FORAGING PATTERNS OF NORTHERN FULMARS IN ALASKA INFERRED FROM FATTY ACID SIGNATURE ANALYSIS

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A

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Abstract

Diets of Northern Fulmars (*Fulmarus glacialis*) in the North Pacific are poorly known, and thus relationships of fulmars to supporting food webs and their potential sensitivity to ecosystem variability, such as that driven by a changing climate, also are uncertain. I employed a new technique, fatty acid (FA) signature analysis, to examine dietary differences among fulmars at three colonies in Alaska. I predicted that 1) signatures of adipose tissue and stomach oils would differ because the time scale each depot reflects differ and/or because adipose tissue FAs may be influenced by predator metabolism, while stomach oil FAs may be influenced by differential uptake; 2) fulmar diets would differ between colonies located in distinct oceanographic settings, which create unique habitats for prey assemblages; 3) diets would differ temporally within colonies because of inter-annual variability in the physical environment resulting in variation of prey FA signatures; and 4) diets of adult fulmars and their chicks would be similar because they feed by regurgitation. I found that FA signatures of adipose tissue were significantly different than those of stomach oil; there were conspicuous spatial and temporal differences in adipose tissue signatures; but diets of adults may differ from those of chicks.

Table of Contents

Page

List of Figures

Page

Chapter 1.

Chapter 2.

List of Tables

Page

Chapter 1.

Chapter 2.

Preface

This thesis is dedicated to my parents, Jack C.M. Wang and Jane W.J. Wang, who taught me the value of hard work, persistence, and always giving 110%. I also dedicate this thesis to my brother, G. Sam Wang, and sister, Kristina L. Wang, for supporting me in my adventures and for keeping me grounded: I thank my family for all their love and encouragement.

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Introduction

Since the mid-1970's, the combined effects of environmental changes and commercial fishing pressures have resulted in fluctuations in the composition of marine communities in the North Pacific Ocean (NRC 1996, Francis et al. 1998). Changes in the N. Pacific Ocean have been implicated in the decline in abundance of some prey species and coincided with changes in abundance and productivity of seabirds and marine mammals (e.g. Trites 1992, Piatt & Anderson 1996, Springer 1998). Observed declines have prompted scientists to investigate causal links between changes in diet composition and population dynamics of marine mammals and seabirds.

Effects of climate change on species at higher trophic levels have been documented worldwide. Over the past 50 years, ocean warming has caused the reduction of sea-ice extent and a decrease in secondary production in sub-Antarctic waters (e.g. Taylor & Wilson 1990, Weimerskirch et al. 2003, Barbraud & Weimerskirch 2001). Probably in response to these changes some seals and seabirds in the Southern Ocean have declined severely, while others have increased. During an El Niño event in the 1990s, die-offs of seabirds in Alaska and of pinnipeds from the west coast of North American down to South America occurred (e.g. Mendenhall 1997). Black Guillemots (*Cepphus grylle*) nesting on Cooper Island in Barrow increased from fewer than 18 individuals in 1975 to 225 in 1989 due to a longer snow-free breeding seasons resulting from global warming (Divoky 1998).

Time scales of climate change range from interannual fluctuations with, impacts such as contrasting patterns of reproductive performance of planktivorous and piscivorous alcids (Kitaysky & Golubova 2000), decadal cycles such as the Pacific Decadal Oscillation (PDO) in the N. Pacific (Mantua et al. 1997), and even longer-term changes (Sugimoto & Tadokoro 1997). The PDO is the alternation of the Aleutian Low pressure system between two quasi-stable states of intensity and spatial variability over a period of 15-30 years (Trenberth & Hurrell 1994, Mantua et al. 1997). Changes between the two states result in dramatic shifts in the biological community (e.g. Francis et al. 1994, Hare & Mantua 2000). Examples of such bottom-up regulation include the response of salmon production across the N. Pacific to changing levels of secondary and primary production, the latter driven by physical conditions created by the PDO (e.g. Francis & Hare 1994, Mantua et al. 1997). Comparable changes in abundance and productivity of seabirds in Alaska are also thought to reflect environmental fluctuations (e.g. Piatt & Anderson 1996, Springer 1998).

Most seabirds are high trophic level consumers in marine food webs and can serve as sensitive indicators of changes in marine ecosystems (Furness & Todd 1984, Cairns 1987, Montevecchi 1993, Montevecchi & Myers 1995, Springer 1998). Many seabird species respond to fluctuations in forage fish populations and respond by changing their diets (Furness & Cooper 1982, Springer et al. 1984, Springer & Speckman 1997). Reproductive success of seabirds can be affected by seasonal and annual changes in prey availability (Vermeer 1992, Kitaysky & Golubova 2000).

When prey availability is low, seabirds may increase foraging effort, skip a year of breeding, emigrate, suffer increased mortality and slower chick growth, and populations may experience reduced breeding success (Springer et al. 1996, Cairns 1987, Furness & Barrett 1991, Furness & Nettleship 1991, Hamer et al. 1991, Springer & Speckman 1997).

Northern Fulmars

Northern Fulmars are an abundant and conspicuous seabird that exemplifies many of the above phenomena. Diets of Northern Fulmars in the N. Pacific Ocean are poorly known, however, and thus relationships of fulmars to supporting food webs and the species' sensitivity to ecosystem variability are also highly uncertain. Fulmars are opportunistic and generalist predators and consume a wider variety of prey than many other species of seabirds in Alaska. In the N. Pacific, fulmars feed on cephalopods, zooplankton, lantern fishes (Myctophidae), Scyphozoan jellyfish, juveniles of commercial fish species, e.g., walleye pollock (*Theragra chalcogramma*), and other forage species that are critical to pelagic food webs and sensitive indicators of environmental change, e.g., capelin (*Mallotus villosus*) and Pacific sandlance (*Ammodytes hexapterus*) (Preble & McAtee 1923, Bradstreet 1985, Hunt et al. 1981a, Hunt et al. 1981b, Harrison 1984, Sanger 1986, Hills & Fiscus 1988, Gould et al. 1997, Hatch & Nettleship 1998, Hatch unpubl.). The ability of fulmars to exploit a wide-variety of prey with no obvious reliance on specific species allows for the speculation that their generalist, opportunistic feeding habits help to

buffer them from changes in physical conditions and associated food webs (e.g. Piatt & Anderson 1996).

In addition to live-caught prey items, fulmars also follow fishing vessels in pursuit of offal, which is composed of fish refuse (livers, entrails, and whole fish discards; Fisher 1952, Hunt et al. 1981b, Hatch 1993). Breeding numbers and ranges of Atlantic fulmars have expanded dramatically over the last 200 years and the importance of commercial fisheries in that growth has been debated (Hatch & Nettleship 1998). Phillips et al. (1999) concluded that the general pattern for Atlantic fulmars in more southerly populations was to consume more discards than fulmars in the northern populations: however, their ability to exploit commercial fisheries for food resources is evident across their range. Use of offal is not well documented in the Pacific (Fitzgerald & Kuletz 2003), yet fulmars may be consuming up to 75% of the total discards from some commercial fisheries (A. Schultz, commercial fisherman, *pers, comm.*).

Implications of fulmars relying on commercial fisheries include potential population expansions in the Pacific and potential crashes in populations if commercial fishery activities are reduced, with or without coincident decreases in abundance of natural prey. Decreased commercial fishery activity may, however, diminish bycatch mortality and thus offset the loss of food potentially obtained during fisheries operations. Current population trends of fulmars are of interest because this species is the largest single component of seabird bycatch in Alaska—59% of the seabird bycatch in the Bering Sea-Aleutian Islands fishery for groundfish and 46% of

the bycatch in the Gulf of Alaska fishery (observations from 1993 – 2002; Fitzgerald & Kuletz 2003).

The population of fulmars in North America is estimated at 2.1 million individuals, with 70% occurring in Alaska (Hatch & Nettleship 1998). There are four major Alaskan breeding colonies: 1) Chagulak Island (52˚35' N, 171˚10' W) located in a deep oceanic basin in the central Aleutian Archipelago with populations estimated at 500 000; 2) St. Matthew and Hall Islands (60˚30' N, 172˚45' W) located in the middle of the large continental shelf in the central Bering Sea with approximately 450 000 individuals; 3) the Semidi Islands (56˚05' N, 156˚45' W) on the continental shelf in the western Gulf of Alaska, where the local oceanography is influenced primarily by the Alaska Coastal Current and the Alaska Stream and where about 440 000 individuals occur; and 4) the Pribilof Islands, principally St. George Island (56˚35' N, 170˚35' W) near the edge of the continental shelf in the southeastern Bering Sea, which hosts an estimated 80 000 individuals (Hatch & Hatch 1983, Hatch 1993). Overall, the populations of fulmars in the N. Pacific are though to be stable (Hatch & Nettleship 1998). The population on St. Paul Island in the Pribilof Islands appears to be increasing, while the population on nearby St. George Island has been decreasing since 1992. There is no discernable trend in the Semidi Islands (Dragoo et al. 2003, Larned & Sapora 2005), and no trend data exist for St. Matthew/Hall or Chagulak islands. In 2003, fulmars colonized Attu Island in the Aleutian Archipelago (Byrd & Williams 2004).

Stomach oil

Northern Fulmars belong to the order Procellariiformes, most members of which are unique among seabirds because they produce stomach oils (Lewis 1966, Warham 1977, Roby et al. 1993, Roby et al. 1997, Taylor et al. 1997). Early studies hypothesized that stomach oil was produced in the glandular wall of the proventriculus and was used for preening (Rosenheim & Webster 1926, Matthews 1949), or that it was an ingested preen gland secretion (Carter & Malcolm 1927, Carter 1928). However, close resemblances were noted between fulmar stomach oil and other oils found in marine systems (Lovern 1938, Kritzler 1948), and further studies on its composition and variability between individuals indicated that it originates from the diet.

Stomach oils are formed in the proventriculi of both by both adults and chicks by a combination of specialized gastric anatomy and physiology (Roby et al. 1989, Place et al. 1989, Roby et al. 1992, Roby et al. 1993). Aqueous dietary components are rapidly emptied from the proventriculus, while neutral lipids are retained (Roby et al. 1989). Oil production was hypothesized to be an adaptation that allows breeding adults to enhance provisioning rates while foraging on distant and dispersed food supplies (Ashmole 1971, Warham 1977, Laugksch & Duffy 1986, Roby et al. 1993, Obst & Nagy 1993, Roby et al. 1997; Taylor et al. 1997). This hypothesis was supported in an experiment demonstrating that stored oil in the proventriculus has energetic advantages for seabirds that frequently experience periods of fasting

because it reduces the metabolic costs of storing fat reserves from assimilated fatty acids (FAs) and later re-mobilizing them for use during fasting (Roby et al. 1997).

Stomach oils are also used by both adults and chicks as a defense mechanism against predators such as falcons, eagles, gulls, ravens, foxes, and humans (Fisher 1952, Warham et al. 1976, Warham 1977, Clarke 1989). Experimental studies and field observations have shown that birds soiled with oil ejected from as far away as 3 meters experienced reduced flight and insulation capabilities that often resulted in death (Fisher 1952, Broad 1974, Clarke 1977, Mearns 1983). Historically, humans consumed stomach oil for medicinal purposes and used it as a lubricant and fuel for oil lamps (Travers & Travers 1873, Warham 1977).

The color of stomach oil is correlated with diet (Warham et al. 1976) and ranges from colorless, to shades of yellow, orange, red, amber, deep reddish-brown and even green, which may be contributed from bile (Fisher 1952, Lewis 1969, Warham 1977). Colorless oils may represent lipids of meso- and bathypelagic fishes (Lewis 1969), while red samples contain carotenoids and esterified astaxanthin pigments found in planktonic crustaceans or in squids that consumed crustaceans (Fisher 1952, Lewis 1969).

The composition of stomach oil was first described early in the last century, but the analyses were incomplete by modern standards, with only partial chemical compositions of the oil identified (Smith 1911, Carter & Malcolm 1927, Rosenheim & Webster 1927, Carter 1928). Later studies showed that the chemical composition of stomach oil includes hydrocarbons, monoester waxes, diacylglycerol ethers,

triglycerides, diglycerides, monoglycerides, alcohols, cholesterols, and free FAs as well as more polar lipids (Lewis 1966, Lewis 1969, Cheah & Hansen 1970a, Cheah & Hansen 1970b, Clarke & Prince 1976, Warham et al. 1976). The lipid composition of stomach oils reflects not only the composition of recent meals but also the relative solubility of each class of lipids in the stomach oils already accumulated (Place et al. 1989). Thus, there are two distinct lipid sources to consider in a dietary analysis of Procellariiform birds using fatty acids, stomach oil and adipose tissue (Lewis 1969, Cheah & Hansen 1970a, Clarke & Prince 1976, Imber 1976; Watts & Warham 1976, Warham 1977, Jacob 1982, Place et al. 1989). Some studies compared the lipid composition of stomach oil and adipose tissue of Procellariiformes (Rosenheim & Webster 1926, Lovern 1938; Cheah & Hansen 1970a, Bishop et al. 1983, Horgan & Barrett 1985, Clarke 1989), and Connan et al. (2005) recently used lipid classes in stomach oils of Procellariiformes to infer diets.

Diet studies

Traditionally, information on diets of breeding seabirds has been collected by screening burrows to obtain chick meals of puffins (Hatch & Sanger 1992); by various methods of live capture to cause adults and chicks to regurgitate (Duffy & Jackson 1986); by visual identification, as used with murres and guillemots to identify meals delivered to chicks (Van Pelt & Shultz 2002, Litzow et al. 2002); and by collecting birds and determining stomach contents by dissection (Duffy & Jackson 1986).

There are several well-known problems associated with these traditional methods. Most methods employed in the past are consumptive—vital meals are taken away from the adults or chicks, or the birds are sacrificed. Visual identification of prey and chick meals from nests give information on prey items of chicks, which may not accurately reflect the diet of the adults (Duffy & Jackson 1986, Annett & Pierotti 1989,Votier et al. 2003). Another problem is that soft-bodied invertebrates or other parts that are not easily detected are often inaccurately represented (Harrison 1984, Duffy & Jackson 1986). Hard parts that persist longer in the digestive system are easier to identify than soft parts and can overemphasize the importance of some prey items (Furness et al. 1984, Jobling & Breiby 1986). Moreover, each observation provides information on only the most recent meal eaten, which may not represent the average diet. Another method for studying diets, the analysis of stable isotopes, provides information on general trophic levels of predators, but the species of prey usually cannot be determined.

In an effort to overcome most of the problems mentioned above, I applied a new method of investigating diets in this study, FA signature analysis. FA analysis is a non-lethal method and is less invasive than most traditional methods of sampling described previously. This type of analysis provides information about the diet integrated over a period of time (e.g. 2 – 4 weeks, Iverson et al. *in prep*) rather than just the most recent meal as with traditional methods.

Lipids have been used as biomarkers and general indicators of diets of predators in marine ecosystems for many years (Sargent et al. 1988), and the analysis

of FAs in reservoir lipids (adipose tissue and blubber) has been used to infer trophic levels, as well as spatial and temporal patterns in foraging behavior, of free living marine mammals and seabirds (Iverson & Oftedal 1992, Iverson 1993, Iverson et al. 1997a, Iverson et al. 1997b, Kirsch et al. 2000, Iverson et al. 2001, Brown et al. 1999, Raclot et al. 1998, Iverson & Springer 2002, Dahl et al. 2003). More recently, diets of marine predators have been described quantitatively using Quantitative Fatty Acid Signature Analysis (QFASA). QFASA is a statistical model that provides quantitative estimates of the proportions of prey species in diets of individual predators (Iverson et al. 2004).

Fatty acid signature analysis

FA signature analysis and QFASA are based on the observation that specific FAs (carbon chain length ≥ 14) from prey are incorporated with little change, or in a predictable manner, into the body fat of marine predators, and thus they can be used as qualitative and quantitative food tracers (Iverson 1993, Iverson et al. 2004). A few FAs can be biosynthesized by animals (Cook 1991), but they can be distinguished from those that are acquired from the diet. FAs are used directly for energy or are re-esterified, primarily to triacylglycerols, and stored in adipose tissue. Although metabolism and biosynthesis of certain FAs occur within the predator, resulting in a FA composition of its adipose tissue that does not match exactly that of its prey, most FAs are deposited with little modification and in a predictable way (Iverson et al. 2004).

Not all FAs are good indicators of diet and appropriate for use in statistical analysis (Iverson et al. 2004). FAs that were used in this study were chosen by how well they indicated diet, and included those with the largest overall variances based on the assumption that the FAs with the largest fluctuations in levels would provide the most useful information in differentiating among fulmars. Sixteen out of 69 identifiable FAs in fulmar adipose tissue and stomach oil samples were used in the analyses, of which 10 arise primarily from diet (18:2n-6, 18:3n-3, 18:4n-3, 20:1n-11, 20:1n-9, 20:4n-6, 20:5n-3, 22:1n-11, 22:1n-9, 22:6n-3) and 6 can be both biosynthesized and acquired from the diet (14:0, 16:0, 18:0, 16:1n-7, 18:1n-9, 18:1n-7); (Iverson et al. 2004). These sixteen FAs are ubiquitous in marine ecosystems and the origins are well known, allowing for the reconstruction of the predator diet (e.g. Ackman et al. 1974, Pascal & Ackman 1976, Ackman 1980, Sargent & Whittle 1981, Sargent et al. 1988, Falk-Petersen et al. 1990, Dunstan et al. 1994).

Objectives

The larger objective of this study was to describe FA signatures of adipose tissue and stomach oil of Northern Fulmars at nesting colonies in Alaska in order to estimate diets. In Chapter 1, I compare the FA signatures of stomach oil and adipose tissue. The finer scale question is if FA signatures from the two lipid sources are detectably different. The larger scale question concerns the biological significance of differences in signatures and implications for diet analysis. In Chapter 2, I use FA signatures to assess spatial (between colonies), temporal (seasonal and inter-annual),

and age class (adult-chick) variability in the diet of fulmars at three colonies located in distinct oceanographic settings in Alaska. I expected that 1) signatures of adipose tissue and stomach oils would differ because of the differing time scale each depot reflects and/or because adipose tissue FAs may be influenced by predator metabolism, while stomach oil FAs may be influenced by differential uptake; 2) fulmar diets would differ between colonies located in distinct oceanographic settings; 3) diets would differ temporally within colonies because of potential interannual variability in the physical environment resulting in temporal shifts in FA signatures of prey species; and 4) diets of adult fulmars and their chicks would be similar because they feed by regurgitation.

