.09
C18:4n-1	0.22 ± 0.02	0.12 ± 0.03
C20:2n-6	0.15 ± 0.01	0.16 ± 0.01
C20:3n-6	0.08 ± 0.01	0.08 ± 0.01
C20:4n-6	0.43 ± 0.04	0.66 ± 0.04
C20:3n-3	0.12 ± 0.01	0.07 ± 0.01
C20:4n-3	0.66 ± 0.03	0.46 ± 0.04
C20:5n-3	12.13 ± 0.71	16.27 ± 0.84
C21:5n-3	0.21 ± 0.01	0.22 ± 0.01
C22:4n-6	0.04 ± 0.00	0.04 ± 0.00
C22:5n-6	0.09 ± 0.01	0.07 ± 0.01
C22:5n-3	0.87 ± 0.10	0.84 ± 0.12
C22:6n-3	5.54 ± 0.72	8.18 ± 0.85
Σ SAT	24.97 ± 0.52	23.78 ± 0.88
Σ MUFA	49.76 ± 1.04	45.44 ± 1.93*
Σ PUFA	25.27 ± 0.93	30.80 ± 1.62**
Σ n-3	21.59 ± 0.90	27.46 ± 1.59**
Σ n-6	2.19 ± 0.05	2.34 ± 0.10
n-3/n-6	9.89 ± 0.43	11.88 ± 0.85*

* $P < 0.05$, ** $P < 0.01$, comparison with herring
Abbreviations: SAT = saturated, MUFA = monounsaturated, PUFA = polyunsaturated

Table 4.4. Fatty acid composition (mass %) of prey species of herring and pollock fed to harbor seals. Values are means ± SE of fatty acids of total herring or pollock, and of individual batches of fish.

	Total			Herring Batch				Pollock Batch		
Fatty acid	Herring		Pollock	H4		H6		P2-3	P5	
n	14		10	8		6		5	5	
SAT										
14:0	8.44 ± 0.28	**	5.12 ± 0.33	8.44 ± 0.38	**	8.44 ± 0.44		4.46 ± 0.38	5.78 ± 0.38	b
16:0	16.08 ± 0.51		15.16 ± 0.61	16.18 ± 0.67		15.94 ± 0.78		16.25 ± 0.80	14.08 ± 0.80	
18:0	1.74 ± 0.17	**	3.29 ± 0.21	1.85 ± 0.13	**	1.59 ± 0.15		3.73 ± 0.37	2.86 ± 0.37	
MUFA										
16:1n-7	6.88 ± 0.37		7.98 ± 0.43	6.63 ± 0.37		7.21 ± 0.43		6.62 ± 0.46	9.35 ± 0.46	bb
18:1n-9	15.21 ± 0.95		12.61 ± 1.12	13.67 ± 1.13		17.26 ± 1.30		14.44 ± 1.39	10.78 ± 1.39	
18:1n-7	3.14 ± 0.34	**	6.46 ± 0.40	3.02 ± 0.30	**	3.31 ± 0.35		7.31 ± 0.69	5.61 ± 0.69	
20:1n-11	10.27 ± 1.26		9.05 ± 1.49	13.16 ± 1.26	**	6.43 ± 1.45	aa	6.03 ± 1.52	12.07 ± 1.52	b
20:1n-9	3.12 ± 0.30		2.91 ± 0.36	2.35 ± 0.22		4.15 ± 0.25	aa	1.90 ± 0.25	3.93 ± 0.25	bb
22:1n-11	12.21 ± 1.14	**	7.28 ± 1.35	13.54 ± 1.64	**	10.43 ± 1.89		5.42 ± 1.38	9.14 ± 1.38	
24:1n-9	0.84 ± 0.10		0.54 ± 0.12	0.59 ± 0.11		1.18 ± 0.12	aa	0.60 ± 0.13	0.49 ± 0.13	
PUFA										
18:2n-6	0.88 ± 0.04		0.78 ± 0.05	0.94 ± 0.04		0.79 ± 0.05		0.78 ± 0.08	0.78 ± 0.08	
20:4n-6	0.48 ± 0.04	**	0.73 ± 0.05	0.48 ± 0.04	**	0.49 ± 0.05		0.80 ± 0.08	0.66 ± 0.08	
20:5n-3	13.55 ± 0.80	**	18.06 ± 0.95	12.35 ± 0.80	**	15.15 ± 0.92	a	20.05 ± 1.32	16.08 ± 1.32	
22:5n-3	0.98 ± 0.11		0.94 ± 0.13	0.80 ± 0.14		1.22 ± 0.17	a	0.95 ± 0.17	0.92 ± 0.17	
22:6n-3	6.19 ± 0.38	**	8.03 ± 0.44	6.02 ± 0.45	*	6.41 ± 0.52		8.58 ± 0.71	7.47 ± 0.71	

*$P < 0.05$,** $P < 0.01$, comparison of total herring vs. total pollock, or H4 herring vs. P2-3 pollock
[a] $P < 0.05$, [aa] $P < 0.01$, comparison of batches H4 vs. H6 herring
[b] $P < 0.05$, [bb] $P < 0.01$, comparison of batches P2-3 vs. P5 pollock
Abreviations: SAT: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid

Table 4.5. Fatty acid composition (mean mass % ± SE) of harbor seal plasma and red blood cells (RBCs) after dietary treatments of herring, pollock or a mixed diet.

