AN ABSTRACT OF THE THESIS OF

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Title: Evaluating the Fatty Acid Signature Technique for Studies of Diet Composition in Piscivorous Waterbirds.

Abstract approved:

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Daniel D. Roby

This research was designed to evaluate the Fatty Acid Signature (FAS) technique as a non-lethal alternative to more traditional, and sometimes destructive, methods of studying the diet composition of piscivorous birds. Specifically we tested the technique with Caspian terns (*Hydroprogne caspia*) which currently nest in large numbers in the Columbia River estuary and are known to consume juvenile salmonids (*Oncorhynchus spp.*) listed under the U.S. Endangered Species Act.

From captive feeding trials conducted with Caspian tern chicks, we determined that FASs of the birds reflected differences in their diets. After 20 days of being fed consistently mixed or monotypic diets of two fish types, chicks displayed different adipose tissue FASs between all 4 diet treatments. When diets were changed, adipose tissue FASs reflected the shift in diet treatments within two weeks. Fatty acid (FA)-specific calibration coefficients (FA level in the consumer divided by FA level in the food) were calculated for Caspian terns fed monotypic diets for 34 days; some calibration coefficients varied in association with diet and age of the terns, and also differed between terns and common murres (*Uria aalge*), whose calibration coefficients were measured in

a separate study. Variation in FA-specific calibration coefficients may be problematic for obtaining accurate estimates of diet composition in piscivorous birds using the Quantitative Fatty Acid Signature (QFASA) technique. We advocate sensitivity analysis to test whether the QFASA models are robust to the magnitude of variation in calibration coefficients detected in this study.

FASs differed among the 3 major fish prey types observed in diets of Caspian terns nesting in the Columbia River estuary during the 2003 breeding season: juvenile salmonids, surf smelt (*Hypomesus pretiosus*), and northern anchovy (*Engraulis mordax*). We detected differences in FASs of nesting Caspian terns between early and late in the nesting season of 2003; these differences were associated with a shift in diet compostion from a diet dominated by juvenile salmonids to a diet dominated by northern anchovy. The FASs of several species of juvenile salmonids, however, exhibited little inter-specific variation, especially between species raised in hatcheries, which comprise the majority of smolts consumed by Caspian terns in the estuary. We found levels of highly-unsaturated FAs (HUFAs) to be higher in wild steelhead smolts than in hatchery-reared steelhead smolts, but HUFA levels in terns did not reflect the changing prevalence of wild steelhead in their diets. This is likely due to contribution of HUFAs to the diets of terns from marine forage fishes. Thus, HUFAs do not appear to be useful indicators of wild steelhead in the diets of these birds.

If the QFASA technique can be validated, it has the potential to provide general information on diet composition for piscivorous birds foraging on broadly different prey types over extended periods. However, due to similarities in FASs of key prey types consumed by Caspian terns in the Columbia River estuary, namely salmonids, obtaining

the precise estimates of diet composition and consumption of different species of salmonids that are requested by resource managers does not seem feasible using the QFASA technique alone. Consequently, more traditional methods of diet composition analysis for Caspian terns (bill load identification, stomach contents analysis) can not be replaced by the FAS analysis technique.

Evaluating the Fatty Acid Signature Technique for Studies of Diet Composition in Piscivorous Waterbirds

by
Anne Mary Myers

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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

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Anne Mary Myers, Author

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Chapter 1

GENERAL INTRODUCTION

Anne Mary Myers

Seabird Diet Studies

Seabird diets can provide valuable information about the status and health of marine ecosystems, including abiotic factors that influence ocean conditions (Baird 1990) and the relative availability of forage fishes to seabirds (Hatch and Sanger 1992; Regehr and Montevecchi 1997) and other upper trophic level marine consumers. Prey availability for colonial nesting seabirds affects foraging trip duration, colony attendance, reproductive success, and, ultimately, demography of seabird populations (Cairns 1987). Measuring the composition of seabird diets can enhance the understanding of marine trophic structure at both local and ecosystem scales, as well as aid in monitoring relative abundance of primary and secondary consumers, including fisheries stocks (Cairns 1992; Diamond and Devlin 2003). Studying and quantifying seabird diets can also provide resource managers with information about food availability for threatened and endangered seabirds (Anderson et al. 1982; Safina et al. 1990), as well impacts to threatened and endangered seabird prey, facilitating informed resource management decisions (Collis et al. 2002; Roby et al. 2003).