I anticipated that this study would advance our understanding of seabird foraging ecology in Alaska, provide new information about fulmar diets in different geographic locations, and would complement such facets of ongoing research as the satellite telemetry and molecular genetics on fulmars in Alaska (S.A. Hatch, unpubl.). By understanding the diet and foraging ecology of fulmars and other marine birds, predator-prey relationships and foraging patterns can be used in assessing the impact of environmental variation on the birds and their ecosystem. With those motivations, I resolved to learn as much as possible about the diets of Northern Fulmars in Alaska using FA signature analysis.

Chapter 1. Fatty acid signatures of stomach oil and adipose tissue of Northern Fulmars (*Fulmarus glacialis*) in Alaska: implications for diet analysis[1]

Abbreviations

BHT - butylated hydroxytoluene; FA(s) - fatty acid(s)

1.1 Abstract

The goal of this study was to use fatty acid signature analysis to estimate diets of Northern Fulmars. We compared the fatty acid composition of two lipid sources, stomach oil and adipose tissue, of individuals breeding at three major colonies in Alaska. Fulmars and related species produce stomach oils that consist of lipids retained from prey and represent prey consumed during the most recent foraging events. Fulmars also store dietary lipids, and those they synthesize *de novo*, in adipose tissue. The proportions of fatty acids in adipose tissue reflect deposition and mobilization integrated over periods of weeks. We found that fatty acid signatures of adipose tissue were significantly different than those of stomach oil, but not all fatty acids were consistently higher or lower in either lipid source. These results are likely

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due to the time scales over which diets are integrated—short-term (stomach oil) versus longer term (adipose tissue), the presence of biosynthesized lipids in adipose tissue, or different deposition and mobilization rates and solubility of individual fatty acids.

Keywords fatty acid signature analysis – *Fulmarus glacialis* – stomach oil – Alaska – diet analysis

1.2 Introduction

Fatty acid (FA) signature analysis of reservoir lipid (adipose tissue and blubber) has been used to infer trophic levels, as well as spatial and temporal patterns in foraging behavior of free ranging marine mammals and seabirds (Iverson 1993; Iverson et al. 1997a; Iverson et al. 1997b; Kirsch et al. 2000; Brown et al. 1999; Raclot et al. 1998; Iverson & Springer 2002; Dahl et al. 2003). Given a comprehensive database of prey FA signatures, it is possible to estimate the proportions of different prey in the diet using Quantitative Fatty Acid Signature Analysis, or QFASA (Iverson et al. 2004). FA signature analysis and QFASA are based on the observation that prey FAs of carbon chain length ≥ 14 are incorporated with little change into the body fat of marine predators, and thus they can be used as qualitative and quantitative food tracers (Iverson 1993; Iverson et al. 2004). Some FAs can be synthesized by animals, but the number and types are limited (Cook 1991). Therefore, biosynthesized FAs can be distinguished from those that are acquired from the diet. FAs are used directly for energy or are re-esterified, primarily to triacylglycerols, and stored in adipose tissue. Although metabolism and biosynthesis of certain FAs occur within the predator, resulting in a FA composition of its adipose tissue that does not match exactly that of its prey, most FAs are deposited with little modification or in a predictable way (Iverson et al. 2004). In this study, we present the analysis of stomach oil and adipose tissue fatty acid signatures of Northern Fulmars (*Fulmarus glacialis*).

Northern fulmars belong to the order Procellariiformes. Members of this order are unique among seabirds because they produce stomach oils (Lewis 1966; Warham 1977; Roby et al. 1993; Roby et al. 1997; Taylor et al. 1997). Close resemblances were noted between the lipid composition of Procellariiform stomach oil and other oils found in marine systems (Lewis 1966; Cheah & Hansen 1970ab) and further studies on the composition of stomach oil and variability between individuals indicated that it originates from the diet (Lewis 1969; Cheah & Hansen 1970a; Clarke & Prince 1976; Imber 1976; Watts & Warham 1976; Warham 1977; Jacob 1982; Place et al. 1989). Stomach oils are formed in the proventriculus of both adults and chicks by a combination of specialized gastric anatomy and physiology (Roby et al. 1989; Place et al. 1989; Roby et al. 1992; Roby et al. 1993), which rapidly empties aqueous dietary components from the proventriculus while retaining the neutral lipids (Roby et al. 1989).

The color of stomach oil has been found to be correlated with diet (Warham et al. 1976). In fulmars, it ranges from colorless, to shades of yellow, orange, red, amber, deep reddish-brown, and green (Figure 1.1). Colorless oils may represent lipids of meso- and bathypelagic fishes (Lewis 1969), while red samples contain carotenoids and esterified astaxanthin pigments found in planktonic crustaceans or in squids that have consumed crustaceans (Fisher 1952; Lewis 1969). Green coloration may arise from a contribution of bile (Fisher 1952; Lewis 1969; Warham 1977).

The composition of stomach oil was first described early in the last century, but those analyses were incomplete by modern standards, investigating only partial

chemical compositions of the oil (Smith 1911; Carter & Malcolm 1927; Carter 1928; Rosenheim & Webster 1927). Later studies determined that the chemical composition of stomach oil includes hydrocarbons, monoester waxes, diacylglycerol ethers, triacylglycerols, diacylglycerols, monoacylglycerols, alcohols, cholesterols, and free FAs, as well as more polar lipids (Lewis 1966; Lewis 1969; Cheah & Hansen 1970a; Cheah & Hansen 1970b; Clarke & Prince 1976; Warham et al. 1976). More recently, Connan et al. (2005) have used lipid classes in stomach oils of Procellariiformes to infer their diets. Previous studies comparing the FA composition of stomach oil and adipose tissue of Procellariiformes (Rosenheim & Webster 1926; Lovern 1938; Cheah & Hansen 1970a; Bishop et al. 1983; Horgan & Barrett 1985; Clarke 1989) relied on small samples and limited quantitative analysis to distinguish FA signatures of the two lipid sources.

The eventual goal of our work is to use QFASA to estimate diets of fulmars in Alaska. In the context of that goal, the objectives of the current study were (1) to characterize the relationship between FAs in adipose tissue and stomach oil of individual Northern Fulmars, and (2) to evaluate how well FAs discriminate between the two lipid sources. We expected that FA signatures from stomach oil and adipose tissue would differ because adipose tissue FAs are comprised of dietary FAs plus those that can be biosynthesized, whereas stomach oils contain only dietary FAs that have not been processed metabolically but may have experienced selective uptake or release (Place et al. 1989). Additionally, FAs from adipose tissue likely provide information on dietary intake integrated over an extended interval (e.g., 2-4 weeks;

Iverson et al. *in prep*) whereas stomach oil FAs should represent only the most recent intake (e.g., 1-2 days: Roby et al. 1989 suggested < 12 hrs for stomach oil to form in 4 week-old Antarctic giant-petrel chicks (*Macronectes giganteus*). We further expected to find no consistent pattern for all FAs in stomach oil versus adipose tissue among individual birds, i.e., relative levels of different FAs in stomach oil and adipose tissue would vary independently due to individual variability in diets and differing rates of mobilization and/or utilization.

1.3 Materials and methods

1.3.1 Study sites and sample collection

Samples from adult fulmars and chicks were collected from three of the four major fulmar colonies in Alaska during the breeding season of 2004: Chowiet Island in the Semidi Islands group in the western Gulf of Alaska (56˚05' N, 156˚45' W), St. George Island in the Pribilof Islands group in the eastern Bering Sea (56˚35' N, 170˚35' W), and Chagulak Island in the central Aleutian Archipelago (52˚35' N, 171˚10' W) (Figure 1.2). Samples were not collected on the same dates at the three islands due to logistical constraints.

Adult birds were captured using a modified dip net, a noose-pole, or by hand from their nests. Adipose tissue and stomach oil samples were collected from adults on Chagulak I. in July (n = 26) and St. George I. in June (n = 27) and August (n = 20). Adipose tissue and stomach oil samples were collected from chicks captured by hand from the nest on Chowiet I. during August (n = 30).

To collect stomach oil, the bird's head was positioned in a Whirl-Pak© immediately upon capture to ensure minimal loss of sample if the bird willingly regurgitated during handling. Collected oil samples averaged 80 ml. Within several hours of being collected, the stomach oil was transferred to glass vials with Teflon caps containing chloroform with 0.01% BHT as antioxidant for storage.

Biopsy was used to obtain synsacral adipose tissue samples from individuals (Enderson & Berger 1968). The sampled area was disinfected with chlorhexidine and a shallow incision approximately 0.5 cm in length was made just through the skin. A sample of approximately 0.1 g was excised from the subcutaneous fat reservoir and placed in a vial of chloroform with 0.01% BHT. The incision sites were closed with Vet-bond © to insure closure and rapid healing, which minimized the chances of infection. All samples were stored frozen until analyzed.

Morphometric measurements were taken from all individuals. Wing-length and mass were used to estimate the ages of chicks using a logarithmic curve fitted to changes in length with age from a sample of known-age chicks (Hatch 1979). Chicks sampled on Chowiet I. in August were estimated to be 22 ± 5.7 (SD) days old on average. The age of chicks at fledging is unknown in Alaska, however the mean age at fledging in Scotland is 53 days (Hatch & Nettleship 1998). On Chowiet I. in 2002, the first fulmar chick was observed to fledge on September 10 and the first observed hatching occurred on July 16, inferring an approximate fledging at 56 days of age.

1.3.2 Lipid extraction and analysis

Lipids were extracted using a modified Folch extraction (Folch et al. 1957; Iverson et al. 2001). FA methyl esters were prepared directly from 100 mg of the pure extracted lipid (filtered and dried over anhydrous sodium sulfate) using 3.0 ml Hilditch reagent (0.5 N H_2SO_4 in methanol) and 1.5 ml methylene chloride with 0.01% BHT, capped under nitrogen, and heated at 100°C for 1 hour. FA methyl esters were then extracted into hexane, concentrated, and brought up to volume (50 mg/ml) with high purity hexane.

Identification and quantification of FA methyl esters were performed on samples using temperature-programmed gas liquid chromatography as described previously (Iverson & Oftedal 1992; Iverson et al. 1997b; Budge et al. 2002; Iverson et al. 2004) on a Perkin Elmer Autosystem II Capillary FID gas chromatograph fitted with a 30m x 0.25 mm id column coated with 50% cyanopropyl polysiloxane (0.25µ film thickness; J&W DB-23; Folsom, California, USA) and linked to a computerized integration system (Turbochrom 4 software, PE Nelson, San Jose, California, USA). All sample chromatograms and FA identifications were individually checked, corrected, and reintegrated as necessary. FAs are expressed as mass percent of total FAs.

The dietary lipids of most animals, and especially carnivores, consist of triacylglycerols (3 FAs esterified to a glycerol backbone), reflecting the primary storage form of lipid in animals. However, in the marine environment a number of fish and invertebrates store their lipids primarily as wax esters comprised of a FA

esterified to a long-chain fatty alcohol (e.g. Benson et al. 1972). Many seabirds have the ability to digest and assimilate dietary wax esters (Roby et al. 1986). The presence of fatty alcohols resulting from the transesterification of wax esters in stomach oil was determined by using thin layer chromatography. Fatty alcohols, and thus wax esters, were not present in adipose tissue samples. In order to account for wax esters, the alcohols of which are deposited as FAs in the adipose tissue, extra precautions were taken to convert wax ester alcohols to their respective FAs when present. The transesterification process converts the FAs in acyl lipids to FA methyl esters, however, fatty alcohols and dimethylacetals are also generated if wax esters are present. Thus, we used a modified Jones' reagent (13.5 g CrO_3, 6.4 ml H_2SO_4, 43.6 ml distilled water) to oxidize the alcohols and dimethylacetals to free FAs, which were methylated and quantitatively recombined with FA methyl esters from the same sample according to Budge & Iverson (2003).

1.3.3 Data analysis

Sixty-nine FAs were identified and quantified in Northern Fulmar lipid samples. FAs that were used in statistical analyses were chosen by how well they reflected diet (Iverson et al. 2004). Selected FAs were those with the highest overall variances, which represented the majority of the signature, and those with overall means of $\geq 0.35\%$ of the total FAs. Seventeen FAs met these criteria. Despite the large overall variance of one of those FAs (22:5n-3), it was excluded from the analyses because it may be an intermediate between 20:5n-3 and 22:6n-3 (e.g.

Ackman et al. 1988). All analyses were thus performed using 16 FAs, of which 10 are considered to be exclusively dietary and 6 could arise from both diet and biosynthesis. The 16 FAs accounted for 90.8% by mass of the total FAs (Table 1.1). Percentages of FAs were transformed into log ratios prior to analysis by first renormalizing the values for the FAs over 100% and then dividing the value for each of the other FAs by the value for a reference FA, 18:0 (Aitchison 1986; Budge et al. 2002; Iverson et al. 2002). The resulting 15 ratios were then log transformed and used in the analyses.

Differences between adipose tissue and stomach oil samples were evaluated using a combination of univariate and multivariate techniques. A MANOVA was performed on a subset of FAs to evaluate differences in FA signatures between the two lipid sources in adults from Chagulak I. in July and St. George I. in June and August. This was followed by an ANOVA using Tukey's multiple comparisons test to detect any pair-wise differences between sampling groups. A paired t-test using a Bonferroni adjustment was used to evaluate differences between individual FAs from stomach oil and adipose tissue in adults on Chagulak I. in July, St. George I. in June and August, and chicks on Chowiet I. in August. Discriminant analyses were performed to reveal patterns of variation between stomach oil and adipose tissue FAs among adults on Chagulak I. in July, St. George I. in June and August, and chicks on Chowiet I. in August. Separate discriminant analyses were also performed to evaluate how well FAs discriminated between adipose tissue and stomach oil from adults on Chagulak I. in July, St. George I. in June and August, and chicks on

Chowiet I. in August. All statistical analyses were performed using the SAS statistical software (SAS 2000). Wilk's λ was used as the test of significance of the discriminant analyses to separate groups. The number of observations correctly classified and the squared Mahalanobis distances to groups were used to evaluate the performance of the discriminant analyses. Classifications were cross-validated using a jack-knife procedure (SAS 2000). The predicted group membership of individuals based on the jack-knife procedure was examined to determine into which group individuals were misclassified. Data are presented as means ± standard errors. Differences are considered significant at $p < 0.05$.

1.4 Results

There were significant differences in FA signatures between stomach oil and adipose tissue among adults on Chagulak I. in July and St. George I. in June and August ($p < 0.0001$, MANOVA, Figure 3). Levels of 14 FAs were significantly different ($p < 0.0033$) between adipose tissue and stomach oil of adults at Chagulak I.; ten were different for adults on St. George I. in June and nine in August; and 15 were different between the two lipid sources for chicks on Chowiet I. (Figure 1.3, Table 1.1). Absolute differences between the mean levels of some individual FAs in stomach oil and adipose tissue were significantly different between sampling groups (Table 1.1).

We also evaluated how well FAs discriminated between adipose tissue and stomach oil independently for all four sampling groups. We found that for all groups,

100% of the variation between adipose tissue and stomach oil was explained using the subset of 16 FAs: 98.2% of adults were correctly classified to lipid source on St. George I. in June and 95.0% in August; 98.1% of adults on Chagulak I. were correctly classified in July; and 96.7% of chicks were correctly classified on Chowiet I. in August (Figure 1.4a). The squared Mahalanobis distances between lipid sources ranged from 39 to 110 standard deviation units ($p < 0.0001$, Table 1.2). Individual observations for the first and second discriminant functions on the same plot revealed similar separation between the two lipid sources for all four sampling groups (Figure 1.4a).

Discriminant analysis showed that the differences in signatures between stomach oil and adipose tissue were similar for adults on Chagulak I. in July and St. George I. in August, while differences in signatures between stomach oil and adipose tissue were best discriminated in adults on St. George I. in June and chicks on Chowiet I. in August (Figure 1.4b). The Squared Mahalanobis distances from group centroids ranged from 5 to 20 standard deviation units ($p < 0.0001$, Table 1.2). The first and second discriminant functions explained 90.4% of the variation in differences in lipid sources among adults on Chagulak I. in July, adults on St. George I. in June and August, and chicks on Chowiet I. in August. The first three discriminant functions were significant ($p < 0.0001$), the first function alone accounted for 52.2% of the variance, and 77.1% of the individuals were correctly classified.

1.5 Discussion

The FA composition of stomach oil and adipose tissue differed substantially within individual adults and chicks in all regions. We found levels of FAs 14:0, 16:0, 16:1n-7, 16:4n-1, 18:4n-3 and 20:5n-3 to be significantly higher in stomach oil than in adipose tissue (Fig. 1.3). In contrast, levels of the isomers of the long-chain monounsaturated FAs 20:1 and 22:1 were generally lower in stomach oils compared to adipose tissue from the same individuals (Table 1.1, Fig. 1.3). Differences in FA signatures of adipose tissue and stomach oil could arise from the different time spans reflected in the samples (i.e., stomach oil represents the last meal whereas adipose tissue represents a longer term integration of intake). The most recent meals would likely have a different FA pattern than the average diet if heterogeneous meals were consumed over time. Additionally, such differences in the composition of the two lipid sources may be caused by varying degrees of direct deposition of individual FAs from the diet. Presumably, stomach oil had undergone no metabolic processing of triacylglycerols and FAs, but was instead a mixture of the triacylglycerols and FAs from the most recent meal. Also, little is known about any selectivity in uptake or release of specific FAs from stomach oil. In contrast, the FAs found in adipose had been digested and assimilated, i.e., released from the glycerol or alcohol backbone, re-esterified and passed through the circulation, then released, taken up and re-esterified into adipose tissue. Although it has been shown that many FAs from the diet are deposited in adipose tissue at an almost 1:1 ratio (Iverson & Springer 2002; Iverson et al. 2004), it is also known that the effects of metabolism within the

predator can affect levels of specific FAs in adipose stores (e.g., Klasing 1998; Iverson et al. 2004; Cooper et al. 2005).