	Plasma			Red blood cells		
Fatty acid	Herring	Pollock	Mixed	Herring	Pollock	Mixed
SAT						
14:0	2.00 ± 0.05 *	1.31 ± 0.03	2.08 ± 0.07	0.92 ± 0.06 *	0.62 ± 0.05	0.71 ± 0.06
16:0	15.91± 0.13 *	14.91 ± 0.13	17.18 ± 0.18	15.61 ± 0.60	14.37 ± 0.74	14.16 ± 0.26
18:0	12.05 ± 0.22	11.66 ± 0.13	9.69 ± 0.22	13.65 ± 0.32	14.11 ± 0.27	14.31 ± 0.35
MUFA						
16:1n-7	3.13 ± 0.06	3.17 ± 0.09	2.77 ± 0.09	0.88 ± 0.04	0.83 ± 0.06	1.00 ± 0.06
18:1n-9	14.82 ± 0.20 *	14.03 ± 0.15	14.93 ± 0.31	7.78 ± 0.30	7.01 ± 0.20	8.27 ± 0.25
18:1n-7	4.69 ± 0.11 *	6.69 ± 0.15	5.66 ± 0.15	4.61 ± 0.21 *	5.76 ± 0.19	3.94 ± 0.13
20:1n-11	2.66 ± 0.12	2.66 ± 0.10	2.83 ± 0.23	3.86 ± 0.34	3.91 ± 0.15	2.95 ± 0.09
20:1n-9	0.66 ± 0.06 *	0.83 ± 0.04	0.69 ± 0.05	1.70 ± 0.11	1.95 ± 0.13	2.87 ± 0.15
22:1n-11	0.69 ± 0.05	0.63 ± 0.04	0.64 ± 0.05	5.89 ± 0.40	5.47 ± 0.26	7.49 ± 0.86
24:1n-9	1.93 ± 0.11	2.15 ± 0.13	1.13 ± 0.05	11.05 ± 0.34	11.51 ± 0.44	12.28 ± 0.47
PUFA						
18:2n-6	1.82 ± 0.05 *	1.23 ± 0.04	1.36 ± 0.07	0.96 ± 0.04 *	0.83 ± 0.08	0.88 ± 0.06
20:4n-6	16.12 ± 0.26 *	13.33 ± 0.29	16.27 ± 0.52	17.38 ± 0.32	17.52 ± 0.47	14.88 ± 0.19
20:5n-3	16.50 ± 0.28	16.53 ± 0.24	17.58 ± 0.48	8.40 ± 0.18	8.14 ± 0.24	7.68 ± 0.19
22:5n-3	2.22 ± 0.07 *	4.77 ± 0.21	3.00 ± 0.09	2.62 ± 0.17	3.20 ± 0.29	2.89 ± 0.44
22:6n-3	4.79 ± 0.09 *	6.10 ± 0.16	4.19 ± 0.08	2.38 ± 0.18	2.43 ± 0.24	2.93 ± 0.26
22:n-3 ratio	0.46 ± 0.22	0.78 ± 0.28	0.72 ± 0.22	1.12 ± 0.05 *	1.34 ± 0.07	0.97 ± 0.10
Σ SAT (%)	29.96 ± 0.19 *	27.86 ± 0.17	28.99 ± 0.19	30.18 ± 0.76	29.10 ± 0.91	29.17 ± 0.49
Σ MUFA(%)	28.58 ± 0.24 *	30.17 ± 0.22	28.62 ± 0.19	35.78 ± 1.74 *	36.44 ± 0.89	38.80 ± 0.47
Σ PUFA (%)	41.45 ± 0.26	41.97 ± 0.17	42.39 ± 0.30	31.73 ± 0.41 *	33.46 ± 0.34	30.18 ± 0.54

*Indicates significant difference between herring and pollock diet
Abbreviations: SAT: saturated fatty acid; MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid

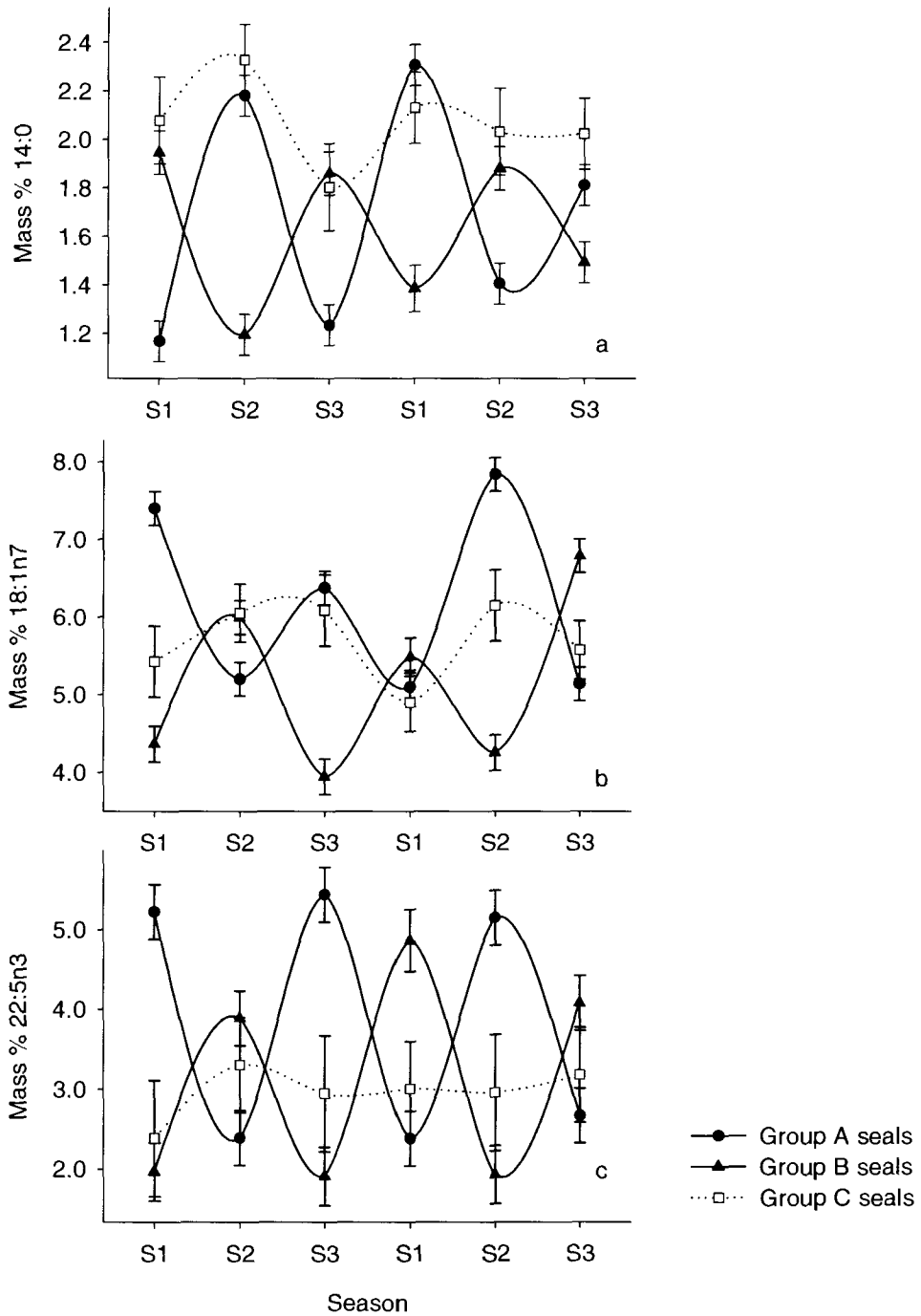

Fig. 4.1. Examples of significant effects of diet on the mass % of a saturated (a), monounsaturated (b) and polyunsaturated (c) fatty acid in harbor seal plasma during six feeding trials representing three seasons. Groups A and B seals were fed alternating diets of herring or pollock with Group A beginning on pollock and Group B beginning on herring. Group C seals were fed equal proportions of herring and pollock (mixed diet) throughout all trials.