Traditional studies of seabird diets involve identifying soft-tissue and diagnostic hard parts from stomach contents, regurgitations, and castings (Gaston and Noble 1985; Springer et al. 1986; Springer et al. 1996; Collis et al. 2002; Roby et al. 2002; Roby et al. 2003). These methods provide valuable information about diet composition, but certain biases are unavoidable, including representation of only the most recent meal(s) consumed and under-representation of prey with easily digested diagnostic parts (Jobling and Breiby 1986; Springer et al. 1996). Additionally, identification of stomach contents often requires lethal sampling of the predator, an unacceptable cost for threatened and

endangered predators, and a collection procedure that is ethically unacceptable to an increasing proportion of the public. Stomachs of collected predators are sometimes empty, needlessly sacrificing subjects without obtaining diet information.

Newer, non-lethal techniques have been developed to assess diet, such as analysis of the ratios of stable isotopes of carbon and nitrogen in muscle tissue (Rau et al. 1992; Gannes et al. 1997; Kelly 2000). Stable isotope ratios can provide information about the trophic level of predators, general foraging location (nearshore vs. offshore, benthic vs. pelagic), and habitat of prey (freshwater vs. marine), but can not provide detailed information on diet composition, such as species composition of prey (Hobson 1993; Sydeman et al. 1997; Nisbet et al. 2002).

In order to evaluate a non-lethal method for investigating seabird diets that might circumvent some of the problems of more traditional diet studies, I used the fatty acid signature (FAS) technique (Iverson 1993) to study Caspian tern diets. The diet composition of this seabird is of particular interest to resource managers in the Pacific Northwest, due to its known reliance on stocks of threatened and endangered juvenile salmonids (Collis et al. 2002; Roby et al. 2002). I compared the fatty acid signatures (FASs) of captively-reared Caspian tern chicks with those of their diets. I also compared FASs of wild adult Caspian terns with those of known prey types as determined by visual observations of bill-loads and stomach content analysis of soft-tissue and hard parts at the East Sand Island, OR breeding colony (CBR 2003).

Fatty Acid Signature Analysis

A non-lethal technique recently employed for diet analysis of marine predators involves comparing fatty acid profiles of predator and prey (Iverson 1993; Iverson and Springer 2002; Iverson et al. 2004). Fatty acids are the primary constituent of most lipids. In marine organisms, fatty acids are most commonly comprised of carbon chains 14 to 24 atoms long, and either saturated (containing no double-bonds) or unsaturated (containing one to six double-bonds). Fatty acids in marine organisms are very diverse, with high levels of polyunsaturated fatty acids that originate from unicellular phytoplankton and seaweeds (Ackman 1980).

Fatty acids are classified using nomenclature developed by the International Union of Pure and Applied Chemistry (IUPAC). For the fatty acid designated 22:6n-3, for example, the first number represents the number of carbon atoms in the chain, the second number is the number of double bonds between carbon atoms, and the last is the position of the first double bond closest to the terminal methyl carbon (Gurr and James 1991). The quantitative profile of all fatty acids in fat reserves is referred to as the fatty acid signature (FAS) of the organism (Iverson 1993). Fatty acids with carbon chains greater than 13 remain structurally intact and effectively unchanged throughout digestion, absorption, and transport via the bloodstream in monogastric animals and are presumably deposited in adipose tissue in a predictable way, allowing some interpretation of diet (Lee et al. 1971; Fraser et al. 1989; Iverson et al. 1995; Kirsch et al. 1998; Kirsch et al. 2000). Once assimilated, fatty acids are either metabolized for energy or re-esterified, primarily into triacylglycerols, and stored in adipose tissue (Iverson et al. 2004). Because animals

can biosynthesize relatively few fatty acids (Cook 1991), it is possible to determine which fatty acids in a predator must have originated from the diet (Iverson et al. 2004).

The analysis of fatty acids has been used to study trophic level and spatial and temporal patterns in foraging behavior of free-ranging marine mammals and seabirds (Iverson 1993; Iverson et al. 1997; Raclot et al. 1998; Iverson and Springer 2002; Dahl et al. 2003). FASs have been used to verify the presence of fish and other prey types in the diet of mammals, such as mink (*Mustela vison*) (Rouvinen and Kiiskinen 1989; Wamberg et al. 1992), polar bears (*Ursus maritimus*) (Colby et al. 1993), arctic foxes (*Alopex lagopus*) (Pond et al. 1995), as well as penguins (Sphenisciformes) (Johnson and West 1973; Raclot et al. 1998). Fatty acid signatures have also been used to study changes in the diet of pinnipeds (Iverson 1993; Iverson et al. 1997; Kirsch et al. 2000). Additionally, the composition of fatty acids found in certain fishes and invertebrates has been used to accurately identify these organisms to the species level (Iverson et al. 1997; Budge et al. 2002; Iverson and Springer 2002). Because some of these fishes and invertebrates are common prey for a host of seabirds and marine mammals, it is possible that quantitative information about the diet composition of these predators may be inferred from their FASs (Iverson et al. 2004).