We observed that wax ester alcohols were only found in Northern Fulmar stomach oil samples and not in their adipose tissue. This observation is consistent with our understanding of the metabolic fate of wax esters in predators that store FAs as triacylglycerols (Budge & Iverson 2003). Many seabirds have the ability to digest and assimilate dietary wax esters efficiently (Roby et al. 1986), and wax ester alcohols contribute significantly to the fatty acid composition of adipose tissue in predators (Budge & Iverson 2003). Therefore, in comparing FA signatures between stomach oil and adipose tissue, we accounted for the wax ester alcohols that can be deposited into the adipose tissue. The technique described in Budge & Iverson (2003) generates a FA signature of prey containing wax esters that is equivalent to that which the predator has available for deposition upon digestion of that prey, and thus we are confident that this procedure did not contribute to any significant differences found between stomach oil and adipose tissue FAs in our fulmar samples.

Although 14:0 can be synthesized by vertebrates, very little of it is released from the fatty acid synthase enzyme complex and, therefore, a high adipose tissue concentration of 14:0 is found only when it is acquired from the diet (Nelson 1992). The low level of 14:0 in adipose tissue of fulmars compared to stomach oil suggests that 14:0 was mobilized directly from stomach oil before it could be deposited into the adipose tissue, or explained by the temporal differences between the lipid sources.

Lower levels of 16:0 in fulmar adipose tissue compared to stomach oil are inconsistent with findings for other species of seabirds (Iverson & Springer 2002), and 16:0 is found to be higher in the blubber of marine mammals than in their prey due to biosynthesis (Kirsch et al. 2000). One explanation for this difference is that although animals may have the capacity for synthesizing lipids, many rarely use their biochemical apparatus for lipid synthesis if their diet includes sufficient fats (Pond 1998). Therefore, lower levels of 16:0 in fulmar adipose tissue may indicate a diet of prey with similarly low levels of 16:0, or of individuals in a non-fasting state. 16:1n-7 is a precursor for the synthesis of other fatty acids (i.e. 18:1n-7, 20:1n-7) and low levels in fulmar adipose tissue could be due to its mobilization directly from stomach oil to produce longer chain FAs rather than using it from adipose tissue. However, this is not very likely because animals that acquire sufficient fats in their diet will not biosynthesize FAs (Pond 1998).

Reduced deposition of 18:4n-3 and 20:5n-3 could also be explained by direct mobilization of these FAs from stomach oil before they can be deposited into adipose tissue. The n-3 and n-6 FAs are considered essential FAs and deficiencies in them can cause detrimental effects in membrane and organ function, which may explain why levels of 18:4n-3 and 20:5n-3 are low in adipose tissue (Innis 1991).

Discriminant analyses revealed a distinct separation between stomach oil and adipose tissue using the subset of indicator FAs (Figure 1.4a). However, discriminant analysis using the differences between levels of individual FAs in oil compared to adipose tissue (Figure 1.4b) indicated otherwise. It showed that (1) a consistent

relationship between adipose tissue and stomach oil FAs does not exist or (2) a consistent relationship does exist but there is significant spatial variation in the diet that accounts for the differences in signatures. Not all FAs occurred at lower or higher levels in stomach oil or adipose tissue consistently in all four sampling groups (Figure 1.3, Table 1.1) suggesting that physiological mechanisms alone do not explain the differences in lipid source signatures and that a combination of diet and physiology influences the differences between FA acid signatures of stomach oil and adipose tissue. We suggest that differences in FA signatures of adipose tissue and stomach oil are due not only to the presence of biosynthesized FAs in adipose tissue, but may also be explained by a combination of two effects: (1) temporal differences, with stomach oil signatures representing a short-term diet and adipose tissue signatures reflecting diet integrated over a longer interval, and (2) different deposition or mobilization characteristics of individual FAs from oil into adipose tissue. In the first case, large variation in FA signatures in stomach oil and adipose tissue would be indicative of a highly variable diet over time, whereas close similarities between the signatures of the two lipid sources would indicate a temporally homogenous diet.

It is advantageous for seabirds that form stomach oil to directly metabolize FAs in the oil rather than to deposit them first in adipose tissue and later mobilize them during periods of fasting, as the latter bears a cost of 25% to 30% of the assimilated energy (Ricklefs 1974; Spady et al. 1976; Roby et al. 1989; Roby et al. 1997). Preferential accumulation of neutral lipids, predominantly triacylglycerols, in stomach oil and the rapid gastric emptying of more polar lipids, such as

phospholipids, rather than a uniform deposition in adipose tissue may be an important determinant of FA signatures in stomach oil compared to adipose tissue. Cooper et al. (2005) have shown that in grey seals (*Halichoerus grypus*), individual dietary FAs likely undergo differential metabolism before their assimilation into chylomicrons, triacylglycerol-rich lipoproteins synthesized in the small intestine of mammals that act as the primary transport lipoproteins for dietary FAs in blood. This results in differences between FA composition of seal blubber and prey. In birds, dietary triacylglycerols are absorbed in the small intestine and incorporated into portomicrons which are the primary transport lipoproteins. Portomicrons are routed through the liver, where elongation and desaturation can occur, along with synthesis of monounsaturated FAs. The liver repackages the dietary lipids and synthesized lipids and the resulting triacylglycerols are carried to peripheral tissue for energy or stored in the adipose tissue for later use (Klasing 1998). Because dietary FAs must pass through the liver, the possibility of extensive modification is greater in birds than in most mammals. It is therefore important to understand how individual dietary FAs are being processed in birds.

This study is the first attempt to compare the FA composition of stomach oil and adipose tissue of Northern Fulmars in detail. FA signature analysis of both stomach oil and adipose tissue has the potential of being extremely informative, with stomach oil providing information on the most recent meals and adipose tissue revealing a diet integrated over a longer period of time. Our investigation has shown that there are differences between stomach oil and adipose tissue signatures; however,

the biological importance of these differences needs to be evaluated. By using QFASA (Iverson et al. 2004) to model the diets of fulmars using stomach oil and adipose tissue and a library of known prey FAs, we will be able to determine how these differences in signatures are biologically significant when making quantitative inferences on diet. In order to accurately estimate the diet of predators using QFASA, calibration coefficients must be calculated through captive feeding studies to account for lipid metabolism (Iverson et al. 2004, Cooper et al. 2005). In addition to modeling the diet of fulmars from their adipose tissue using calibration coefficients calculated for other seabirds (Iverson & Springer 2002), stomach oil compositions without calibration coefficients might be also used to model the diet of fulmars. If the results of the model are comparable, then the implications are that stomach oil and adipose tissue FAs represent the same diet, and the less invasive method of using stomach oil would give the same information as the relatively more invasive adipose tissue collection. Because stomach oil FAs in essence are an intermediate between prey and adipose tissue FAs, it is feasible that the differences between stomach oil and adipose tissue FA signatures may be used to calculate calibration coefficients for fulmars in lieu of a captive study and, at the very least, reveal metabolic information of individual FAs in fulmars that may be applied to other seabirds in future studies.

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Table 1.1. Mean values for fatty acid composition of adipose tissue and stomach oil. Mean diff. = mean differences between values for adipose tissue and stomach oil ± SD.

	Chagulak Is. July (n = 26)				St. George Is. June (n = 27)			
	fat	oil	mean diff.	p-value	fat	oil	mean diff.	p-value
Saturated	(14.68 ± 3.130)	(17.76 ± 2.19)			(18.75 ± 2.509)	(31.18 ± 10.32)		
14:0*	3.19 ± 0.969	4.97 ± 1.247	1.78 ± 1.204[a,b]	<.0001	7.46 ± 2.215	18.79 ± 9.675	11.33 ± 8.887[a]	<.0001
16:0*	8.55 ± 2.009	11.11 ± 1.594	2.56 ± 2.006[a,b]	<.0001	8.66 ± 1.578	11.02 ± 1.692	2.36 ± 2.495[b]	<.0001
17:0	0.10 ± 0.038	0.07 ± 0.017			0.11 ± 0.077	0.06 ± 0.039		
18:0*	2.84 ± 0.564	1.61 ± 0.531	-	-	2.51 ± 0.784	1.30 ± 1.223	-	-
20:0	0.30 ± 0.067	0.15 ± 0.035			0.37 ± 0.070	0.35 ± 0.098		
Monounsaturated	(67.99 ± 7.305)	(57.57 ± 7.22)			(67.14 ± 6.348)	(51.94 ± 10.988)		
16:1n-11	0.29 ± 0.093	0.34 ± 0.064			0.30 ± 0.094	0.21 ± 0.070		
16:1n-7*	3.35 ± 1.319	6.30 ± 1.058	2.94 ± 1.343[a]	<.0001	2.96 ± 0.957	3.57 ± 1.011	0.61 ± 0.919[a,b]	<.0001
18:1n-11	0.53 ± 0.210	0.33 ± 0.154			0.67 ± 0.197	0.23 ± 0.330		
18:1n-9*	13.05 ± 3.63	13.08 ± 3.925	0.04 ± 3.881[a]	<.0001	7.48 ± 4.283	3.15 ± 2.876	4.33 ± 3.835[b]	0.0241
18:1n-7*	2.95 ± 1.7073	3.62 ± 1.093	0.67 ± 0.871[a]	<.0001	1.78 ± 1.264	1.16 ± 1.173	0.63 ± 1.216[b]	0.1215
18:1n-5	0.43 ± 0.091	0.62 ± 0.144			0.45 ± 0.065	0.51 ± 0.077		
20:1n-11*	19.57 ± 3.775	12.02 ± 1.891	7.56 ± 3.097[a]	0.1360	28.78 ± 7.629	22.57 ± 7.908	6.21 ± 10.170[a]	0.0018
20:1n-9*	4.11 ± 0.820	3.92 ± 1.097	0.20 ± 0.902[a]	<.0001	3.77 ± 0.572	2.89 ± 0.524	0.88 ± 0.579[a]	<0.0001
20:1n-7	0.47 ± 0.120	0.29 ± 0.070			0.510 ± 0.157	0.19 ± 0.172		
22:1n-11*	22.97 ± 7.154	17.09 ± 4.729	5.88 ± 5.190[a,b]	0.0030	20.66 ± 4.501	17.75 ± 4.752	2.91 ± 6.761[a]	0.0002
22:1n-9*	1.52 ± 0.479	1.25 ± 0.446	0.27 ± 0.415[a,b]	0.0020	1.20 ± 0.286	0.67 ± 0.306	0.53 ± 0.386[a]	0.1031
22:1n-7	0.30 ± 0.083	0.25 ± 0.083			0.25 ± 0.051	0.16 ± 0.060		
Polyunsaturated	(10.55 ± 3.93)	(15.54 ± 6.46)			(8.31 ± 4.237)	(10.55 ± 7.228)		
16:2n-4	0.24± 0.124	0.47 ± 0.159			0.29 ± 0.092	0.42 ± 0.194		
16:3n-6	0.21 ± 0.084	0.27 ± 0.142			0.11 ± 0.056	0.07 ± 0.055		
16:4n-1	0.18 ± 0.133	0.64 ± 0.711			0.23 ± 0.124	1.01 ± 0.657		
18:2n-6*	0.99 ± 0.076	0.92 ± 0.182	0.07 ± 0.182[a]	<.0001	0.67 ± 0.156	0.36 ± 0.214	0.31 ± 0.162[b]	0.5872
18:3n-3*	0.43 ± 0.131	0.51 ± 0.088	0.08 ± 0.122[a]	<.0001	0.24 ± 0.109	0.19 ± 0.171	0.05 ± 0.110[b]	0.0020
18:4n-3*	1.25± 0.795	2.03 ± 1.305	0.78 ± 1.023[a]	<.0001	0.90 ± 0.528	1.70 ± 1.215	0.80 ± 0.858[a]	<.0001
20:2n-6	0.26 ± 0.062	0.25 ± 0.074			0.14 ± 0.041	0.09 ± 0.070		
20:4n-6*	0.25 ± 0.052	0.29 ± 0.065	0.04 ± 0.068[a]	<.0001	0.23 ± 0.094	0.24 ± 0.307	0.01 ± 0.316[a]	<.0001
20:4n-3	0.31 ± 0.136	0.47 ± 0.102			0.18 ± 0.105	0.24 ± 0.252		
20:5n-3*	2.54 ± 1.581	6.83 ± 3.706	4.29 ± 3.348[a]	<.0001	2.05 ± 1.269	4.61 ± 3.304	2.56 ± 3.236[a]	<.0001
21:5n-3	0.22 ± 0.108	0.33 ± 0.165			0.18 ± 0.103	0.16 ± 0.191		
22:5n-3	1.21 ± 0.347	0.51 ± 0.208			1.26 ± 0.388	0.46 ± 0.519		
22:6n-3*	4.12 ± 1.507	4.31 ± 1.571	0.19 ± 1.416[a]	<.0001	2.98 ± 2.204	2.18 ± 2.275	0.80 ± 2.294[b]	0.1603
24:1n-9	0.96 ± 0.440	1.20 ± 0.571			0.63 ± 0.228	0.42 ± 0.164		

Table 1.1 continued

	St. George Is. August (n = 20)				Chowiet Is. August (chicks n = 30)			
	fat	oil	mean diff.	p-value	fat	oil	mean diff.	p-value
Saturated	(16.00 ± 2.467)	(19.39 ± 1.234)			(15.29 ± 2.785)	(16.02 ± 3.115)		
14:0*	3.443 ± 1.015	5.01 ± 1.484	1.56 ± 0.924[b]	<.0001	2.46 ± 0.588	3.87 ± 0.610	1.41 ± 0.671[a,b]	<.0001
16:0*	9.09 ± 1.711	11.33 ± 1.428	2.24 ± 1.697[a]	<.0001	9.55 ± 1.891	9.81 ± 2.045	0.26 ± 1.903[a]	<.0001
17:0	0.15 ± 0.051	0.55 ± 0.253			0.28 ± 0.052	0.65 ± 0.401		
18:0*	3.32 ± 0.608	2.51 ± 0.897	-	-	3.00 ± 0.547	1.69 ± 0.863	-	-
20:0	0.27 ± 0.071	0.11 ± 0.042			0.25 ± 0.050	0.13 ± 0.032		
Monounsaturated	(59.88 ± 6.809)	(49.06 ± 2.811)			(67.12 ± 5.880)	(62.92 ± 6.906)		
16:1n-11	0.30 ± 0.098	0.33 ± 0.100			0.37 ± 0.101	0.32 ± 0.042		
16:1n-7*	3.86 ± 1.086	5.77 ± 0.718	1.91 ± 1.105[b]	<.0001	3.61 ± 0.992	6.04 ± 0.564	2.43 ± 1.089[a,b]	<.0001
18:1n-11	0.94 ± 0.462	0.91 ± 0.364			0.58 ± 0.198	0.35 ± 0.160		
18:1n-9*	12.80 ± 3.384	12.15 ± 4.641	0.65 ± 3.134[a]	0.0052	18.11 ± 3.222	15.46 ± 3.364	2.65 ± 4.011[a]	<.0001
18:1n-7*	3.61 ± 1.009	4.23 ± 1.354	0.62 ± 0.905[a,b]	<.0001	3.07 ± 1.003	3.12 ± 1.057	0.05 ± 1.002[a]	<.0001
18:1n-5	0.49 ± 0.086	0.56 ± 0.149			0.57 ± 0.108	0.72 ± 0.153		
20:1n-11*	17.44 ± 4.282	12.06 ± 2.686	5.38 ± 4.385[a]	0.6916	17.20 ± 4.169	12.67 ± 2.786	4.52 ± 3.410[a]	0.0031
20:1n-9*	3.54 ± 0.706	2.98 ± 0.538	0.56 ± 0.612[a]	0.1292	5.01 ± 0.720	4.57 ± 0.815	0.44± 0.749[a]	<.0001
20:1n-7	0.47 ± 0.206	0.32 ± 0.318			0.51 ± 0.083	0.37 ± 0.070		
22:1n-11*	17.03 ± 5.327	10.83 ± 3.247	6.20 ± 5.257[b]	0.2989	18.26 ± 4.496	19.196 ± 5.015	0.94 ± 4.650[a]	<.0001
22:1n-9*	1.13 ± 0.418	0.72 ± 0.222	0.41 ± 0.318[a]	0.3270	1.36 ± 0.269	1.49 ± 0.345	0.13 ± 0.309[b]	<.0001
22:1n-7	0.22 ± 0.071	0.16 ± 0.069			0.22 ± 0.045	0.25 ± 0.059		
Polyunsaturated	(16.59 ± 4.105)	(22.62 ± 2.572)			(10.28 ± 3.098)	(11.38± 5.015)		
16:2n-4	0.19 ± 0.034	0.19 ± 0.046			0.35 ± 0.088	0.52 ± 0.162		
16:3n-6	0.29 ± 0.109	0.44 ± 0.125			0.16 ± 0.078	0.26 ± 0.084		
16:4n-1	0.25 ± 0.138	0.59 ± 0.274			0.06 ± 0.052	0.16 ± 0.121		
18:2n-6*	0.98 ± 0.207	0.82 ± 0.141	0.15 ± 0.139[a,b]	0.0151	1.28 ± 0.095	1.083 ± 0.147	0.20 ± 0.168[a]	<.0001
18:3n-3*	0.55 ± 0.221	0.63 ± 0.208	0.08 ± 0.165[a,b]	<.0001	0.53 ± 0.136	0.55 ± 0.109	0.02 ± 0.140[a,b]	<.0001
18:4n-3*	1.96 ± 0.935	3.15 ± 1.245	1.20 ± 0.877[a]	<.0001	0.64 ± 0.391	1.09 ± 0.534	0.46 ± 0.440[a]	<.0001
20:2n-6	0.29 ± 0.040	0.3 ± 0.080			0.3 ± 0.043	0.25 ± 0.044		
20:4n-6*	0.34 ± 0.122	0.43 ± 0.586	0.09 ± 0.543[b]	0.0002	0.33 ± 0.126	0.36 ± 0.113	0.03 ± 0.160[a]	<.0001
20:4n-3	0.47 ± 0.155	0.64 ± 0.289			0.32 ± 0.126	0.41 ± 0.163		
20:5n-3*	4.79 ± 2.186	9.24 ± 1.038	4.45 ± 2.003[a]	<.0001	1.95 ± 1.167	4.10 ± 2.476	2.15 ± 1.895[a]	<.0001
21:5n-3	0.34 ± 0.105	0.49 ± 0.060			0.12 ± 0.071	0.21 ± 0.103		
22:5n-3	1.40 ± 0.492	0.94 ± 0.511			1.26 ± 0.469	0.69 ± 0.476		
22:6n-3*	6.88 ± 1.637	7.43 ± 1.547	0.56 ± 1.708[a,b]	<.0001	4.77 ± 1.326	3.90 ± 1.795	0.87 ± 1.759[a,b]	<.0001
24:1n-9	0.61 ± 0.220	0.58 ± 0.192			0.67 ± 0.185	1.52 ± 0.415		

[a] Values are mean mass percent ± SD of fatty acids (31 out of 69, including reference FA 18:0) which averaged ≥ 0.2% among samples analyzed, with totals in parentheses. *Designates the 16 fatty acids used in analyses. Values for mean diff. are absolute values in percentages: values for the same FAs that do not share a common superscript letter are significantly different ($p < 0.0033$, Tukey's multiple comparison test). P-values are shown for paired t-tests using the Bonferroni adjustment to evaluate differences between adipose tissue and stomach oil.