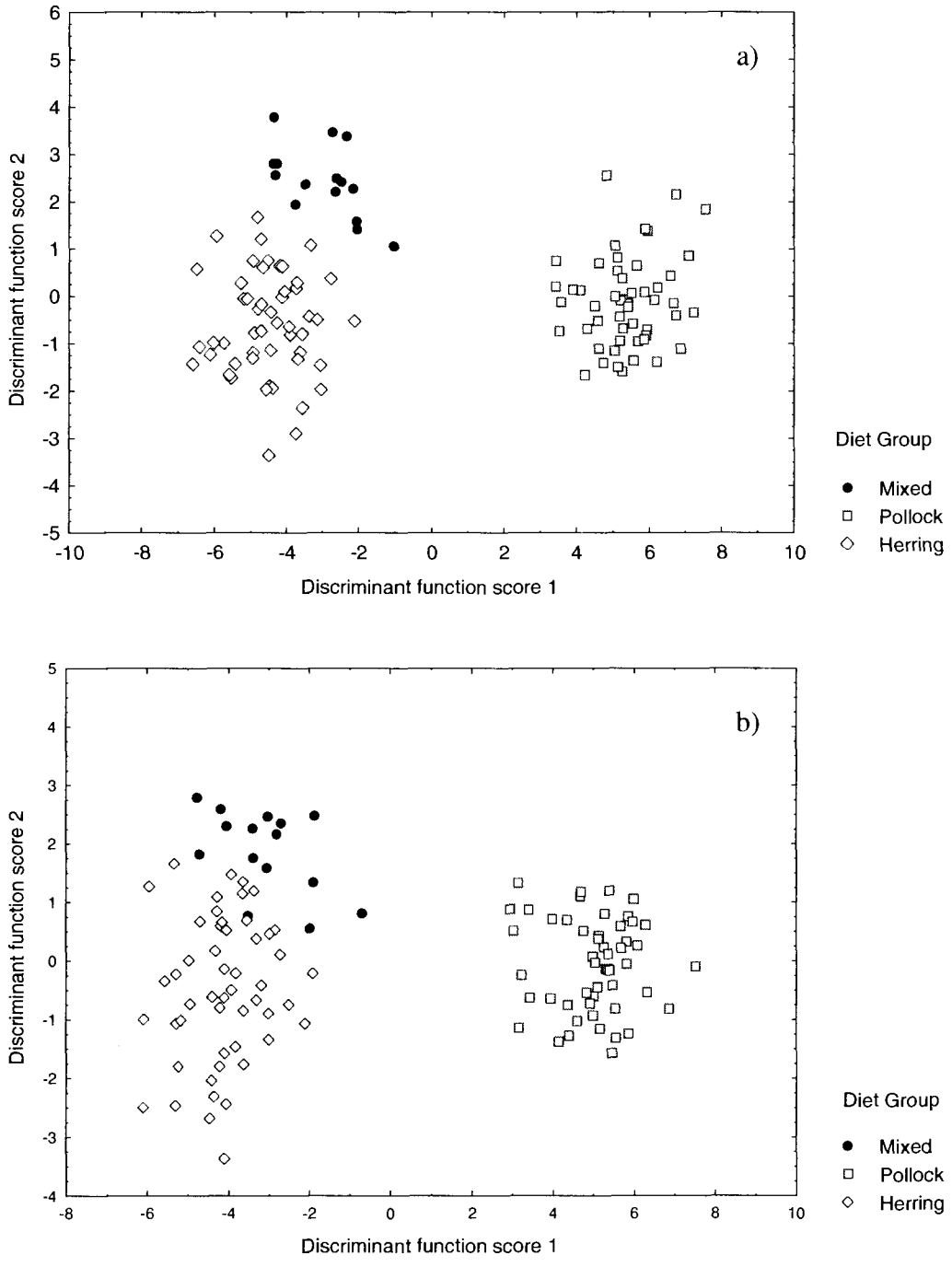

Fig. 4.2. Separation of three diet groups (mixed, pollock and herring) based on linear discriminant scores derived from plasma fatty acid profiles of harbor seals sampled in the last month of four-month feeding trials. Plot a) Original model using 11 fatty acids. Plot b) Reduced model using only the 8 fatty acids that were significantly different in fish diets.

Table 4.6. Classification matrixes obtained from the discriminant analysis of the plasma fatty acid profiles of captive harbor seals fed a mixed, pollock or herring diet in the last month of four-month feeding trials. a) Original model using 11 fatty acids. b) Reduced model using only the 8 fatty acids that were significantly different in fish diets

a) Original model

	Mixed (n=15)	Pollock (n=51)	Herring (n=51)	% Correct
Mixed	15	0	0	100.0
Pollock	0	51	0	100.0
Herring	0	0	51	100.0
Total	15	51	51	100.0

b) Reduced model

	Mixed (n=15)	Pollock (n=51)	Herring (n=51)	% Correct
Mixed	14	0	1	93.3
Pollock	0	51	0	100.0
Herring	3	0	48	94.1
Total	17	51	49	96.6

Table 4.7. Results of test of DFA model on "new" plasma samples. Classification matrix obtained from the discriminant analysis of the plasma fatty acid profiles of captive harbor seals fed a mixed, pollock or herring diet in the (a) first two months and (b) third month of four-month feeding trials. Predictor discriminant functions were derived from the discriminant model in Fig. 4.2a.

a) First two months *

	Mixed (n=24)	Pollock (n=62)	Herring (n=59)	% Correct
Mixed	22	0	2	91.6
Pollock	9	32	21	51.6
Herring	16	1	42	71.2
Total	47	33	65	66.2

b) Third month

	Mixed (n=10)	Pollock (n=30)	Herring (n=27)	% Correct
Mixed	10	0	0	100.0
Pollock	1	28	1	93.3
Herring	3	0	24	88.9
Total	14	28	25	92.5

* Includes samples from the transition period from one diet to the next

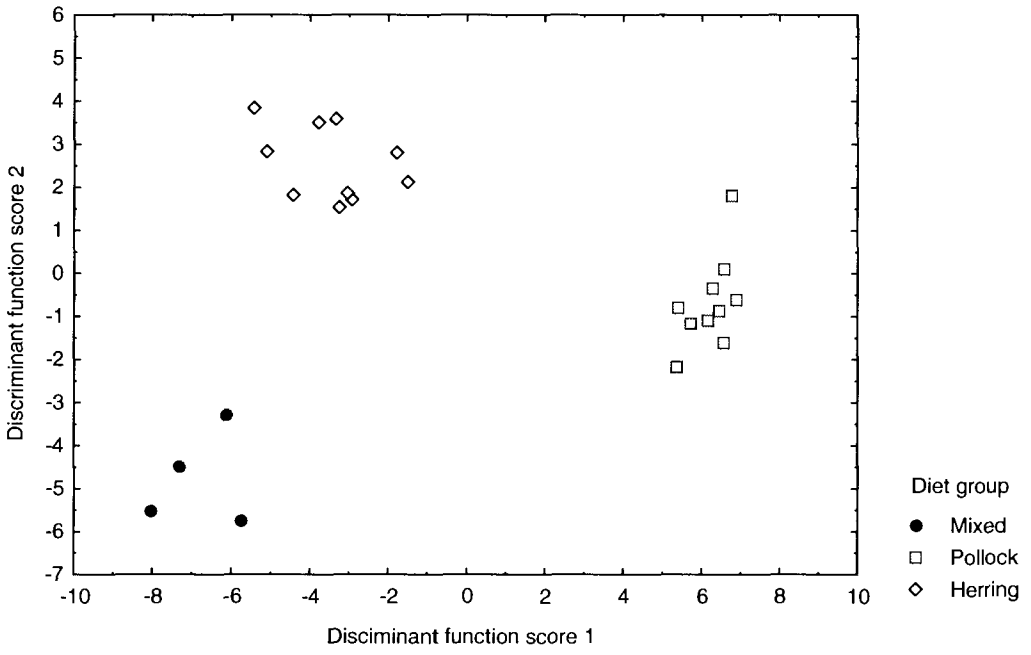

Fig. 4.3. Separation of three diet groups (mixed, pollock and herring) based on linear discriminant scores derived from RBC fatty acid profiles of harbor seals sampled at the end of four-month feeding trials.

Table 4.8. Classification matrix obtained from the discriminant analysis of the RBC fatty acid profiles of captive harbor seals fed a mixed, pollock or herring diet at the end of four-month feeding trials.

	Mixed (n=4)	Pollock (n=10)	Herring (n=10)	% Correct
Mixed	4	0	0	100.0
Pollock	0	10	0	100.0
Herring	0	0	10	100.0
Total	4	10	10	100.0

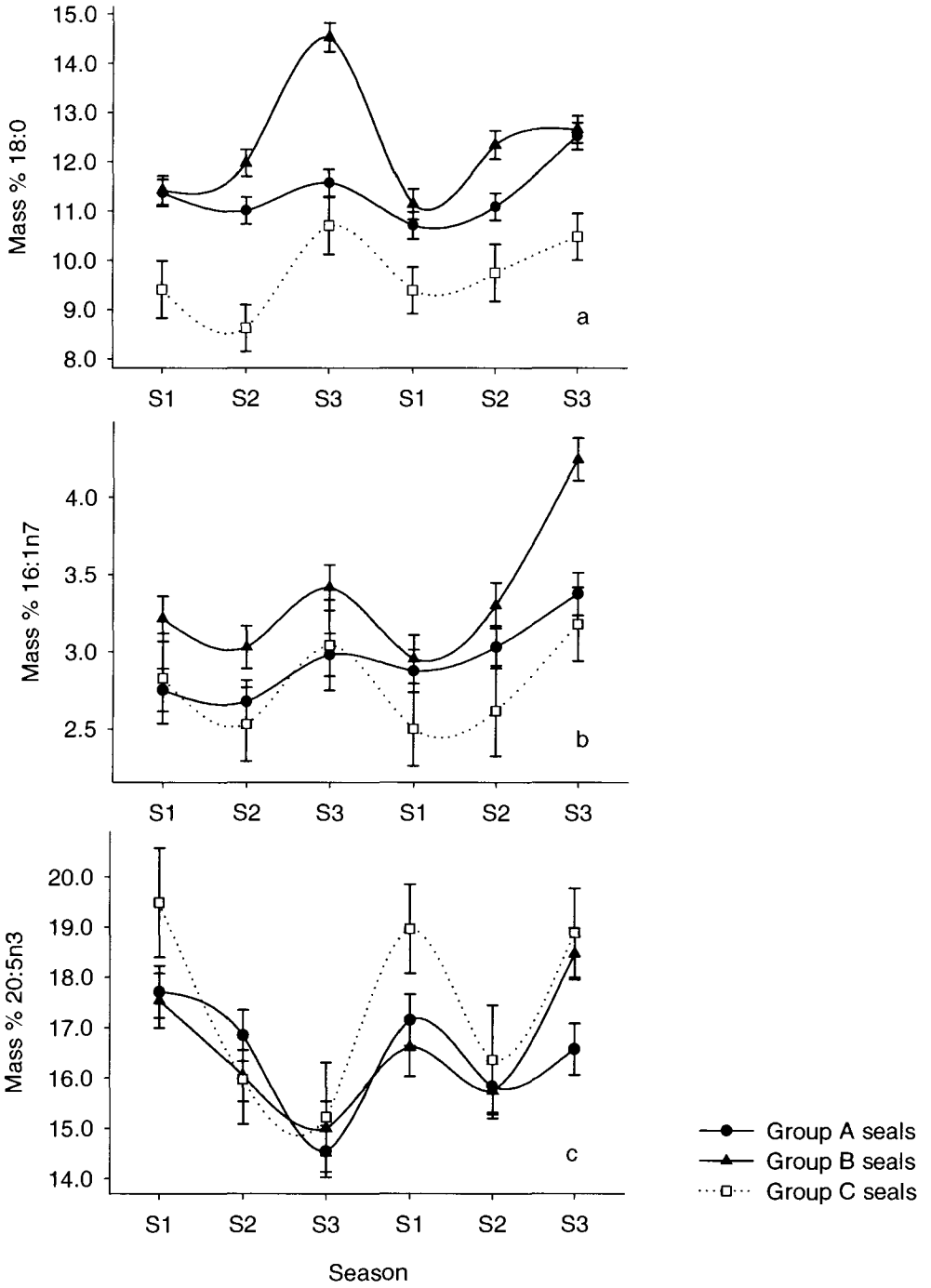

Fig. 4.4. Example of the significant effect of season on the mass % of a saturated (a), monounsaturated (b) and polyunsaturated (c) fatty acid in harbor seal plasma during six feeding trials representing three seasons. Groups A and B seals were fed alternating diets of herring or pollock with Group A beginning on pollock and group B beginning on herring. Group C seals were fed equal proportions of herring and pollock (mixed diet) throughout all trials.