Table 1.2. Squared Mahalanobis distances from discriminant analysis. Distances to group centroids in standard deviation units ($p < 0.0001$): (a) for mean absolute differences between stomach oil and adipose tissue, and (b) between lipid source signatures for adults on Chagulak I. in July, St. George I. in June and August 2004, and chicks on Chowiet I. in August 2004.

(a)	Group = mean differences between stomach oil and adipose tissue			
From Group	Chowiet I. Aug.	Chagulak I. Jul.	St. George I. Aug.	St. George I. Jun.
Chowiet I. Aug.	0.00	10.35	14.12	18.94
Chagulak I. Jul.	10.35	0.00	4.44	12.26
St. George I. Aug.	14.12	4.44	0.00	13.96
St. George I. Jun.	18.94	12.26	13.96	0.00
(b)	*Adipose tissue*			
From lipid source	Chowiet I. Aug.	Chagulak I. Jul.	St. George I. Aug.	St. George I. Jun.
Stomach oil				
Chowiet I. Aug.	52.92	-	-	-
Chagulak I. Jul.	-	36.28	-	-
St. George I. Aug.	-	-	26.94	-
St. George I. Jun.	-	-	-	30.38

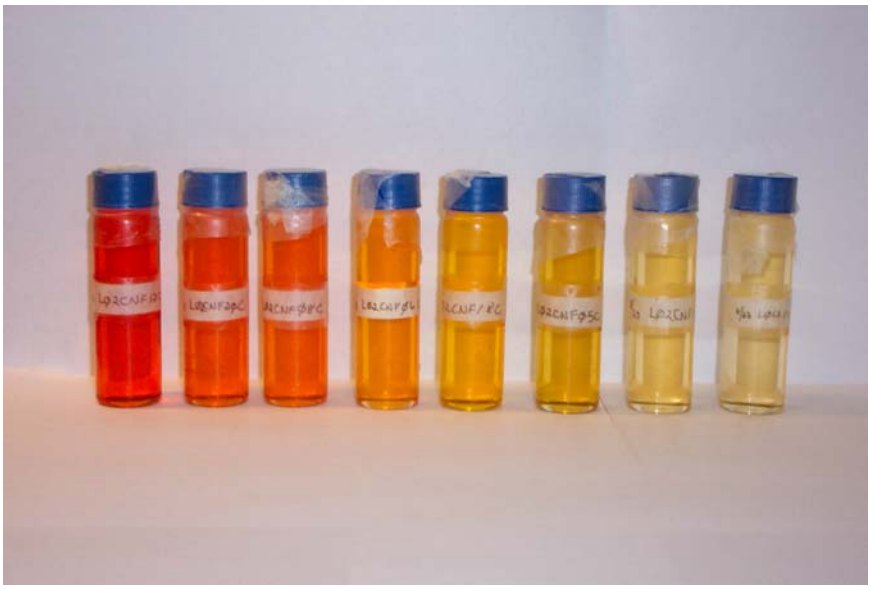

Figure 1.1. Range of coloration of stomach oil. Samples collected from Northern Fulmars in Alaska. Most samples displayed shades of yellow, orange, or red. Occasional greenish samples (not shown) probably contained bile (Fisher 1952; Lewis 1969; Warham 1977).

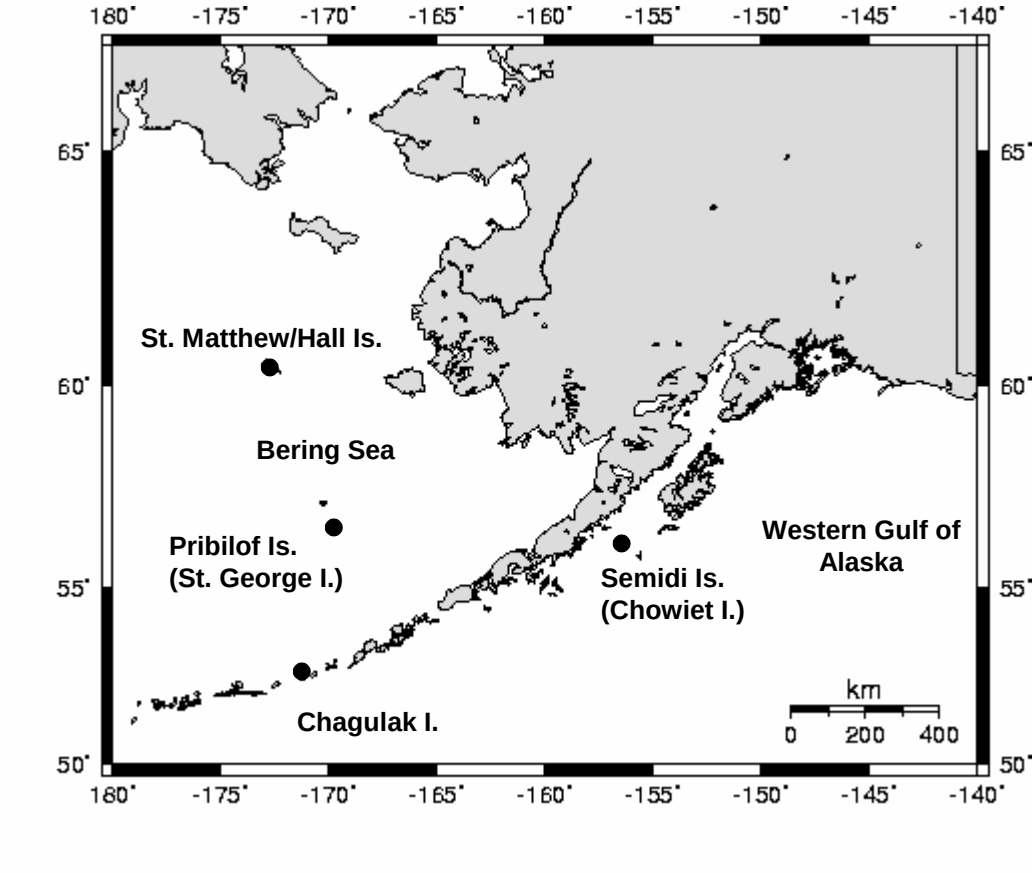

Figure 1.2. Locations of the four major fulmar colonies in Alaska.
1) Semidi Islands (56˚05' N, 156˚45' W) in the western Gulf of Alaska; 2) Chagulak Island (52˚35' N, 171˚10' W) in the eastern Aleutian Islands; 3) Pribilof Islands (56˚35' N, 170˚35' W) in the eastern Bering Sea; and 4) St. Matthew and Hall islands (60˚40' N, 173˚10' W) in the northern Bering Sea. Studies were not undertaken at the latter site.

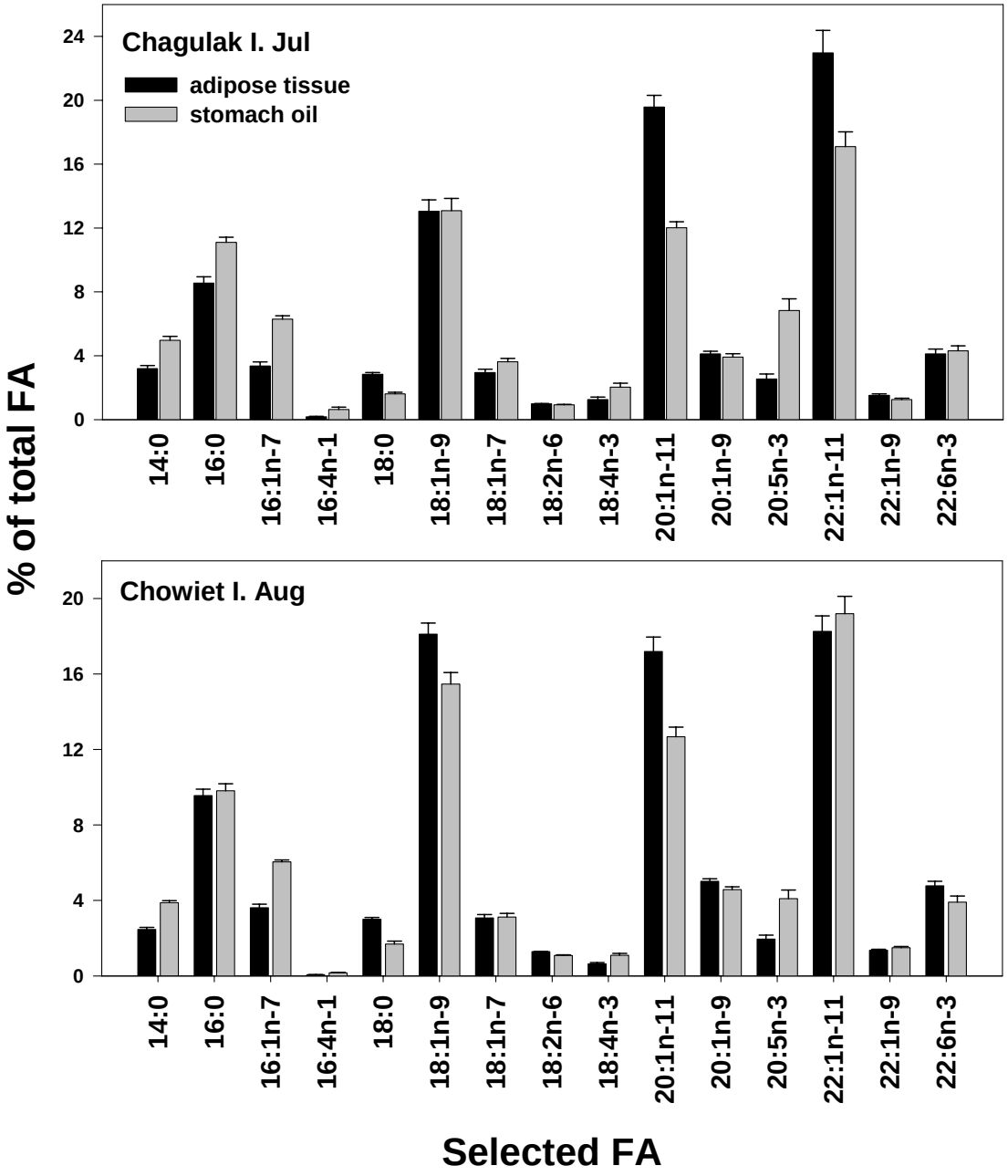

Figure 1.3. Mean values of stomach oil and adipose tissue signatures. The 16 most abundant FAs in Northern Fulmar lipids, including reference FA 18:0, illustrating characteristic differences in patterns between lipid sources for adults on Chagulak I. in July (n = 26), St. George I. in June (n = 27) and August (n = 20), and chicks on Chowiet I. in August (n = 30) 2004. Adipose tissue and stomach oil samples were collected from the same individuals. Bars are means and vertical lines are 1 SE.

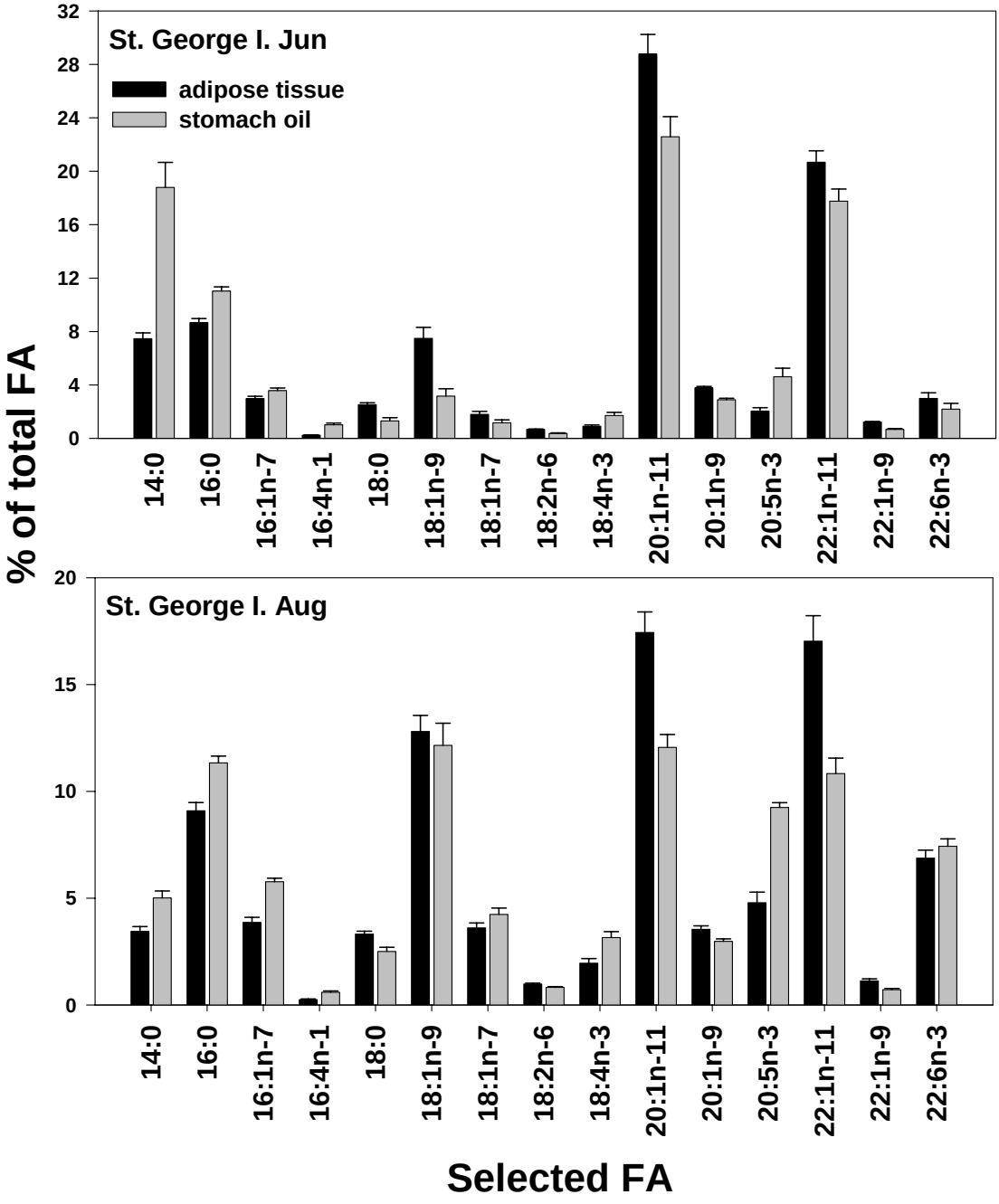

Figure 1.3 continued

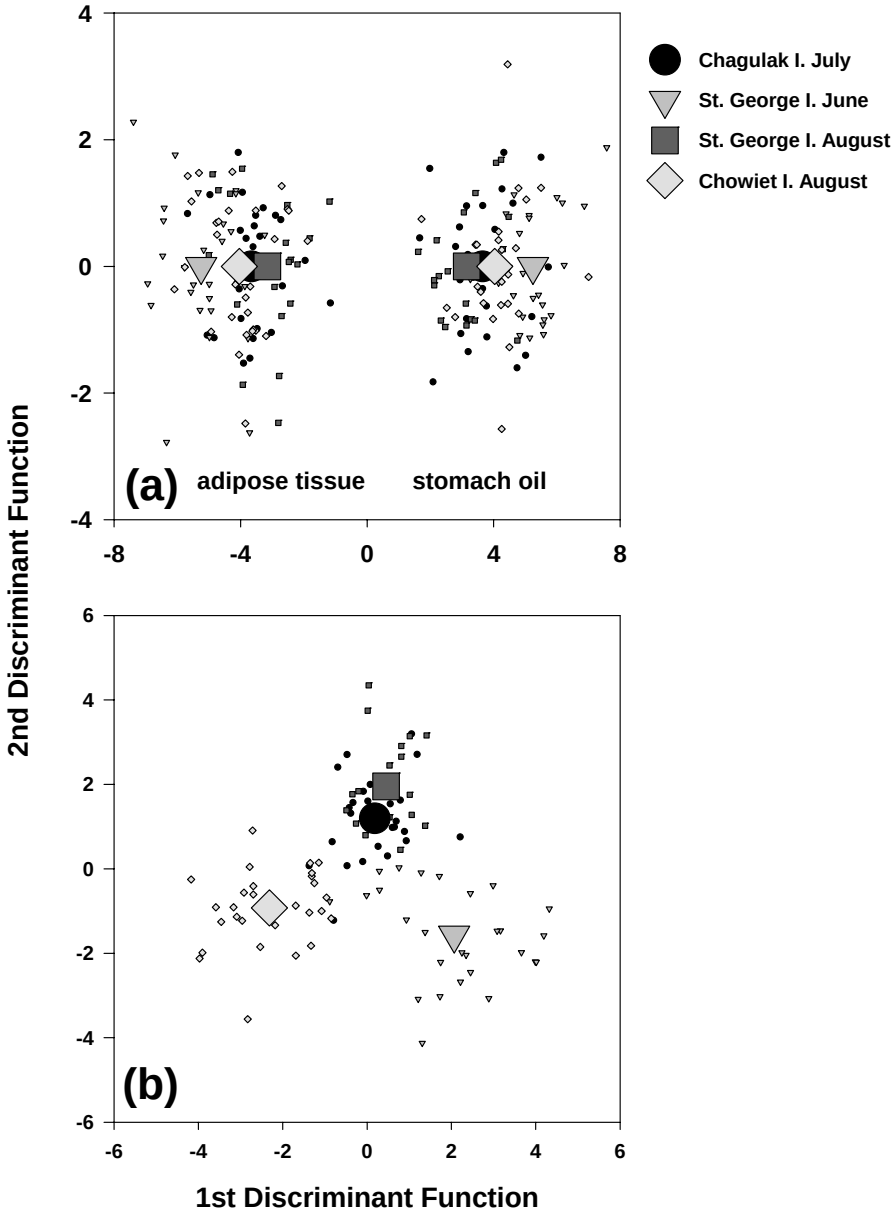

Figure 1.4. Discriminant scores of mean differences between lipid sources (a) Individual discriminant analysis to evaluate how well our subset of FAs discriminated between adipose tissue and stomach oil for adult Northern Fulmars on Chagulak I. in July (n = 26), St. George I. in June (n = 27) and August (n = 20), and chicks on Chowiet I. in August (n = 30) 2004. (b) Discriminant analysis to evaluate how well the mean differences in fatty acid signatures of stomach oil and adipose tissue separated adults on Chagulak I. in July (n = 26), St. George I. in June (n = 27) and August (n = 20), and chicks on Chowiet I. in August (n = 30) 2004.

Chapter 2. Fatty Acids in Northern Fulmars (*Fulmarus glacialis*) breeding in Alaska: a qualitative comparison of diet[2]

2.1 Abstract

Variations in the diet composition of a generalist predator may be indicative of changes in the ecosystem. Diets of Northern Fulmars (*Fulmarus glacialis*) in the North Pacific Ocean are poorly known, and thus relationships of fulmars to supporting food webs and their potential sensitivity to ecosystem variability also are uncertain. We employed fatty acid signature analysis of adipose tissue from adults and chicks to examine dietary differences among fulmars breeding at the three largest colonies in Alaska. We found conspicuous differences between fatty acid signatures of adult fulmars at the three locations within years, and between seasons at individual colonies. Inter-annual differences in signatures at individual colonies were not as great as seasonal and spatial differences. We conclude that differences in FA signatures reflect differences in diet composition, probably because the colonies are located in distinct oceanographic settings, which create unique habitats for prey assemblages, and inter-annual variation in diet reflects variation in the physical environment. Comparison of adults and chicks revealed similarities in differences between age class signatures in 2003 and 2004,

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suggesting that adults fed chicks a diet different than that they consumed themselves.

Keywords fatty acids, Northern Fulmar, *Fulmarus glacialis*, Alaska, diet analysis

2.2 Introduction

Northern fulmars (*Fulmarus glacialis*) are opportunistic and generalist predators. In the North Pacific Ocean, fulmars feed on cephalopods, zooplankton, lantern fishes (Myctophidae), Scyphozoan jellyfish, juveniles of commercial fish species, e.g., walleye pollock (*Theragra chalcogramma*), and other forage species that are critical to pelagic food webs and sensitive indicators of environmental change, e.g., capelin (*Mallotus villosus*) and Pacific sandlance (*Ammodytes hexapterus*) (Preble & McAtee 1923, Bradstreet 1985, Hunt et al. 1981a, Hunt et al. 1981b, Harrison 1984, Sanger 1983, Hills & Fiscus 1988, Gould et al. 1997, Hatch & Nettleship 1998, Hatch unpubl.). Fulmars also follow fishing vessels for offal (Fisher 1952, Hunt et al. 1981b, Hatch 1993). However, diets of fulmars at nesting colonies in summer are poorly known in the N. Pacific. Thus, relationships of fulmars to supporting food webs, and the sensitivity of fulmars to ecosystem variability, such as that driven by a changing climate or by commercial fisheries, also are highly uncertain.

The population of fulmars in North America is estimated at 2.1 million individuals with 70% occurring in Alaska (Hatch & Nettleship 1998). There are four major breeding colonies in Alaska: 1) Chagulak Island (52˚35' N, 171˚10' W), located in a deep oceanic basin in the central Aleutian Archipelago, has a population estimated at 500 000; 2) St. Matthew and Hall Islands (60˚30' N, 172˚45' W) located in the middle of the large continental shelf in the central Bering Sea has approximately 450 000 individuals; 3) the Semidi Islands (56˚05' N, 156˚45' W) in the western Gulf of Alaska, where the local oceanography is primarily influenced by the Alaska Coastal Current and

the Alaska Stream has approximately 440 000 individuals; and 4) the Pribilof Islands (56˚35' N, 170˚35' W), located near the continental shelf edge in the southeastern Bering Sea, have approximately 79 700 individuals (Hatch & Hatch 1983, Hatch 1993, Fig. 2.1). The locations of these colonies in distinct oceanographic settings, i.e., in distinct habitats, provide opportunities for characteristic prey assemblages and food webs to form. By understanding the foraging ecology and diets of fulmars and other marine birds in diverse habitats, predator-prey relationships and foraging patterns can be used in comparative ways as measures of the impact of environmental variation on the birds and the ecosystem.

There are several well-known problems and biases associated with traditional methods of estimating seabird diets, such as analysis of stomach contents, including differential retention of prey hard parts and the need to sacrifice animals (Furness et al. 1984; Harrison 1984; Duffy & Jackson 1986; Jobling & Breiby 1986; Annett & Pierotti 1989; Votier et al. 2003). Most of the problems are eliminated by using fatty acid (FA) analysis to estimate diets. The concept of using lipids as biomarkers and general indicators of diets of predators in marine ecosystems has been applied for many years (Sargent et al. 1988), and the analysis of FAs in reservoir lipids (adipose tissue and blubber) has been used to infer trophic levels, as well as spatial and temporal patterns in foraging behavior, of free ranging marine mammals and seabirds (Iverson & Oftedal 1992, Iverson 1993, Iverson et al. 1997a, Iverson et al. 1997b, Kirsch et al. 2000, Iverson et al. 2001b, Brown et al. 1999, Raclot et al. 1998, Iverson & Springer 2002, Dahl et al. 2003).

FA signature analysis is based on the knowledge that prey species have characteristic FA patterns, or signatures, and that specific FAs of carbon chain length ≥ 14 from prey are incorporated with little change into the body fat of marine predators and can thus be used as qualitative and quantitative diet tracers (Iverson 1993, Iverson et al. 2004). Some FAs can be synthesized by animals, but the number is limited (Cook 1991) and they can be distinguished from those that are acquired only from the diet. Once ingested, FAs are used for energy directly or are re-esterified, primarily to triacylglycerols, and stored in adipose tissue. Although metabolism and synthesis of certain FAs occur within the predator, resulting in a FA composition of its adipose tissue that does not match exactly that of its prey (Iverson et al. 2004), most FAs are deposited with little modification and in a predictable way.

The objectives of this study were to use FA signatures to assess spatial (between colonies), temporal (seasonal and inter-annual), and age class (adult-chick) variability in the diet of fulmars at three colonies in Alaska. We predicted that the diets would: 1) differ among colonies because they are located in distinct marine habitats with different prey assemblages; 2) differ inter-annually because of variability in the physical environment and the production and availability of prey; 3) differ seasonally due to availability of prey whose presence and abundance respond to seasonal physical and biological cycles; and 4) not differ between adults and chicks because adult fulmars feed their chicks by regurgitation of the meal they have just consumed, and theoretically chicks receive an aliquot of the homogenized meal.

2.3 Materials and Methods

2.3.1 Sample collection

Samples of adipose tissue were collected from adult fulmars on Chowiet I. (Semidi Islands) during the incubation stage in May 2003 (n = 30) and 2004 (n = 25), and during the chick-rearing stage in August 2003 (n = 27) and 2004 (n = 31); on St. George I. (Pribilof Islands) during incubation in June 2003 (n = 29) and 2004 (n = 30), and during the chick-rearing stage in August 2003 (n = 9) and 2004 (n = 26); and on Chagulak I. during incubation in July 2004 (n = 30). Samples of adipose tissue were collected from chicks and one respective parent on Chowiet I. during August 2003 (n = 8 pairs) and 2004 (n = 25 pairs). Samples were not collected on the same dates at the three islands due to logistical constraints. Fulmars at St. Matthew I. and Hall I. were not sampled.

Adult birds were captured using a modified dip net or a noose-pole, or by hand at the nest. Chicks were captured by hand from the nest. A live biopsy technique was used to obtain synsacral adipose tissue samples from individuals (Enderson & Berger 1968; Iverson & Springer 2002). The area was disinfected with chlorhexidine and an incision of approximately 0.5 cm in length was made through the skin. A small sample of adipose tissue of approximately 0.1 g was excised from the fat layer and placed in a vial of chloroform with 0.01% BHT as an antioxidant. Vet-bond© was applied on the incision to insure closure and rapid healing.

Morphometric measurements were taken from all individuals. Wing-length and mass were used to estimate the ages of chicks (Hatch 1979). All samples were stored frozen until analyzed.

2.3.2 Lipid extraction and analysis

Lipids were extracted using a modified Folch extraction (Folch et al. 1957, Iverson et al. 2001a). FA methyl esters were prepared directly from ≤ 100 mg of the pure extracted lipid (filtered and dried over anhydrous sodium sulfate) using 3.0 ml Hilditch reagent (0.5 N H2SO4 in methanol) and 1.5 ml methylene chloride with 0.01% BHT, capped under nitrogen, and heated at 100°C for 1 hour. FA methyl esters were then extracted into hexane, concentrated, and brought up to volume (50 mg/ml) with high purity hexane.

FA methyl esters were quantified using temperature-programmed gas liquid chromatography as described previously (Iverson & Oftedal 1992, Iverson et al. 1997b, Budge et al. 2002, Iverson et al. 2004) on a Perkin Elmer Autosystem II Capillary FID gas chromatograph fitted with a 30m x 0.25 mm id column coated with 50% cyanopropyl polysiloxane (0.25µ film thickness; J&W DB-23; Folsom, California, USA) and linked to a computerized integration system (Turbochrom 4 software, PE Nelson, San Jose, California, USA). Fatty acids and isomers were identified using known standard mixtures (Nu Check Prep., Elysian, Minnesota, USA), silver-nitrate (argentation) chromatography, and GC-mass spectrometry (Hewlett-Packard 6890 Gas Chromatograph, 1:20 split injection, Micromass Autospec oa-TOF mass spectrometer, operated at 1000 resolution

and scanning masses 120 to 450 [Hewlett Packard, Palo Alto, California, USA]). GC columns were kept in good condition by changing septa daily, cleaning the injector liner regularly, use of a guard column, and frequent replacement. All sample chromatograms and fatty acid identifications were individually checked and corrected and reintegrated as necessary. FAs are expressed as mass percent of total FAs and are designated by short-handed nomenclature of carbon chain length, number of double bonds and location (n-x) of the double bond nearest the terminal methyl group.

2.3.3 Data analysis

Not all FAs are indicative of diet and appropriate for use in statistical analysis (Iverson et al. 2004). FAs that were used in our analyses were chosen by how well they indicated diet, which included those with the largest overall variances and overall means ≥ 0.2% of the total FAs. Seventeen FAs met these criteria and represented the majority of the signature. Despite the large overall variance of one of these, 22:5n-3, it was not used as it may be an intermediate between 20:5n-3 and 22:6n-3 (e.g. Ackman et al. 1988). Thus, all analyses were performed using 16 FAs, of which 10 come from diet alone and 6 arise from both biosynthesis and diet: together these FAs accounted for 90.9% by mass of the total FAs (Table 2.1). Percentages of the 16 FAs were renormalized over 100% and the values were divided by the value for a reference FA, 18:0 (Aitchison 1986). The resulting 15 ratios were then log transformed and used in the analyses. The sample size of any group used in discriminant analysis must be greater than the number of variables

used (Stevens 1986, Legendre & Legendre 1998), and, therefore, only groups with 16 or more samples were included in the analyses.

Differences in FA signatures among adult fulmars and between adults and chicks were evaluated using a combination of univariate and multivariate techniques. Due to sample size restrictions mentioned above, data from adults on St. George I. in August 2003 (n = 9) and chicks and adults on Chowiet I. in August 2003 (n = 8 pairs) were only included in the principle component analysis (PCA). The seven ratios resulting from the eight FAs with the highest overall variance were used to conduct a paired t-test for chicks and adults on Chowiet I. in August 2003. Because most samples were not repeated, each data set (e.g., Chowiet I. in May 2004) was treated as an individual sampling group and we performed a MANOVA followed by ANOVA using Tukey's multiple comparisons test with the Bonferroni adjustment to detect pair-wise differences in mean concentrations of FAs between groups. A 2-factor MANOVA was used to evaluate overall year or month effects of FA concentrations for adults sampled on Chowiet I. in May and August 2003 and 2004. A 1-factor MANOVA was used to evaluate any overall month effects for adults sampled on St. George I. in June and August 2004, and to evaluate any overall year effects for adults sampled on St. George I. in June of 2003 and 2004. PCA and discriminant analyses were used to evaluate how well our subset of FAs discriminated among all sampling groups. Scores for individual samples from PCA were used as response variables in an ANOVA to analyze the effect of each sampling group on FAs.

We used a paired t-test with a Bonferroni adjustment to evaluate differences between individual FAs from adults and chicks on Chowiet I. during August 2003 and 2004. PCA and discriminant analysis were also used to evaluate how well FAs discriminated between adults and chicks. To assess the effect of age on FA composition, the samples' scores on the PCs were used as response variables in an ANOVA.

Wilk's λ was used as the test of significance of the discriminant analyses to separate among groups of adults at the three colonies and between adults and chicks on Chowiet I. in August 2004. The number of observations correctly classified was used to evaluate the performance of the discriminant analyses. Classifications were cross-validated using a jack-knife procedure (SAS 2000). The predicted group membership of individuals based on the jack-knife procedure was examined to determine into which group individuals were misclassified. All statistical analyses were performed in the SAS program (SAS 2000).

Chick age (days) was estimated from wing length (mm) using a logarithmic curve fitted to changes in length with age from a sample of known-age chicks (Hatch 1979).

2.4 Results

2.4.1 Differences among adults

Sixty-nine FAs were identified and quantified in fulmar adipose tissue samples. Given this large number, we present summary data for only those FAs we used in the analyses (see Table 1). Complete data, including all FAs quantified, are available upon request from the corresponding author.

The FA composition varied substantially across time and among colonies (Table 2.1, Fig. 2.2). We found a significant difference in adult FA signatures between years and months on Chowiet I. ($p < 0.0001$, 2-factor MANOVA). Overall FA signatures were also different between years for adults on St. George I. in June 2003 and 2004 ($p < 0.0001$, MANOVA), however only one FA (18:2n-6) was significantly different ($p < 0.0033$, Tukey's multiple comparison with Bonferroni adjustment, Table 2.1), indicating that signatures were actually quite similar between groups. FA signatures also differed between adults sampled on St. George I. in June and August 2004 ($p < 0.0001$, MANOVA), with five individual FAs differing between groups (Table 2.1).

Although the most abundant FAs were generally similar across sampling groups, levels of individual FAs varied greatly among colonies and over time. All adults exhibited high levels of 20:1n-11 (15.0 – 29.1%) and 22:1n-11 (17.9 – 31.3%) (Table 2.1, Fig. 2.2). Higher levels of 20:1n-9 (6.5 – 7.0%) were found in adults on Chowiet I. in May 2003 and 2004, and in May 2004 the birds were characterized by a unique signature having significantly lower levels of 20:5n-3 (0.2%) and 22:6n-3 (1.2%) than the mean of

all adults. FA 18:1n-9 (7.4 – 9.1%) was lowest in fulmars from Chowiet I. in August 2004 and St. George I. in June 2003 and 2004 (Table 2.1, Fig. 2.2).

The PCA produced four eigenvalues > 1.0. In combination, these PCs explained 88.3% of the variance (PC1:41.7%, PC2:20.3%, PC3: 15.5%, PC4: 10.8%). A plot of the mean scores ± SE on PC1 and PC2 shows separation between sampling groups with PC1 separating all adults from Chowiet I. from adults on St. George I. and Chagulak I., and PC2 separating individuals from Chowiet I. in August 2003 and 2004 and St. George I. in August 2003 from all other sampling groups (Fig. 2.4a). Based on the FA loadings along PC1, the separation of sampling groups was due mainly to the combination of differences in levels of 22:1n-9, 22:1n-11, 20:1n-9 (generally higher in adults on Chowiet I.) and 18:4n-3, 20:5n-3 (lower in adults on Chowiet I.). FA loadings along PC2 show that differences in levels of 14:0, 16:0, and 16:1n-7 also contributed to the separation among adults. All four PCs were significant ($p < 0.0001$, ANOVA), and using Tukey's multiple pair-wise comparisons, none of them were able to distinguish patterns in differences among the three colonies or between each individual group, i.e., individual FAs were not always higher or lower at Chagulak I., Chowiet I., or St. George I.

Results of the discriminant analysis showed that the first discriminant function separated groups by colonies and the second discriminant function roughly separated signatures of birds sampled early in the breeding season (May and June) from those sampled later in the breeding season (July and August) (Fig. 2.5a). This analysis also demonstrated the inter-annual similarities in FA signatures among individuals from Chowiet I. in May and August 2003 and 2004. Although FA signatures, in general,

overlapped between colonies and sampling periods, 81.8% of original grouped cases, and 76.1% of cross-validated grouped cases were correctly classified to their respective sampling groups (Table 2.3). The first six discriminant functions were significant and accounted for 99.1% of the variation ($p \leq 0.0001$): the first and second accounted for 69.2% of the variation (first: 45.6%, second: 23.6%).

2.4.2 Differences between adults and chicks

The ages of chicks sampled on Chowiet I. in 2003 and 2004 were estimated to be 14 ± 5.8 days (range 9 – 25) and 21 ± 5.9 days (range 13 – 32), respectively, and were significantly different between years ($p = 0.0031$). FA composition varied significantly ($p < 0.0001$, MANOVA) between adults and chicks from Chowiet I. in August 2004 (Table 2.2, Fig. 2.3), and eight FAs were significantly different between age classes ($p < 0.0033$, paired t-test, Bonferroni adjustment): 16:0, 16:1n-7, 18:1n-9, 18:1n-7, 18:2n-6, 18:3n-3, 20:1n-9 and 20:4n-6 were higher in chicks than in adults. Although levels of 22:1n-11 averaged 29% and 27% in adults compared to only 15% and 18% in chicks in 2003 and 2004, respectively, these differences were not significant.

The PCA produced three eigenvalues > 1.0, which in combination accounted for 82.5% of the variation (PC1:37.5%, PC2:29.2%, PC3:15.8%). A plot of the mean scores ± SE on PC1 and PC2 showed a clean division between adults and chicks (Fig. 2.4b). PC1 and PC2 were both significant, but using Tukey's multiple pair-wise comparisons, only PC1 was able to distinguish differences between adults and chicks in 2004 and differences between chicks in both years ($p < 0.0001$, ANOVA). Nevertheless,

differences between adults and chicks in 2003 and differences between adults in both years were not significant.