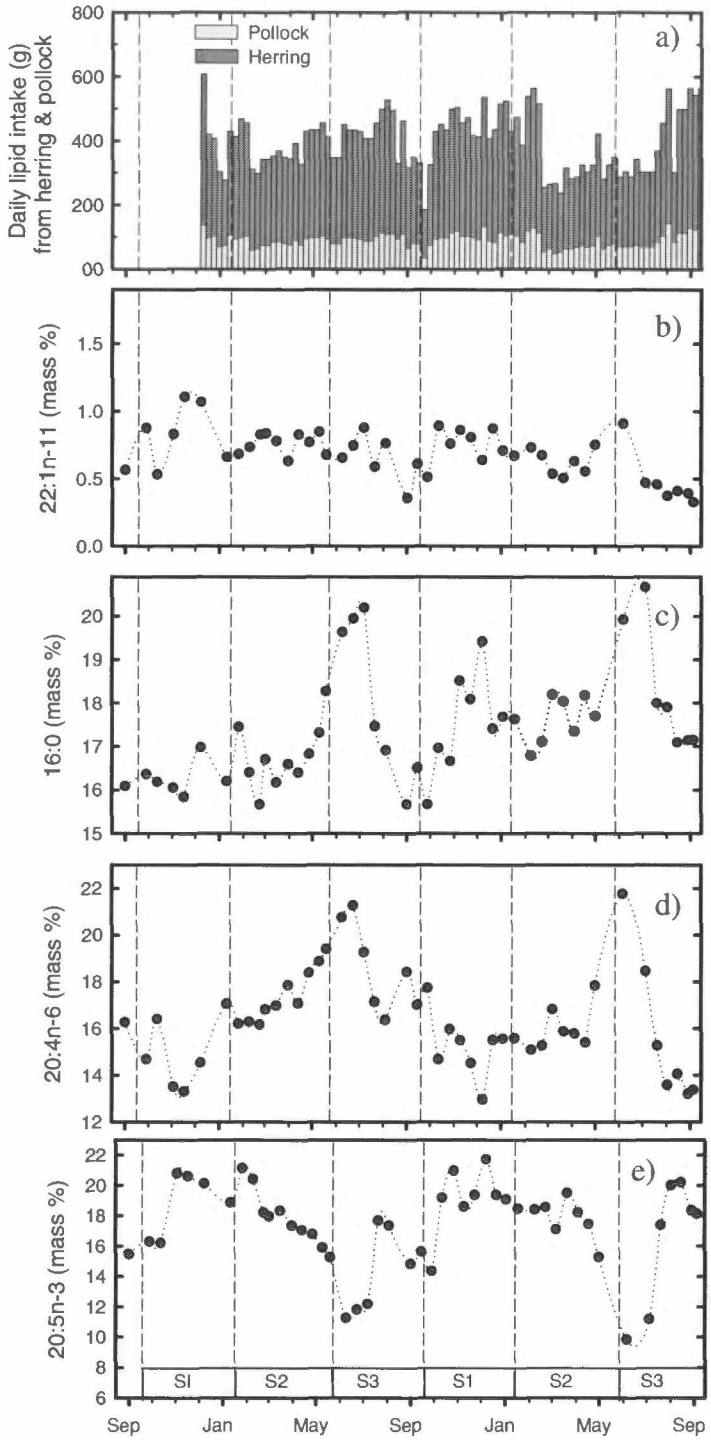

Fig. 4.5. Variability of plasma fatty acids in a harbor seal (Snapper) fed a continuous mixed diet. (a) lipid intake (g/d). (b) No seasonal response. (c-e) Apparent seasonal response. Vertical dashed lines included as a reference to diet switching in other seals.

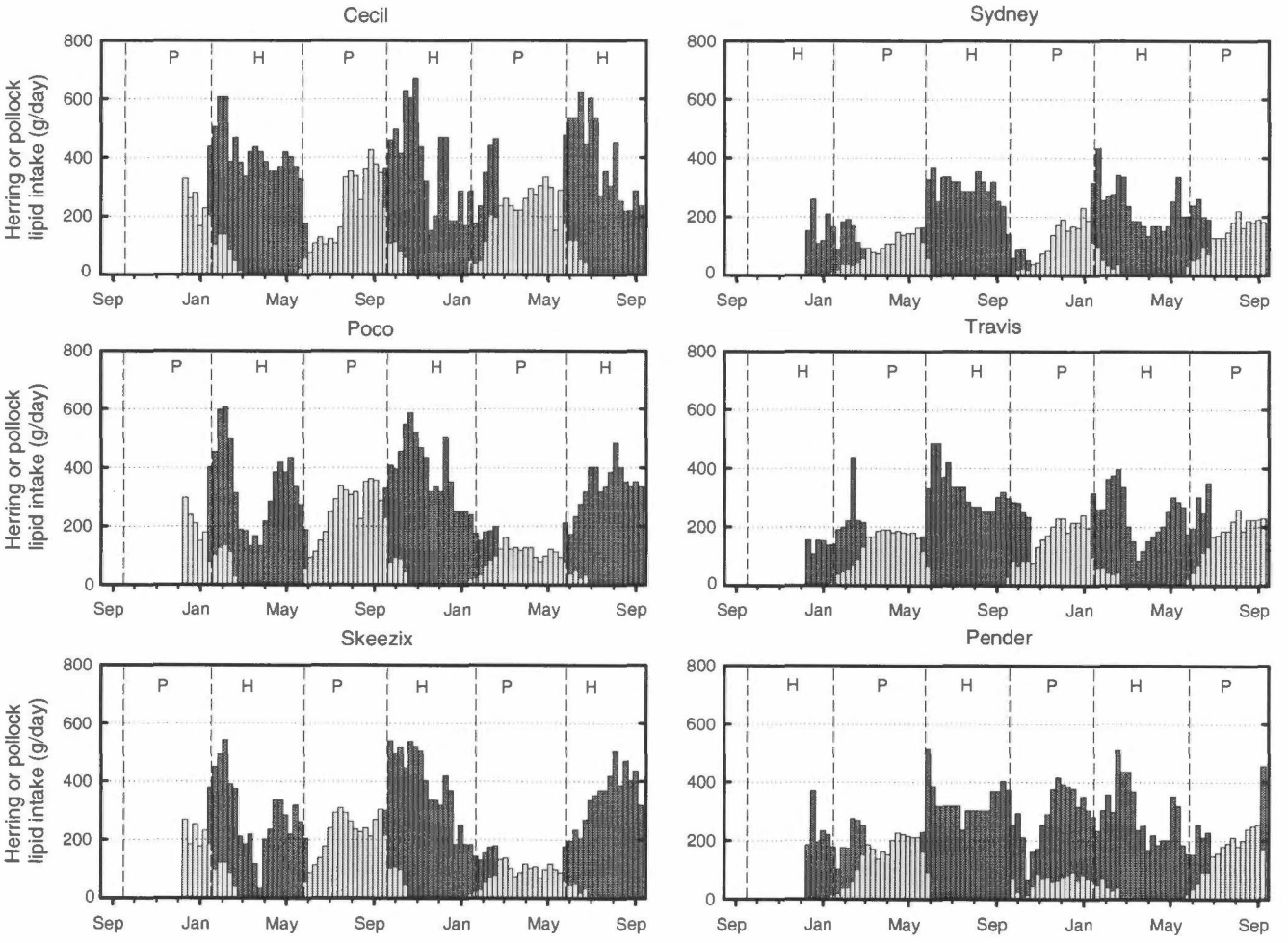

Fig. 4.6. Mean lipid intake (g/day) of individual harbor seals on alternating diets of herring (H, dark bars) and pollock (P, light bars). Each bar represents one week. Group A seals (left) started on pollock and group B seals (right) started on herring. Vertical dashed lines indicate approximate end of each four-month feeding trial.

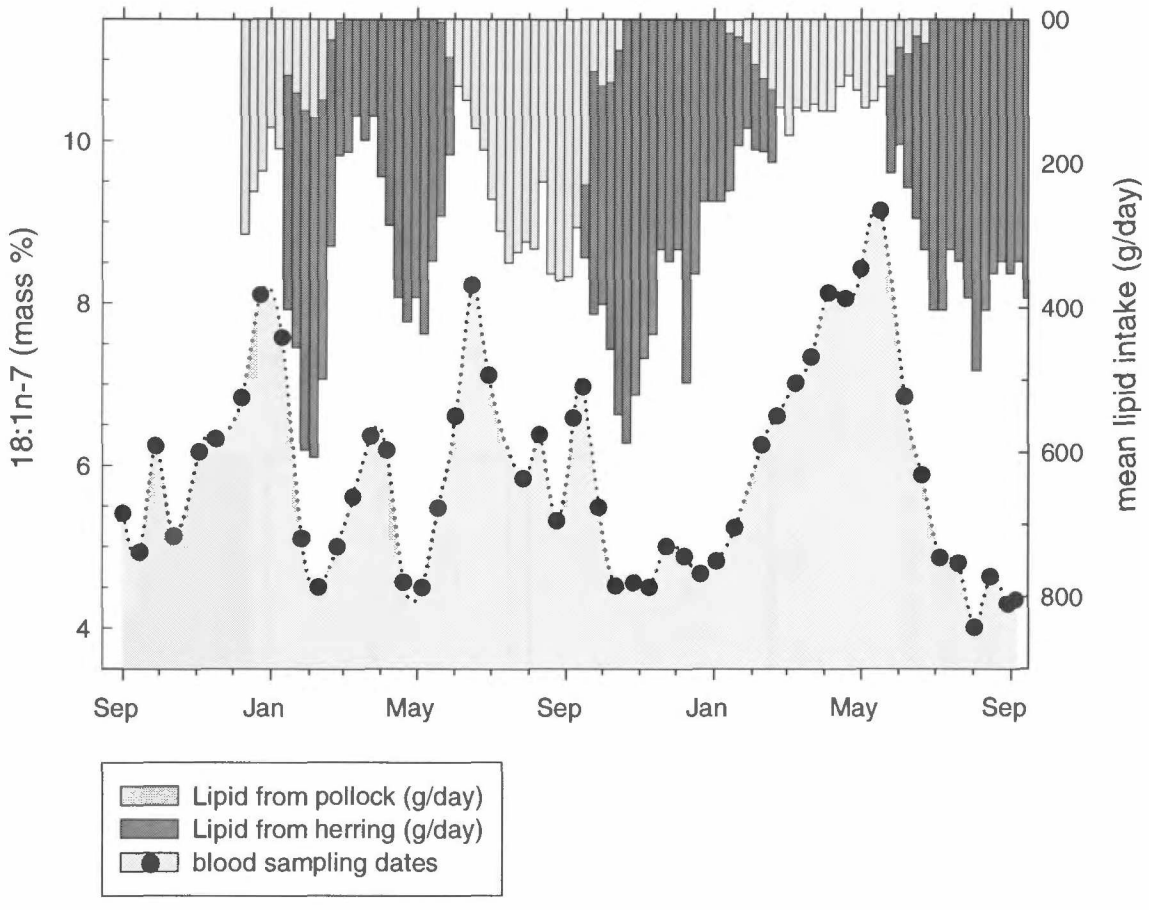

Fig. 4.7. Effect of mean total lipid intake (bars; g/day) on the mass percentage of 18:1n-7 (closed circles) in plasma from harbor seal Poco. Plasma values of 18:1n-7 were lower on the herring diet and higher on the pollock diet, but both values increased with decreasing total lipid intake. Note: Lipid intake axis is reversed. Area under blood sampling dates is highlighted to illustrate relationship only. Intake data were unavailable for September-early December 1998.