Results of the discriminant analysis demonstrated the strong significance of overall differences among the 16 FAs between age classes (Fig. 5b). The first discriminant function accounted for 100% of the variation ($p < 0.001\%$): 100% of original grouped case and 96.0% of cross-validated individuals were correctly classified as adults or chicks.

2.5 Discussion

Differences in FA signatures of adult fulmars at the three locations within years and between seasons at individual colonies supported our predictions that 1) fulmar diets would differ between colonies; 2) diets would differ between years within colonies, and 3) diets would differ between seasons within colonies. Signatures of chicks differed significantly in 2004 from those of adults, which did not support prediction 4) that diets of adult fulmars and their chicks would be similar.

2.5.1 Differences among adults

FA profiles of fulmars varied conspicuously among the three colonies—Chowiet I. on the continental shelf of the western Gulf of Alaska was most different from St. George I. on the shelf of the eastern Bering Sea, and Chagulak I. in the Aleutian Archipelago and ecotone separating the basins of the Bering Sea and the Gulf of Alaska lay midway between. Likewise, seasonal differences were pronounced at both Chowiet I.

and St. George I. We also found inter-annual differences at Chowiet I. and St. George I., but those differences were not as great as the spatial differences between colonies and seasonal differences at Chowiet I. and St. George I.

Although FA signatures of adults on Chowiet I. during May 2003 and 2004 were statistically different, the two groups fell into the same quadrant in the discriminant analysis (Fig. 2.4a). Signatures in August 2003 and 2004 were significantly different, but also fell into the same quadrant. Furthermore, the Mahalanobis distance between group centroids in the discriminant analysis was not great for adults in August 2003 and 2004, which minimizes the biological significance of the statistical difference. The Mahalanobis distance was greater in May 2003 and 2004, indicating diets likely did differ somewhat more between springs of the two years.

Adults on St. George I. in June 2003 and 2004 had almost identical FA signatures, with only 18:2n-6 being statistically different between the two years. However, levels of 18:2n-6 in birds sampled on St. George I. in June 2003 and 2004 were just 0.63% and 0.67%, respectively, which may not be biologically significant, and the Mahalanobis distance was very short, which supports the indication that the same, or a very similar, diet was consumed by adults in June of both years.

Previous studies compiled by Hatch & Nettleship (1998) have shown seasonal and annual shifts in the diets of Northern Fulmars in the Atlantic and Pacific, and also longer-term variation in their diet. Compared to other members of the Procellariiformes, Northern Fulmars forage relatively close to their breeding colonies. During the pre-incubation and incubation stage, foraging trips can last over 4 – 5 days, and Atlantic

fulmars have been recorded to forage as far as 1000 km from their breeding colonies before egg-laying and 40-200 km during incubation. Observations on the daily activity of adults indicate that most foraging during the chick-rearing stage occurs < 100 km from the breeding colonies (Furness & Todd 1984, Hatch & Nettleship 1998). These observations suggest that because foraging ranges differ between the breeding stages, then diets may also differ between the breeding stages. This is consistent with our results that show seasonal changes in the diets of fulmars at Chowiet and St. George islands. In other words, largely overlapping foraging ranges at early stages (pre-incubation) should result in somewhat similar diets. During the chick-rearing stage the diets should be distinct, as birds forage closer to their colonies and diets should thus reflect oceanographic settings of that region. In addition, the inter-annual differences we found in the diets of adults on Chowiet I. (August 2003 versus August 2004) could be explained by the differences in chick ages and associated differences in adult foraging patterns.

Our results also compliment what is currently known about assemblages of prey species in the Western Gulf of Alaska, Bering Sea, and Aleutian Is., and about the trophic dependencies of other species of seabirds in these regions. Although diets of conspecific seabirds overlap extensively across the broad northern N. Pacific, there are still notable regional differences. For example, squids are abundant and widespread throughout the N. Pacific and are known to be consumed by fulmars and many other species of seabirds that feed at the edge of and off the continental shelf (DeGange & Sanger 1986, Sanger 1987, Hills & Fiscus 1988, Hatch & Nettleship 1998). On the other hand, whereas capelin is a common and important forage species of piscivorous seabirds in the Gulf of Alaska, it is

much less common at the Pribilof Is. and rare in the Aleutian Is. (Hunt et al. 1981a, Sanger 1983, DeGange & Sanger 1986, Sanger 1987, Springer & Byrd 1989, Hatch & Sanger 1992, Springer et al. 1996). Similarly, most piscivorous seabirds at the Pribilof Is. consume principally juvenile walleye pollock, which is the most abundant species of forage fish in that habitat on the eastern Bering Sea shelf. Pollock is common, but less so, in the Gulf of Alaska and rare in the Aleutians. Diet studies of fulmars have also shown a lower diversity of fish prey in high-arctic waters than in the low-arctic or boreal zones, and higher diversity in the north-east Pacific waters than anywhere else fulmars forage (Hatch & Nettleship 1998), which also supports our findings of spatial differences in diet between fulmars at the three colonies.

2.5.2 Differences between adults and chicks

Although we did not find significant differences between FA signatures of chicks and adults in 2003, the qualitative differences between age classes were similar to those found between chicks and adults in 2004 (Table 2). We attribute the lack of power to detect any differences between signatures in 2003 to a small sample size (n = 8 pairs), and thus the strict interpretation of these results in 2003 should be done cautiously.

FA signatures of adult fulmars on Chowiet I. differed from those of chicks, significantly so in 2004. This may be explained by: 1) adults actually feeding their chicks a different diet than they were consuming themselves, or 2) the use of essential FAs from stomach oil in Procellariiform chicks rather than adipose tissue.

The first possibility, that adults fed their chicks different prey than they consumed themselves, contradicts information compiled by Hatch & Nettleship (1998), who report that "as far as is known" the food of the young is "similar to that of adults." Furthermore, Bishop et al. (1983) found large differences between four FAs in adipose tissue of short-tailed shearwater (*Puffinus tenuirostris*) adults compared to chicks, and also that there was less variability in the FA composition of the adipose tissue in chicks than in adults. This reflected differences in their modes of existence, e.g., adult shearwaters have a higher turnover rate of adipose tissue FAs because they are continuously foraging and dietary FAs are deposited into the adipose tissue to a limited extent, while chicks accumulate large quantities of adipose tissue as an energy reserve and to fuel tissue synthesis. The authors also found variability between stomach oil and adipose tissue signatures and suggested that the digestibility of prey and the amount of time it remains in the stomach of the adult before it is fed to a chick would affect the lipid composition of the meal. That is, if the adults collected their food a considerable distance from the colony, some digestion would have occurred in the stomach prior to the chick being fed and could have affected what the adult deposited versus what was fed to the chicks. Partial digestion of food items could explain the differences in signatures even if adults fed their chicks the same prey they consumed, meaning that differences between adult and chick signatures are not biologically meaningful. On the other hand, data collected on body masses of four species of Procellariiformes returning to their breeding colonies suggested that adults use short trips almost exclusively to gather prey for their chicks, while longer trips were used to replenish energy consumed during foraging for chick

meals (Weimerskirch et al. 1994). This provides evidence that adults may have fed chicks different prey items than they consumed for themselves. Furthermore, the ages of chicks sampled in 2003 and 2004 were significantly different. Because there was less temporal overlap in fat deposition between chicks and adults in 2003 (chicks in 2004 were older), the age difference between years may have contributed to the differences between signatures of adults and chicks in 2004.

With regard to the second possibility, stomach oil is another source from which FAs can be mobilized. Storage of lipids in the proventriculus has energetic advantages for seabirds that frequently experience periods of fasting, because it reduces the need to synthesize fat reserves from assimilated FAs and later re-mobilize these stores during fasting with the associated metabolic cost (Roby et al. 1989, Roby et al. 1997). Chicks at the nest will utilize FAs from their stomach oil before utilizing FAs stored in their adipose tissue. This results in differences in FA profiles between stomach oil and adipose tissue (Chapter 1), and thus signatures from adult and chick stomach oil may be more similar than stomach oil and adipose tissue from the same bird. In fact, Hendriks et al. (2000) found that the FA profiles of crop contents of adult and chick grey-faced petrels (*Pterodroma macroptera*) were indeed almost identical, suggesting that stomach oil comparisons should be made rather than comparisons of adipose tissue signatures. However, unlike other Procellariiformes with wider foraging ranges, adult fulmars only incidentally feed their chicks stomach oil. Instead, whole or partially digested prey are more commonly passed between adults and chicks (Imber 1976, Hatch & Nettleship

1998), which suggests that comparing stomach oil between adults and chicks may not be applicable for fulmars.

In summary, FA signatures of adult Northern Fulmars varied seasonally and between the three colonies, likely reflecting changes in prey distributions and abundances between geographic regions (habitats) and over seasons, as well as possible variability in the foraging behavior and foraging ranges of adults during different stages of the breeding season. Inter-annual variation in signatures was much less prominent. Life history patterns of fulmars during the breeding season support our findings of seasonal variation in the diets of fulmars on Chowiet I. and St. George I. in 2003 and 2004. Differences in fatty acid profiles we found between adults and chicks on Chowiet I. in August 2004, but no differences in 2003, could be explained by several factors and requires further investigation.

It will be important to build a library of FA signatures of prey for fulmars to use to estimate diets quantitatively with QFASA (Iverson et al. 2004), and also to evaluate the spatial and temporal variability of prey to be used in this model. Further studies should also examine the signatures from fresh regurgitations of adults and compare them to the stomach oil and adipose tissue signatures of chicks, which will aid in the understanding of how signatures change from one lipid source to another.

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Table 2.1. Mean values for fatty acid composition of adult adipose tissue.

	Chowiet Island			
	May 2003 (n = 30)	Aug 2003 (n = 27)	May 2004 (n = 25)	Aug 2004 (n = 31)
Saturated	(11.03 ± 2.387)	(13.79 ± 5.204)	(10.17 ± 2.520)	(15.09 ± 3.983)
14:0*	1.83 ± 0.434[b,c]	1.94 ± 0.836[c]	1.73 ± 0.451[b,c]	2.52 ± 0.630[c,d]
16:0*	5.81 ± 1.663[b]	7.55 ± 3.676[b,c]	5.40 ± 1.743[b]	7.32 ± 2.574[c]
17:0	0.31 ± 0.185	0.51 ± 0.226	0.16 ± 0.063	0.43 ± 0.205
18:0*	2.24 ± 0.460	2.90 ± 0.853	2.03 ± 0.319	3.67± 0.991
20:0	0.36 ± 0.076	0.37 ± 0.113	0.33 ± 0.087	0.40 ± 0.084
Monounsaturated	(78.14 ± 5.127)	(70.02 ± 10.11)	(83.16 ± 3.248)	(66.96 ± 7.514)
16:1n-11	0.31 ± 0.129	0.38 ± 0.221	0.17 ± 0.061	0.70 ± 0.342
16:1n-7*	1.88 ± 0.899[a,b]	2.31 ± 1.560[b,c]	1.94 ± 1.018[a,b]	2.11 ± 0.858[b]
18:1n-11	0.77 ± 0.259	0.65 ± 0.344	0.60 ± 0.161	0.56 ± 0.187
18:1n-9*	12.62 ± 3.816[a,c]	11.91 ± 5.615[d,e]	13.19 ± 2.937[a]	9.05 ± 5.436[b]
18:1n-7*	1.55 ± 0.650[a,b]	2.69 ± 1.618[a,c]	1.41 ± 0.354[a,b]	1.88 ± 0.985[b]
18:1n-5	0.42 ± 0.104	0.34 ± 0.132	0.48 ± 0.155	0.58 ± 0.196
20:1n-11*	18.91 ± 3.388[a,b]	14.95 ± 4.869[a]	23.63 ± 3.337[b]	18.42 ± 4.384[a]
20:1n-9*	6.96 ± 0.670[b]	5.26 ± 1.907[c]	6.51 ± 0.248[b]	4.03 ± 0.828[a]
20:1n-7	0.55 ± 0.097	0.65 ± 0.202	0.42 ± 0.062	0.73 ± 0.179
22:1n-11*	30.32 ± 5.362[b,c]	27.34 ± 10.753[a,b]	31.34 ± 4.017[b]	26.52 ± 7.478[a]
22:1n-9*	2.76 ± 0.412[d]	2.50 ± 1.000[c,d]	2.43 ± 0.264[d]	1.48 ± 0.411[a,b]
22:1n-7	0.41 ± 0.069	0.42 ± 0.324	0.40 ± 0.055	0.31 ± 0.072
Polyunsaturated	(10.71 ± 3.029)	(16.10 ± 5.466)	(6.63 ± 1.079)	(17.89 ± 4.594)
16:2n-4	0.34 ± 0.082	0.27 ± 0.184	0.06 ± 0.028	0.32 ± 0.118
16:3n-6	0.08 ± 0.048	0.14 ± 0.113	0.39 ± 0.052	0.077 ± 0.063
16:4n-1	0.04 ± 0.044	0.07 ± 0.085	0.01 ± 0.006	0.03 ± 0.044
18:2n-6*	1.01 ± 0.127[a]	0.85 ± 0.183[b,c]	0.97 ± 0.14[a]	1.04 ± 0.278[b,c]
18:3n-3*	0.26 ± 0.094[a,b]	0.33 ± 0.148[a,b]	0.19 ± 0.081[a,b]	0.42 ± 0.132[a,b]
18:4n-3*	0.46 ± 0.224[b]	0.76 ± 0.530[b]	0.14 ± 0.062[c]	0.89 ± 0.382[b]
20:2n-6	0.25 ± 0.053	0.28 ± 0.106	0.19 ± 0.045	0.39 ± 0.107
20:4n-6*	0.38 ± 0.076[a]	0.52 ± 0.185[a]	0.27 ± 0.037[a,c]	0.61 ± 0.268[a]
20:4n-3	0.15 ± 0.077	0.30 ± 0.159	0.07 ± 0.045	0.45 ± 0.189
20:5n-3*	1.04 ± 0.708[b]	3.02 ± 2.716[a,d]	0.21 ± 0.131[c]	2.41 ± 1.378[b,d]
21:5n-3	0.09 ± 0.046	0.22 ± 0.115	0.05 ± 0.011	0.17 ± 0.071
22:5n-3	1.15 ± 0.494[a]	1.66 ± 0.682[b,c]	0.43 ± 0.201[e]	2.55 ± 0.760[a,b]
22:6n-3*	2.85 ± 1.522[b,d]	4.92 ± 2.121[a,b]	1.16 ± 0.487[c]	5.68 ± 1.811[a,b]
24:1n-9	1.34 ± 0.303	1.29 ± 0.519	1.41 ± 0.283	1.10 ± 0.349

Table 2.1. continued

	St. George Island			
	June 2003 (n = 29)	Aug 2003 (n = 9)	June 2004 (n = 30)	Aug 2004 (n = 26)
Saturated	(18.19 ± 2.688)	(19.59 ± 3.335)	(19.29 ± 3.091)	(16.57 ± 3.303)
14:0*	5.69 ± 1.936[a]	2.62 ± 0.938	7.38 ± 2.389[a]	3.4 ± 1.081[b,d]
16:0*	8.74 ± 2.013[a,b]	11.45 ± 2.431	8.49 ± 1.688[a]	8.97 ± 2.105[a,b]
17:0	0.21 ± 0.113	0.17 ± 0.126	0.12 ± 0.075	0.14 ± 0.048
18:0*	2.77 ± 0.842	4.48 ± 0.996	2.52 ± 0.777	3.25 ± 0.619
20:0	0.35 ± 0.083	0.27 ± 0.079	0.38 ± 0.088	0.27 ± 0.075
Monounsaturated	(67.94 ± 8.217)	(57.64 ± 7.333)	(69.61 ± 6.259)	(63.32 ± 8.506)
16:1n-11	0.28 ± 0.086	0.30 ± 0.045	0.30 ± 0.103	0.29 ± 0.093
16:1n-7*	3.08 ± 1.549[a,c]	4.82 ± 1.309	2.84 ± 0.994[a]	3.87 ± 1.320[a]
18:1n-11	1.05 ± 0.500	0.58 ± 0.314	0.66 ± 0.196	0.89 ± 0.424
18:1n-9*	8.10 ± 4.023[b,f]	19.19 ± 4.23	7.41 ± 4.193[b,e]	12.65 ± 3.317[d,e,f]
18:1n-7*	2.37 ± 1.581[a,c]	5.66 ± 1.633	1.73 ± 1.216[b,c]	3.52 ± 1.122[a]
18:1n-5	0.37 ± 0.061	0.41 ± 0.066	0.45 ± 0.076	0.49 ± 0.104
20:1n-11*	26.00 ± 9.521[b,c]	9.44 ± 4.804	29.09 ± 7.454[b]	17.67 ± 4.498[a,c]
20:1n-9*	4.09 ± 0.532[a,c]	4.48 ± 3.069	3.79 ± 0.711[a,c]	3.58 ± 0.741[a]
20:1n-7	0.52 ± 0.217	0.68 ± 0.255	0.54 ± 0.263	0.47 ± 0.186
22:1n-11*	19.99 ± 5.888[a]	10.05 ± 6.115	20.87 ± 4.450[a,c]	17.85 ± 7.655[a]
22:1n-9*	1.34 ± 0.312[a,b,c]	1.03 ± 0.439	1.22 ± 0.362[a,b,c]	1.16 ± 0.506[a]
22:1n-7	0.23 ± 0.053	0.24 ± 0.071	0.25 ± 0.073	0.23 ± 0.093
Polyunsaturated	(13.79 ± 6.679)	(22.71± 4.178)	(11.04 ± 4.662)	(20.07 ± 5.458)
16:2n-4	0.09 ± 0.041	0.18 ± 0.035	0.28 ± 0.099	0.19 ± 0.036
16:3n-6	0.29 ± 0.125	0.29 ± 0.095	0.11 ± 0.054	0.29 ± 0.124
16:4n-1	0.29 ± 0.189	0.20 ± 0.144	0.22 ± 0.123	0.25 ± 0.152
18:2n-6*	0.63 ± 0.327[d]	0.88 ± 0.053	0.67 ± 0.152[c]	0.95 ± 0.198[b,c]
18:3n-3*	0.29 ± 0.197[a]	0.37 ± 0.118	0.23 ± 0.108[a]	0.52 ± 0.222[b]
18:4n-3*	0.79 ± 0.570[b]	0.88 ± 0.551	0.86 ± 0.523[a,b]	1.88 ± 0.963[a]
20:2n-6	0.15 ± 0.060	0.24 ± 0.037	0.14 ± 0.041	0.28 ± 0.044
20:4n-6*	0.26 ± 0.113[b]	0.52 ± 0.127	0.23 ± 0.095[b]	0.33 ± 0.112[b,c]
20:4n-3	0.21 ± 0.140	0.29 ± 0.104	0.18 ± 0.103	0.45 ± 0.174
20:5n-3*	3.56 ± 2.450[a,d]	5.95 ± 2.694	1.97 ± 1.258[a,b]	4.71 ± 2.402[a]
21:5n-3	0.25 ± 0.149	0.39 ± 0.093	0.17 ± 0.010	0.33 ± 0.126
22:5n-3	1.47 ± 0.764	1.95 ± 0.559	1.35 ± 0.590	1.33 ± 0.45
22:6n-3*	3.93 ± 2.129[b,d]	8.60 ± 1.974	2.95 ± 2.112[d]	6.53 ± 2.083[a]
24:1n-9	0.42 ± 0.131	0.55 ± 0.207	0.64 ± 0.251	0.68 ± 0.384