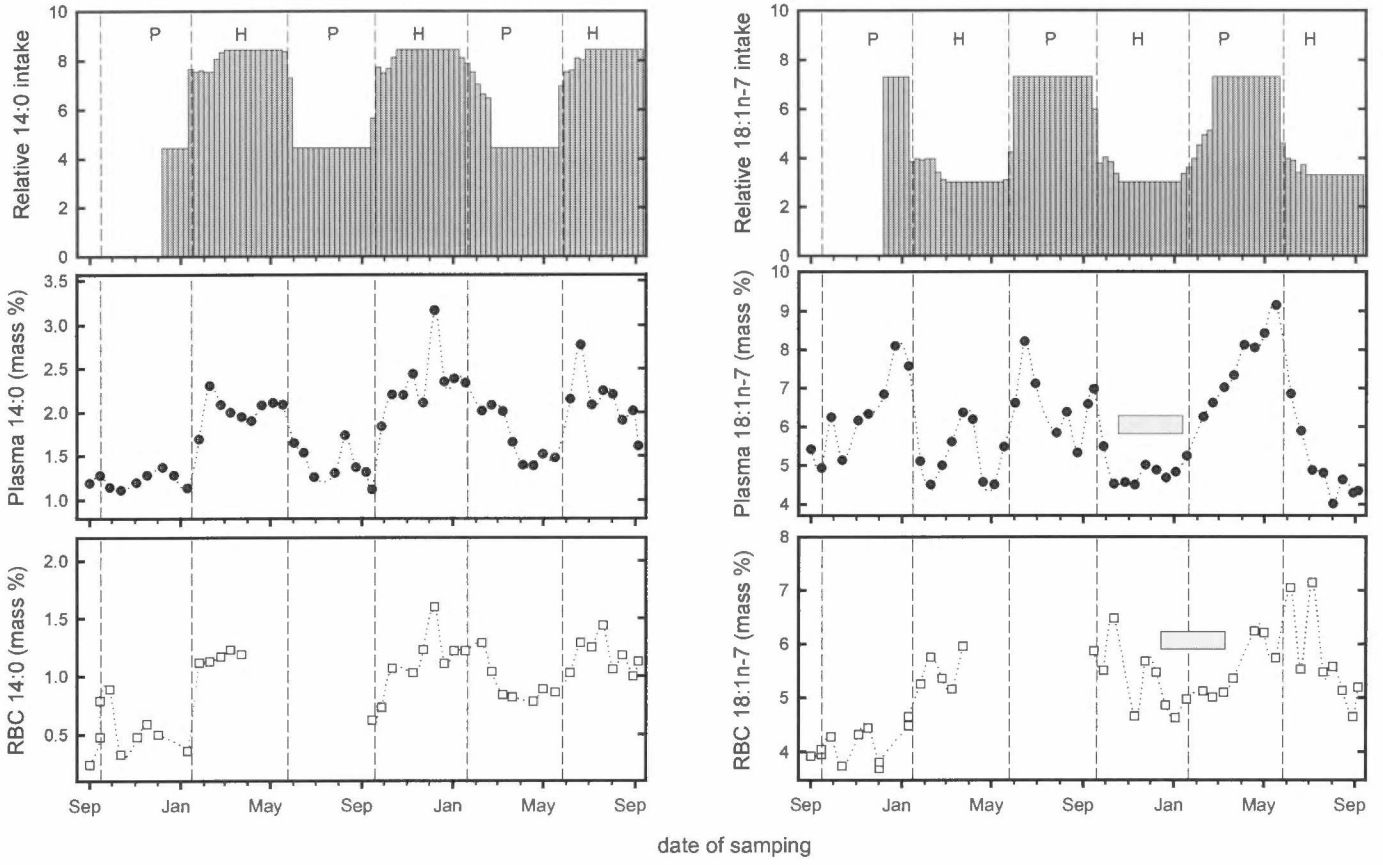

Fig. 4.8. Time series of relative fatty acid intake (top) of 14:0 (left) and 18:1n-7 (right) by harbor seal Poco over six feeding trials and the response of plasma (middle) and RBC (bottom) fatty acids to diet switching. Vertical dashed lines indicate end of feeding trials. Horizontal bars illustrate approximate two-month delay of RBC fatty acid response compared to plasma. Relative intake calculated from the mean mass percentage of the given fatty acid for each diet type multiplied by the percent lipid contribution of that diet type to the whole diet. Each bar equals one week. Intake data were unavailable during most of trial 1.

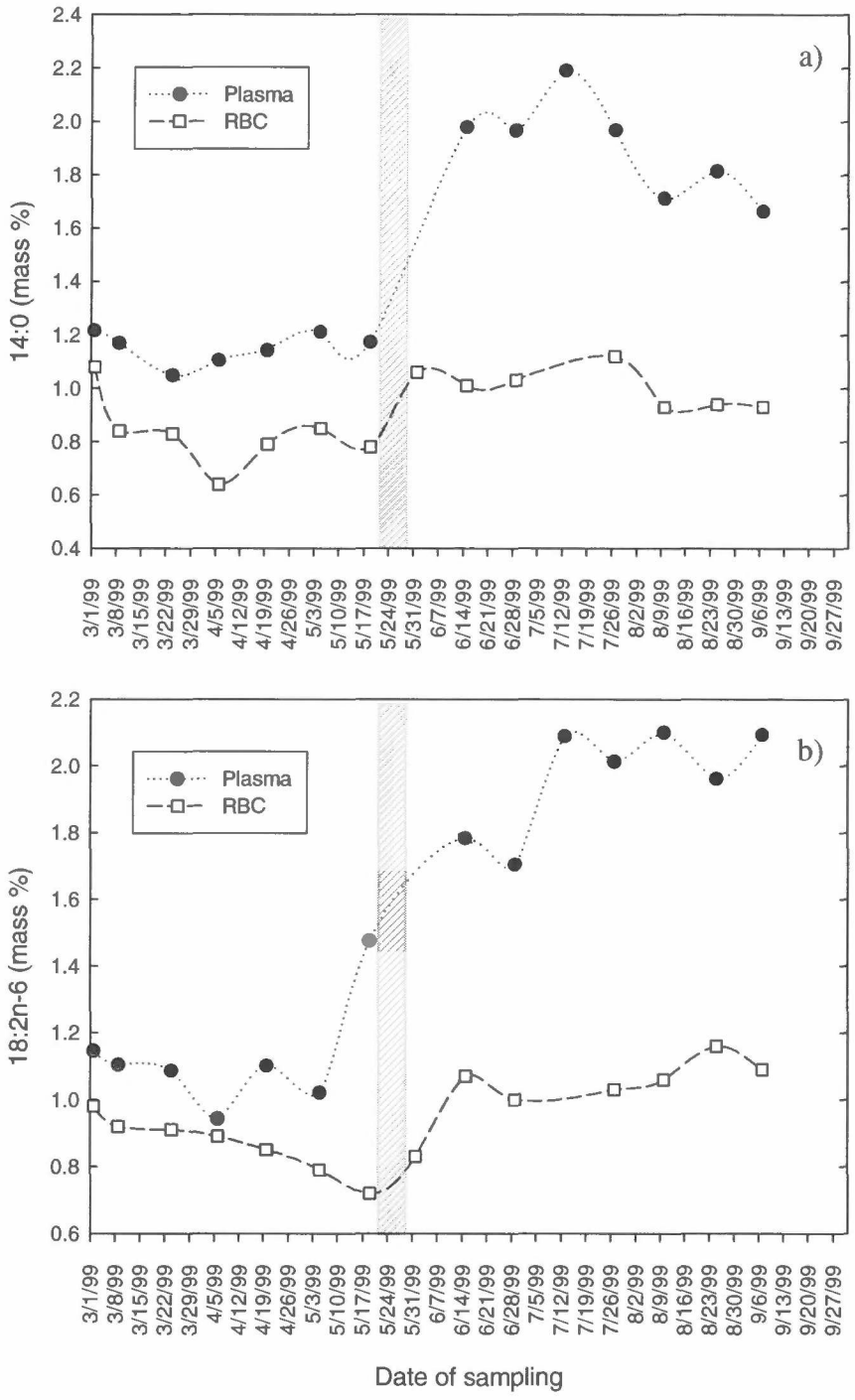

Fig. 4.9. Comparison of the response of plasma (closed circles) and RBC (open squares) fatty acids (a) 14:0 and (b) 18:2n-6 from harbor seal Travis in relation to diet switching from pollock to herring. Grey hatched bar represents the 8-day transition period between trials 2 and 3.