Table 2.1. continued

	Chagulak Island July 2004 (n = 30)
Saturated	(14.53 ± 4.284)
14:0*	2.99 ± 1.14[b]
16:0*	7.98 ± 2.662[a,b]
17:0	0.09 ± 0.039
18:0*	2.70 ± 0.707
20:0	0.31 ± 0.081
Monounsaturated	(71.76 ± 8.590)
16:1n-11	0.28 ± 0.106
16:1n-7*	3.08 ± 1.493[a,c]
18:1n-11	0.51 ± 0.212
18:1n-9*	12.28 ± 4.367[c,d]
18:1n-7*	2.73 ± 1.209[a,c]
18:1n-5	0.41 ± 0.126
20:1n-11*	20.37 ± 4.630[a,b]
20:1n-9*	4.11 ± 0.763[a,c]
20:1n-7	0.47 ± 0.119
22:1n-11*	25.03 ± 9.432[a,b]
22:1n-9*	1.63 ± 0.599[b,c]
22:1n-7	0.33 ± 0.108
Polyunsaturated	(13.66 ± 4.539)
16:2n-4	0.23 ± 0.135
16:3n-6	0.21 ± 0.085
16:4n-1	0.17 ± 0.142
18:2n-6*	0.94 ± 0.191[b]
18:3n-3*	0.40 ± 0.159[b,c]
18:4n-3*	1.14 ± 0.806[a,b]
20:2n-6	0.26 ± 0.063
20:4n-6*	0.25 ± 0.049[b]
20:4n-3	0.29 ± 0.146
20:5n-3*	2.33 ± 1.63[a,b]
21:5n-3	0.21 ± 0.113
22:5n-3	1.18 ± 0.360
22:6n-3*	3.79 ± 1.714[b,d]
24:1n-9	1.11 ± 0.632

[a] Values are mean mass percent ± SD of fatty acids (31 out of 69) which averaged ≥ 0.1% among samples analyzed with totals in parentheses. *Designates the 16 FAs used in analyses. Those values for the same FAs that do not share a common superscript letter are significantly different from each other ($p < 0.0033$, birds on St. George I. in August 2003 were not included in this analysis due to small sample size).

Table 2.2. Mean values for fatty acid composition of adult and chick adipose tissue

	Chowiet August 2003 (n = 8)			
	Adults	Chicks	mean diff.	p-value
Saturated	(12.99 ± 3.506)	(17.98 ± 3.853)		
14:0*	1.81 ± 0.509	2.40 ± 0.786		
16:0*	6.77 ± 2.170	10.65 ± 2.378	3.89 ± 3.113	0.007
17:0	0.51 ± 0.190	0.37 ± 0.053		
18:0*	2.94 ± 0.917	3.63 ± 0.947	-	-
20:0	0.39 ± 0.103	0.30 ± 0.082		
Monounsaturated	(70.28 ± 8.19)	(63.72 ± 8.364)		
16:1n-11	0.50 ± 0.296	0.38 ± 0.049		
16:1n-7*	1.79 ± 0.590	3.20 ± 0.937	1.41 ± 1.113	0.022
18:1n-11	0.82 ± 0.554	0.52 ± 0.330		
18:1n-9*	10.20 ± 4.15	19.852 ± 2.633	9.65 ± 2.883	0.040
18:1n-7*	2.17 ± 0.825	3.41 ± 1.209	-	-
18:1n-5	0.39 ± 0.166	0.51 ± 0.084		
20:1n-11*	15.58 ± 3.473	13.13 ± 4.793	2.45 ± 6.389	0.225
20:1n-9*	5.38 ± 0.965	4.80 ± 0.999	0.58 ± 1.019	0.150
20:1n-7	0.70 ± 0.210	0.60 ± 0.096		
22:1n-11*	29.16 ± 7.276	14.86 ± 4.765	14.30 ± 8.503	0.019
22:1n-9*	2.60 ± 0.776	1.43 ± 0.306	-	-
22:1n-7	0.38 ± 0.105	0.23 ± 0.045		
Polyunsaturated	(16.69 ± 4.964)	(18.22 ± 5.260)		
16:2n-4	0.32 ± 0.222	0.31 ± 0.078		
16:3n-6	0.09 ± 0.037	0.17 ± 0.070		
16:4n-1	0.03 ± 0.028	0.08 ± 0.070		
18:2n-6*	0.91 ± 0.147	1.29 ± 0.084	-	-
18:3n-3*	0.34 ± 0.130	0.52 ± 0.162	-	-
18:4n-3*	0.75 ± 0.377	0.76 ± 0.547	-	-
20:2n-6	0.34 ± 0.125	0.31 ± 0.055		
20:4n-6*	0.65 ± 0.236	0.51 ± 0.199	-	-
20:4n-3	0.34 ± 0.179	0.34 ± 0.122		
20:5n-3*	2.45 ± 1.200	2.95 ± 2.276	-	-
21:5n-3	0.18 ± 0.050	0.20 ± 0.124		
22:5n-3	1.94 ± 0.882	1.54 ± 0.413		
22:6n-3*	5.42 ± 2.267	6.95 ± 2.200	1.53 ± 3.980	0.4978
24:1n-9	1.28 ± 0.335	0.72 ± 0.187		

Table 2.2. continued

	Chowiet August 2004 (n = 25)			
	Adults	Chicks	mean diff.	p-value
Saturated	(14.84 ± 4.218)	(16.12 ± 2.691)		
14:0*	2.50 ± 0.668	2.41 ± 0.488	0.09 ± 0.687	0.006
16:0*	7.12 ± 2.711	9.54 ± 1.861	2.42 ± 2.906	<.0001
17:0	0.46 ± 0.209	0.29 ± 0.051		
18:0*	3.60 ± 1.084	2.92 ± 0.535	-	-
20:0	0.39 ± 0.080	0.25 ± 0.055		
Monounsaturated	(67.42 ± 8.074)	(70.29 ± 5.579)		
16:1n-11	0.72 ± 0.351	0.38 ± 0.104		
16:1n-7*	2.08 ± 0.859	3.69 ± 0.933	1.61 ± 0.207	<.0001
18:1n-11	0.56 ± 0.188	0.59 ± 0.220		
18:1n-9*	8.54 ± 5.435	18.23 ± 3.092	9.69 ± 6.019	<.0001
18:1n-7*	1.72 ± 0.830	3.03 ± 0.960	1.31 ± 1.229	<.0001
18:1n-5	0.60 ± 0.199	0.58 ± 0.106		
20:1n-11*	18.86 ± 4.524	17.39 ± 4.094	1.46 ± 5.250	0.310
20:1n-9*	3.95 ± 0.842	5.09 ± 0.706	1.14 ± 1.005	<.0001
20:1n-7	0.73 ± 0.189	0.51 ± 0.080		
22:1n-11*	27.27 ± 7.638	18.45 ± 4.176	8.83 ± 7.884	0.144
22:1n-9*	1.48 ± 0.425	1.37 ± 0.265	0.11 ± 0.419	0.226
22:1n-7	0.31± 0.073	0.23 ± 0.034		
Polyunsaturated	(17.68 ± 4.887)	(13.52 ± 3.168)		
16:2n-4	0.32 ± 0.099	0.35 ± 0.071		
16:3n-6	0.07 ± 0.048	0.15 ± 0.073		
16:4n-1	0.02 ± 0.025	0.05 ± 0.042		
18:2n-6*	1.05 ± 0.308	1.30 ± 0.096	0.25 ± 0.324	<.0001
18:3n-3*	0.42 ± 0.138	0.53 ± 0.122	0.15 ± 0.307	<.0001
18:4n-3*	0.88 ± 0.398	0.54 ± 0.292	0.34 ± 0.394	0.010
20:2n-6	0.40 ± 0.104	0.29 ± 0.040		
20:4n-6*	0.62 ± 0.286	0.32 ± 0.13	0.30 ± 2.957	<.0001
20:4n-3	0.46 ± 0.202	0.31 ± 0.119		
20:5n-3*	2.36 ± 1.454	1.75 ± 1.052	0.61 ± 1.463	0.340
21:5n-3	0.17 ± 0.066	0.11 ± 0.063		
22:5n-3	2.53 ± 0.814	1.26 ± 0.475		
22:6n-3*	5.53 ± 1.862	4.61 ± 1.299	0.92 ± 1.874	0.6839
24:1n-9	1.11 ± 0.369	0.67 ± 1.83		

[b] Values are mean mass percent ± SD of fatty acids (31 out of 69) which averaged ≥ 0.1% among samples analyzed with totals in parentheses. *Designates the 16 FAs used in analyses in 2004, only 8 fatty acids were used for analysis of birds in 2003. P-values are shown for paired t-test using the Bonferroni adjustment to evaluate differences between adipose tissue in adults and chicks on Chowiet I. in August 2003 and 2004.

Table 2.3. Predicted group classification from discriminant analysis: Chowiet I. May and August 2003, 2004, St. George I. June 2003, 2004 and August 2004, and Chagulak I. July 2004 ($n \geq 15$); 75.9% (173 of 228) of cross-validated grouped cases, correctly classified (Wilk's $\lambda < 0.0001$, see Fig. 4a).

	Predicted group membership								
	Chowiet Is. May 2003	Chowiet Is. August 2003	Chowiet Is. May 2004	Chowiet Is. August 2004	St. George Is. June 2003	St. George Is. June 2004	St. George Is. August 2004	Chagulak Is. July 2004	Total
Chowiet Is. May 2003	27	2	1	0	0	0	0	0	30
Chowiet Is. August 2003	2	21	0	2	0	0	1	1	27
Chowiet Is. May 2004	0	0	24	0	0	0	0	1	25
Chowiet Is. August 2004	1	2	1	25	0	0	1	1	31
St. George Is. June 2003	0	0	0	0	23	4	1	1	29
St. George Is. June 2004	0	0	0	0	2	22	5	1	30
St. George Is. August 2004	0	0	1	2	0	0	17	6	26
Chagulak Is. July 2004	1	3	1	0	0	0	11	14	30
Total	31	28	28	29	25	26	36	25	228

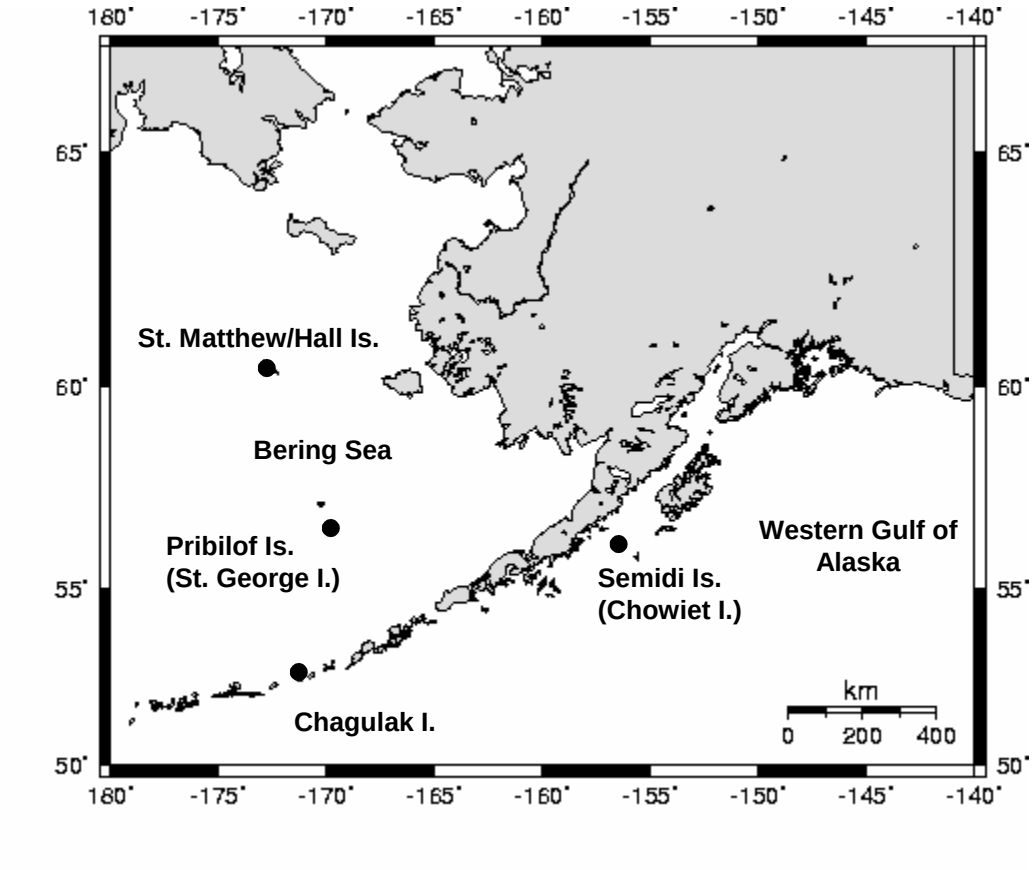

Figure 2.1. Locations of the four major fulmar colonies in Alaska. 1) Chowiet Island (56˚05' N, 156˚45' W) in the Semidi Islands group in the western Gulf of Alaska; 2) Chagulak Island (52˚35' N, 171˚10' W) in the eastern Aleutian Islands; 3) St. George Island (56˚35' N, 170˚35' W) in the Pribilof Islands group in the eastern Bering Sea; and 4) Hall Island (60˚40' N, 173˚10' W), adjacent to St. Matthew Island in the northern Bering Sea. Studies were not undertaken at Hall I.

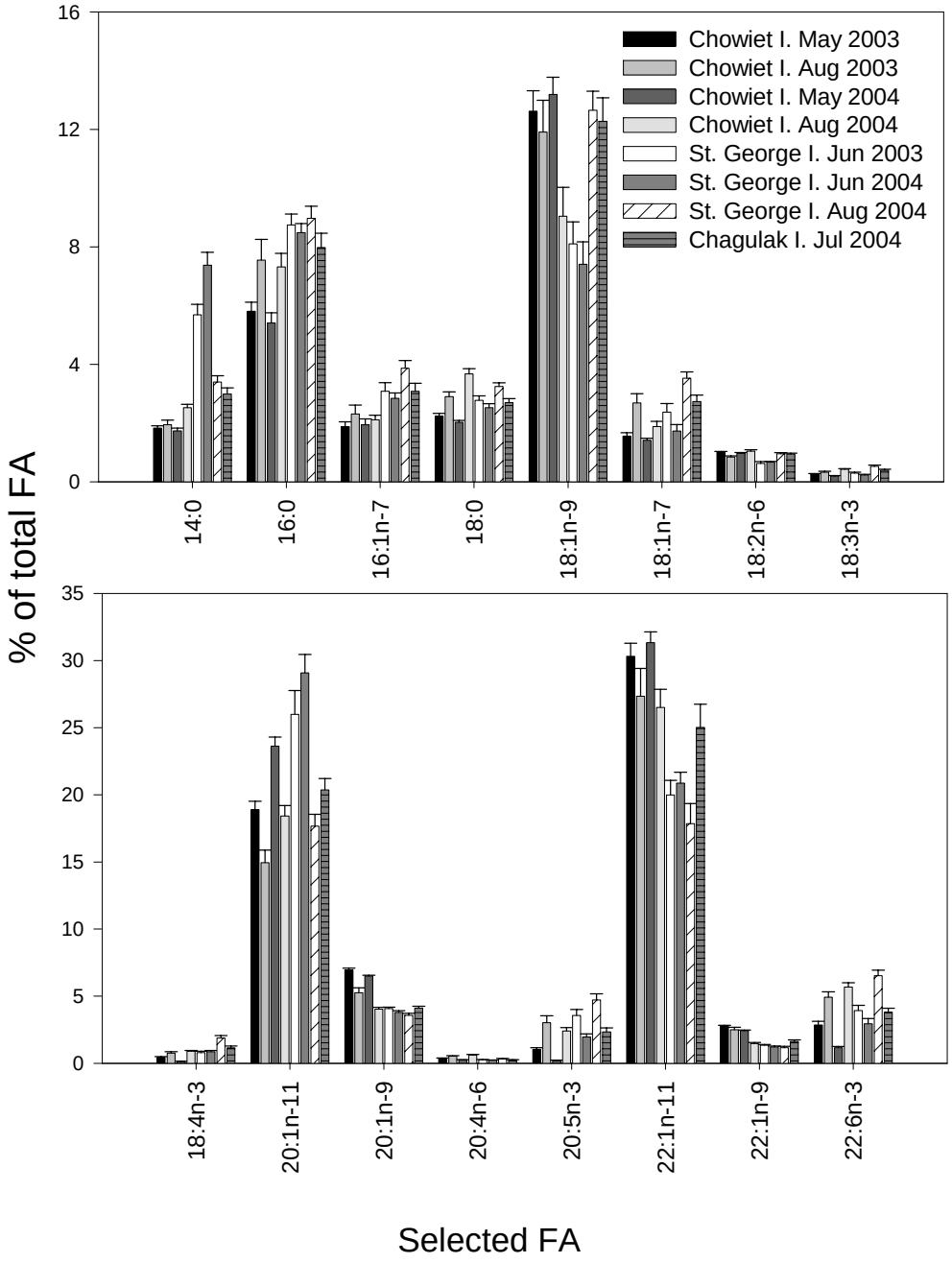

Figure 2.2. Mean values of fatty acids in adult fulmars. The 16 FAs with the greatest overall variance in Northern Fulmar adipose tissue, including reference fatty acid 18:0, illustrating differences between samples. Bars are means and vertical lines are 1 SE. Adults on Chowiet I. during May 2003 (n = 30) and 2004 (n = 27), and August 2003 (n = 25) and 2004 (n = 31); St. George I. during June 2003 (n = 29) and 2004 (n = 30), and August 2004 (n = 26); and Chagulak I. during July 2004 (n = 30).

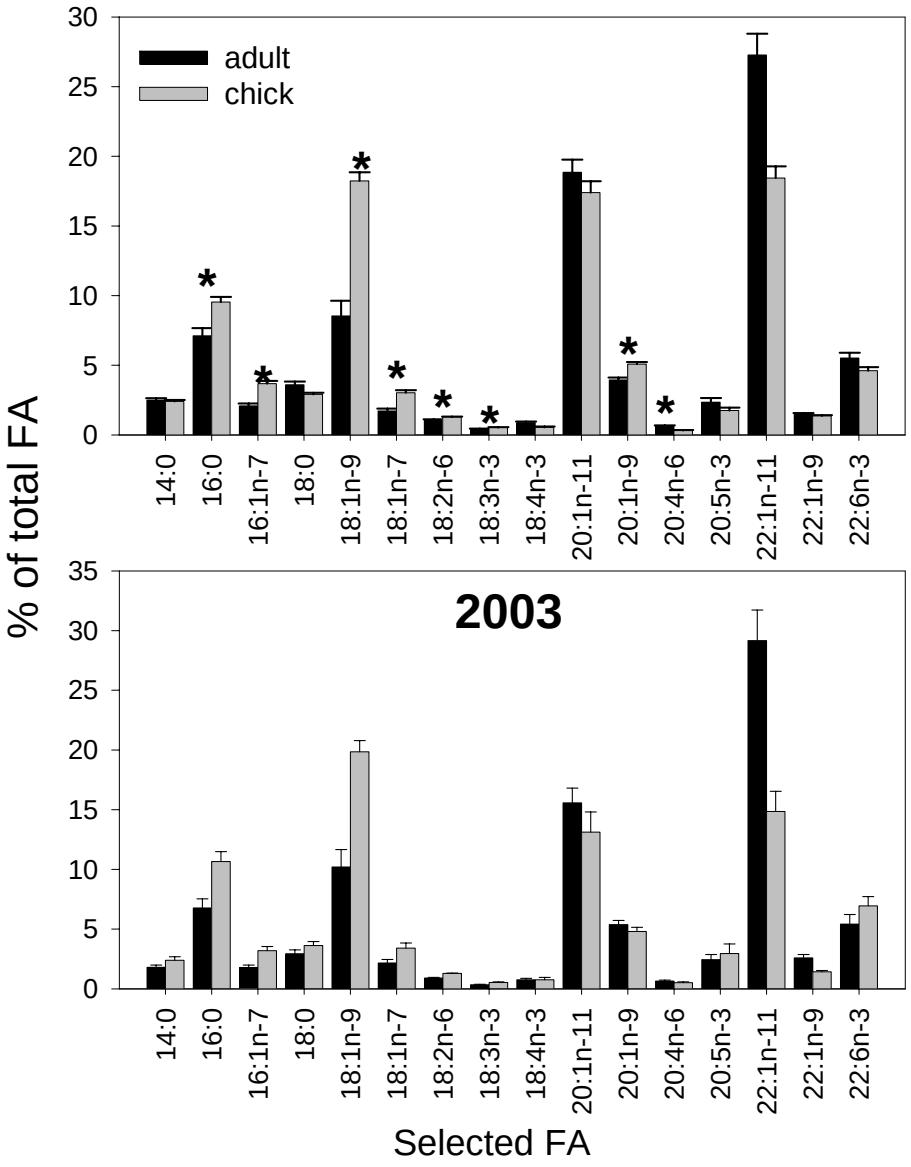

Figure 2.3. Mean values of fatty acids in adult-chick pairs. The 16 FAs with the greatest overall variance in Northern Fulmar adipose tissue, including reference fatty acid 18:0, illustrating differences between samples. Bars are means and vertical lines are 1 SE. Adults and chicks on Chowiet I. during August 2003 (n = 8 pairs) and 2004 (n = 25 pairs). We found significant differences between eight fatty acids in 2004 ($p < 0.0033$, paired t-test) indicated by * above bars.

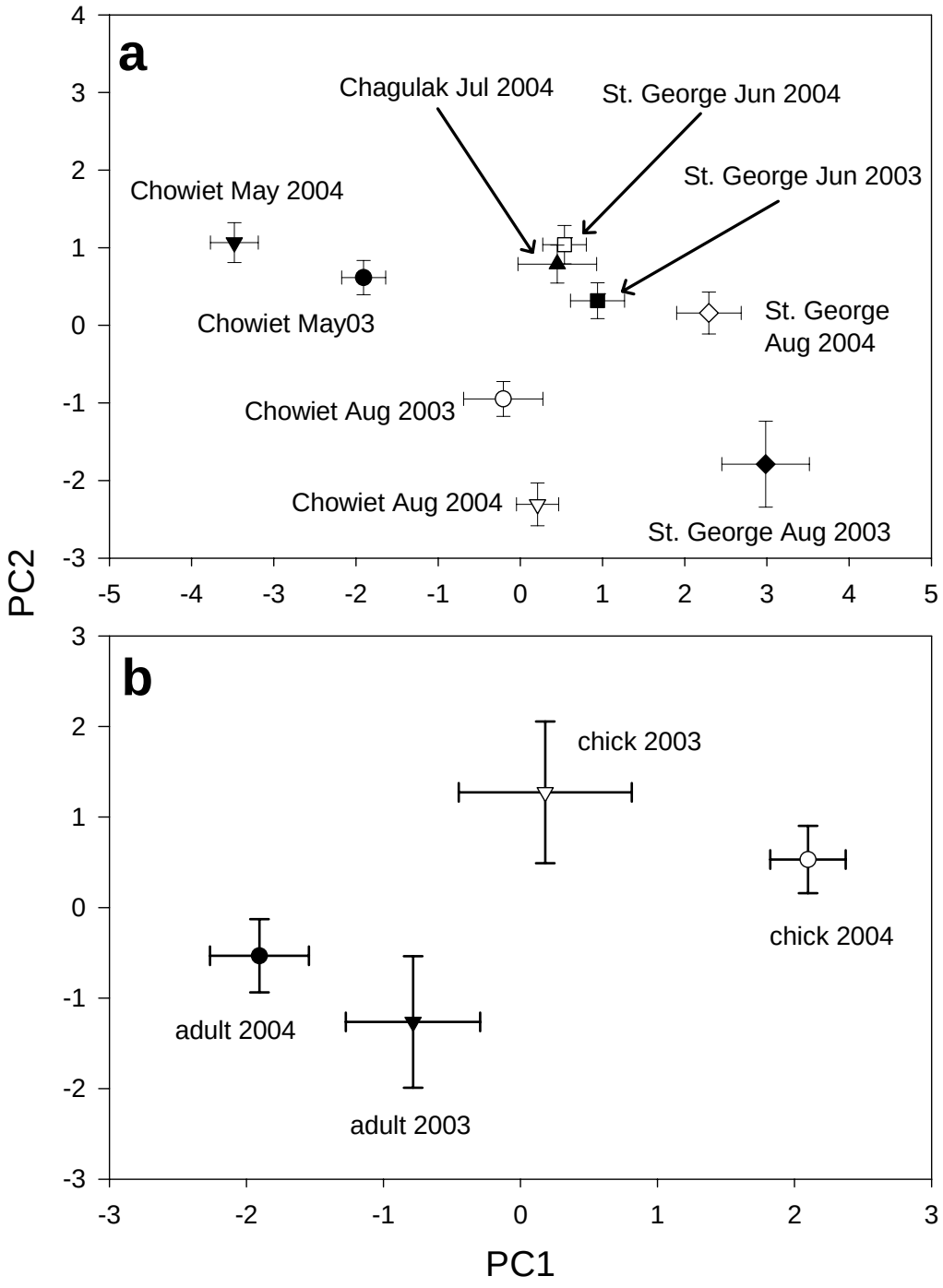

Figure 2.4. (a) First and second principle components for adult fulmars. (b) First and second principle components for adult-chick pairs. Values are means ± SE.

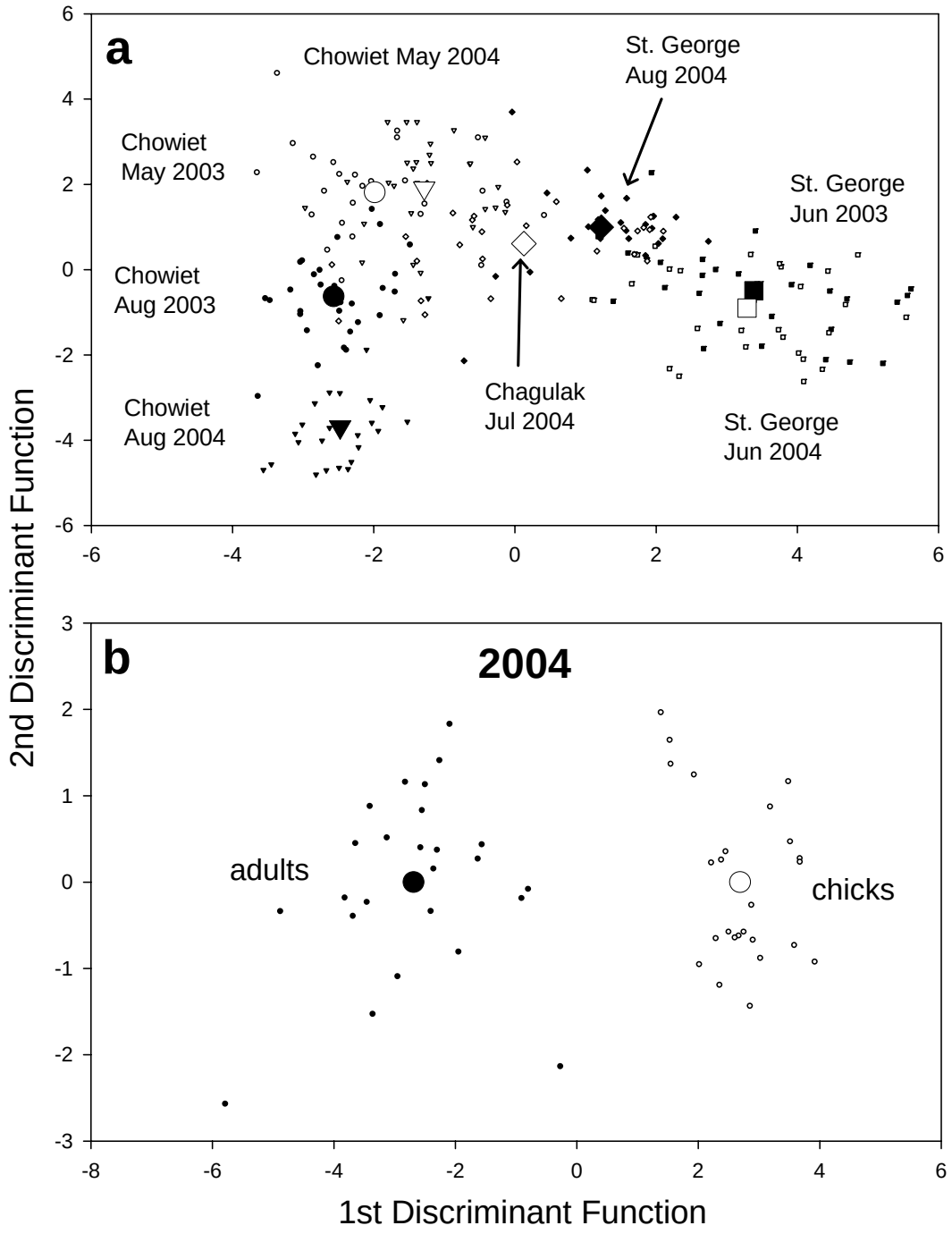

Figure 2.5. (a) First and second discriminant functions for adult fulmars. (b) First and second discriminant functions for adult-chick pairs. Values are means and individual observations.

Conclusions

I compared FA signatures between stomach oil and adipose tissue of Northern Fulmars, and assessed spatial (between colonies), temporal (seasonal and inter-annual), and age class (adult-chick) variability in their diets. I found that FA signatures of adipose tissue were significantly different than those of stomach oil, which supported prediction 1, that signatures of adipose tissue and stomach oils would differ because of the differing time scale each depot reflects and/or because adipose tissue FAs may be influenced by predator metabolism, while stomach oil FAs may be influenced by differential uptake. There were conspicuous spatial and temporal differences in adipose tissue signatures, which supported prediction 2, fulmar diets would differ between colonies located in distinct oceanographic settings and prediction 3, that diets would differ temporally within colonies because of potential interannual variability in the physical environment resulting in temporal shifts in FA signatures of prey species. Adipose tissue signatures in chicks differed significantly from those of adults in 2004, and similar patterns existed in 2003, contrary to prediction 4, that diets of adult fulmars and their chicks would be similar because they feed by regurgitation.

FA composition of both stomach oil and adipose tissue have the potential of being extremely informative, with stomach oil providing information on the most recent meals and adipose tissue revealing a diet integrated over a longer period of time. Both time scales are of interest to biologists. The results of my research have shown that there are differences between stomach oil and adipose tissue signatures, but the biological significance of these differences needs to be evaluated. My results also showed that FA

signatures of adult Northern Fulmars varied seasonally and at the three colonies located in distinct oceanographic settings in Alaska, likely reflecting changes in prey distributions and abundances both between geographic regions and over seasons.

Although I did not find significant differences between chicks and adults in 2003, the differences in the levels of individual FAs between adults and chicks were similar to those found in 2004. I attribute the lack of power to detect any differences between signatures in 2003 to a small sample size (n = 8 pairs). The simplest explanation for the differences in FA profiles between adults and chicks on Chowiet I. in 2004, and likely 2003, is that adults were feeding their chicks a different diet than they were consuming themselves. However, other factors which may explain these differences, such as the preferential use of essential FA from stomach oil in chicks, require further investigation.

In the absence of known prey signatures for reference, the interpretation of FA data is subjective and qualitative. Nonetheless, by using multivariate statistical tools, such as principle components and discriminant analysis, the relative differences between signatures can be assessed. Such use of FA signatures to evaluate diet qualitatively raises the question: how many FAs must differ from one signature to another to conclude that the implied differences in diet are biologically significant? This question can be answered by quantitatively assessing the diet of predators using QFASA. My post-thesis research will include estimations of the relative proportions of prey in the diets of fulmars using QFASA. The results from a QFASA model will also help to interpret the significance of differences in signatures by addressing the issue posed above regarding statistical difference versus biological significance.

In addition to modeling the diets of fulmars using adipose tissue alone, I will also model the diets using stomach oil FA signatures. This will allow me to determine how the differences in signatures between the two lipid sources affect an estimate of diet. If the results of models are comparable, then the implications would be that stomach oil and adipose tissue FAs represent the same diet, and the less invasive method of using stomach oil would provide sufficient information to characterize a fulmar's diet composition. Because stomach oil FAs in essence are an intermediate between prey and adipose tissue FAs, it is feasible that the differences between stomach oil and adipose tissue FA signatures may be used to calculate calibration coefficients for fulmars in lieu of a captive study, and at the very least reveal metabolic information of individual FAs in fulmars that may be applied to other seabirds in future studies.

A short-coming of this study was the unbalanced sampling design available for use in Chapter 1. Because samples were collected opportunistically and fulmars do not always eject stomach oil when handled, I lacked the number of samples from some colonies and age classes needed to assess potentially important interactions. Differences in signatures between the two lipid sources could be explained by either a difference in diet or by patterns of FA mobilization from stomach oil and adipose tissue. Differences between adult and chick signatures, in particular, may result from the use of essential FAs in the stomach oil of chicks rather than from the adipose tissue. To address these issues and determine the rates of FA mobilization from stomach oil, a captive study on adults and chicks is needed. By feeding captive birds known diets (homogenous and mixed), and by using radioisotopes, the fate of fatty acids in food and stomach oil can be

determined (e.g. Budge et al. 2004, Roby et al. 1989). To accurately estimate the diets of wild fulmars, we need to understand FA metabolism, including transitions that occur between food, stomach oil, and adipose tissue FAs.

Additional studies are also needed to address the impact of commercial fisheries on fulmars in Alaska. For example, we should know the amount of offal consumed by fulmars in relation to naturally available prey, and how populations and breeding success respond to the exploitation of commercial byproducts.

From previous studies we know that in an ecosystem, FA signatures of prey are not constant because of variability between age classes, seasons, and geography: nevertheless, variability among species is expected to be much higher than variability within species (Iverson et al. 1997b, Iverson et al. 2002, Budge et al. 2002). However, we do not know how much within-species variability exists over time, or between ecologically distinct regions such as the Bering Sea and the Gulf of Alaska. Currently, the assumption is that prey species in the Gulf of Alaska exhibit the same FA signature as the same species in the Bering Sea and the North Pacific, and there is no significant seasonal change in FA signatures in a particular prey species. However, if there is significant spatial, seasonal, and/or inter-annual variability in prey, a new prey library will need to be built every season and every year from areas of concern, both of which are expensive and time consuming. These issues of temporal and spatial variability in FA composition of forage species are currently being addressed in Dr. Sara Iverson's laboratory at Dalhousie University.

In another ongoing study (Regime Forcing and Ecosystem Reponse [ReFER]), a protocol was developed for using QFASA to estimate the diets of four seabird species on St. Paul I., St. George I., and Bogoslof I. in Alaska (Iverson & Springer 2002). Results from my research, including post-thesis work, will complement the ReFER project by further revealing differences and similarities in diets of seabirds in Alaska. I hope to reveal differences in seabird FA metabolism by comparing calibration coefficients among the different seabird species, and to possibly use fulmar calibration coefficients on closely related species such as storm-petrels.

In conclusion, I found that the diets of Northern Fulmars agreed with most of my predictions, but further work is desirable to thoroughly validate these results. I have shown that FA signature analysis is a powerful tool for characterizing and comparing foraging patterns of seabirds. This approach to diet analysis, including QFASA, can significantly increase our understanding of foraging ecology, diet composition, and predator-prey relationships of fulmars and other marine birds, all of which yield measures of the impact of environmental variation on the birds and their ecosystem.

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