Table 4.9. Summary of significant differences found in harbor seal plasma and dietary fish fatty acids. Data are from Tables 4.4 and 4.5.

Fatty acid	Plasma and fish statistics in agreement	Significant in fish But not in plasma	Significant in plasma But not in fish
SAT			
14:0	X		
16:0			X
18:0		X	
MUFA			
16:1n-7	X		
18:1n-9			X
18:1n-7	X		
20:1n-11		X	
20:1n-9			X
22:1n-11		X	
24:1n-9	X		
PUFA			
18:2n-6			X
20:4n-6	X[a]		
20:5n-3		X	
22:5n-3			X
22:6n-3	X		

[a] Significant difference found in both plasma and fish, but trends were opposite.

Note: For dietary fish comparisons, only batches H4 and P2-3 were used in this analysis because these batches were fed to seals during 4-5 of 6 trials. Does not account for differences that may have been caused by batch differences of H6 and P5 in trial 6.

5 Lipids as biochemical markers in marine mammal physiological and ecological research: current status and future projects

The large increase in the number of studies examining the amount, type and/or flux of lipids in marine mammals since the late 1980's is clear evidence of the importance of lipid measurement in understanding the fundamentals of how marine mammals manage their energy resources and how they are adapted to the aquatic environment. Lipids are the currency of energy exchange in the ocean, serving as natural biochemical markers with which to evaluate life-history patterns, predator-prey relationships and health of populations and ecosystems. This dissertation has contributed significantly to our understanding of patterns of lipid deposition and mobilization in the blubber of bowhead whales and improves our knowledge of this species' foraging ecology, population health and adaptations to life in the arctic oceans (See chapter 2). Additionally in chapter 3, I have highlighted some of the difficulties in interpreting blubber samples from stranded whales. Finally in chapter 4, I have advanced our knowledge of fatty acid metabolism in harbor seals under different diet regimes and validated the use of fatty acid profiles for determining differences in diet history. While great progress has been made in marine mammal lipid research, many questions remain. I will address a handful of issues that I believe are important in enhancing current knowledge and integrating physiological and ecology research.

The function of blubber as an energy store and the response of blubber to changing environmental and nutritional conditions remains a major focus of lipid studies in marine mammals. The high demand of deuterium oxide used to estimate body composition in pinnipeds is a testament to that fact. However, while our understanding of how pinnipeds manage their energy stores is increasing dramatically, current studies in cetaceans are limited. Results from this thesis represent a large increase in our knowledge of bowhead whale energy stores (see Chapter 2) and one of only a handful of studies to address current nutritional status in a large baleen whale outside of historical commercial whaling data. The need for non-lethal methods to estimate lipid energy stores for

condition assessment in free-ranging pelagic cetaceans is great. Aerial photographs of seasonal changes in width to length relationships in gray whales (Perryman and Lynn 2002) is a great start to accomplishing this goal and should be repeated in other species. Efforts are also currently underway to measure blubber thickness by ultrasound in known individual Atlantic right whales (Moore et al. 2001). Recent advances in measuring steroid hormones indicative of reproductive status from blubber samples (Mansour et al. 2002) holds promise for determining pregnancy from blubber biopsies in free-ranging cetaceans. Efforts should be made to couple condition studies such as those above with photo-ID and/or steroid hormone studies so that we can begin to address changes in condition of large cetaceans in response to environmental change and with respect to population dynamics. The work completed on bowhead condition in this thesis is in line with this type of goal in that it was designed as a part of a large multidisciplinary program. Future efforts will be made to combine blubber data from this thesis with studies on age, energetics, metals, contaminants, morphometrics and histopathology, providing a more complete picture of bowhead whale health and condition (Willetto et al. 2002).

The utilization of fatty acid profiles has become a powerful tool to chemically describe the foraging ecology and diets of free-ranging marine mammals. Yet interpretation of fatty acid profiles is evolving as techniques become more sophisticated and more details are examined. With the exception of chylomicron fatty acids, fatty acid profiles from various tissues represent the integration of dietary input over time, not just the most recent meal. This feature leads to two important questions. Firstly, how quickly do fatty acids represent dietary intake and what is the length of the "dietary window" they represent. Secondly, what is an appropriate sample? Evidence from captive harbor seals (this thesis, chapter 4) suggests that fatty acid profiles from plasma and red blood cells represent short-term (weeks) and long-term (months) integration of diet, respectively. However, even within a tissue type, fatty acid turnover can vary according to lipid intake, altering the dietary window. For example, under high fat intake, such as in suckling seal pups, blubber fatty acids represented diet (milk) fatty acids within 2-4 days (Iverson et al.

1995). However, at low dietary fat inputs, turnover of fatty acids in juvenile seal blubber was slower, representing only 25-50% of prey fatty acids in one month's time (Kirsch et al. 2000). It is evident that additional controlled dietary studies are needed to address the time period a fatty acid profile represents in blubber under multiple intake levels so that we can better understand results obtained from wild populations. Furthermore, after an animal has ceased to forage, such as during the molting fast in phocids, do blubber fatty acids continue to represent the prior foraging bout or are specific fatty acids mobilized?

In addressing the appropriateness of sample type I am restricting this discussion to blubber because of its wide use in current diet studies and recent changes in sampling procedures. It has long been established that cetacean blubber can be highly stratified (Ackman et al. 1975), but evidence of significant fatty acid stratification in pinnipeds has only recently been reported (Best et al. 2003). Previously phocid blubber has been described as uniform (Jangaard and Ke 1968) or having small differences between layers (Kakela et al. 1993). Traditionally, whole thickness blubber samples from phocids were used for tracing diet (Iverson et al. 1997). Because of evidence of greater metabolic activity in inner layers leading to a greater turnover of fatty acids and a more rapid diet representation, focus has shifted to analyzing inner layers only. The lack of uniformity within the field will make comparisons between studies more difficult. For example, the definition of inner blubber is currently arbitrary, often based on dividing sections in halves or thirds, or in cetaceans is sometimes based on structural features. Also, the middle layer is often ignored. Yet expansion of adipocytes indicates that the middle layer is important for lipid deposition in at least pregnant bottlenose dolphins (Struntz et al. 2004). Also, high levels of long-chain polyunsaturated fatty acids generally indicative of diet were found in middle layers of minke whales (Olsen and Grahl-Nielsen 2003). Specific details of fatty acid stratification in phocids have not been systematically examined. It is obvious that the physiology of different blubber layers—how and when lipids move in and out of layers is important for diet interpretations and applying the appropriate time-scales to fatty acid profiles in foraging ecology studies.

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