Some differential metabolism of assimilated dietary fatty acids occurs, however, and predator fatty acid composition does not match that of the prey (Kirsch et al. 1998; Iverson et al. 2004). In order to infer taxonomic composition of the diet from predator FASs, Iverson et al. (2004) developed the concept of fatty acid-specific "calibration coefficients." Calibration is needed to account for differential metabolism, deposition, and biosynthesis of fatty acids by predators, which causes levels of certain fatty acids to

be higher or lower compared with availability in the diet. Calibration coefficients are used to account for differential lipid metabolism or storage by predators in statistical models for predicting diet from FASs, as in Quantitative Fatty Acid Signature Analysis (QFASA) (Iverson et al. 2004). Quantitative Fatty Acid Signature Analysis models compare the FASs of predator adipose tissue to the quantitative mixture of FASs from various prey types in order to infer the proportional contribution of each prey type to the diet of the predator (Iverson et al. 2004). Models are utilized because patterns in predator FASs are generally difficult to interpret superficially, especially when the number of prey choices is large, when significant within-species variability in FASs of prey or predator exist, and when aspects of differential FAS metabolism in a predator must be considered (Iverson et al. 2004).

Applying the Fatty Acid Signature Technique and QFASA

Quantitative Fatty Acid Signature Analysis (QFASA) is a challenging and complex analysis based on various assumptions. It is important to anticipate the potential constraints for the technique and understand that it may not be appropriate for all types of quantitative diet studies. First, variation exists in FASs among individual prey owing to variation in prey size, time of sampling, and prey diet. Second, prey types must be reliably distinguishable from one another based on their FASs for the method to be effective. Whether this technique may be applied to diet studies of wild breeding seabirds depends on the variability both within and among prey types, the temporal shift in FASs during the breeding season, and the efficacy of the sampling protocol in capturing this variability. Third, there is intraspecific and interspecific variability in lipid

content of prey. Prey with a higher fat content will contribute proportionally more to a predator's FAS than a prey item with a lower fat content (Iverson et al. 2004). Fat content of forage fishes may vary greatly, both within and among species (Anthony et al. 2000); intraspecific variation is most pronounced seasonally (Iverson and Springer 2002), but also varies with reproductive status, size, and location (Anthony et al. 2000).

In addition to factors influencing variability in FASs of prey, there are also factors affecting FASs of predators. Individual predators may display temporal variation in preferential metabolism or deposition of certain fatty acids in adipose tissue. This might relate to longitudinal variation in diet, nutritional status, or metabolic rate. Also, it is important to understand the role of biosynthesized fatty acids and how they may complicate the interpretation of dietary FASs. Though dietary fatty acids with ≥14 carbon chain lengths can be incorporated into adipose stores with little modification to their structure, providing information about dietary sources, the degree of accumulation of individual fatty acids (FAs) in adipose stores of birds can be augmented due to biosynthesis from other dietary components (proteins, carbohydrates), or reduced through selective metabolism of certain FAs, compared with levels in the prey (Klasing 1998; Iverson and Springer 2002; Iverson et al. 2004). Another potential confounding factor for the fatty acid signature technique is determining the length of time that a FAS from adipose tissue of a predator integrates dietary FASs of prey. This could depend on whether the individual is rapidly fattening or fasting, and the particular species studied (Iverson et al. 2004). It is not clear how long dietary FASs will persist in a predator after a switch or change in diet composition (Iverson et al. 2004). A concern with modeling the diets of predators using QFASA is whether the technique can detect prey that are a

small proportion of or are rare occurrences in the diets of predators. Prey types that contribute a small proportion of total lipids in the diet will generally not be detected by QFASA but may still make up a significant proportion of the total number of prey consumed (Iverson et al. 2004).

An important consideration is how, where, and when fatty acids should be sampled from a predator to get a true and unbiased whole–body FAS because not all adipose tissues behave in the same way. Adipose tissue is dynamic; adipocytes store or mobilize triacylgylcerols, depending on energy balance in the organism. Only those sites that represent the most metabolically active fat stores should be sampled for analyses of FASs, such as the large adipose depots used primarily for lipid storage (Iverson et al. 2004). In seabirds, the assumption has been that many lipid storage depots do not differ in their FAS. Iverson and Springer (2002) found that mesenteric, breast, and subcutaneous synsacral fat sampled from individual red-legged kittiwakes (*Rissa brevirostris*), black-legged kittiwakes (*R. tridactyla*), common murres (*Uria aalge*), and thick-billed murres (*U. lomvia*) were similar within an individual. Since then, studies have used synsacral subcutaneous adipose tissue to measure representative FASs of seabirds (Iverson and Springer 2002, Wang 2005).

Study Species and Management Implications

The East Sand Island Caspian tern (*Hydroprogne caspia*) colony is the largest known colony for this species in the world (Cuthbert and Wires 1999; Suryan et al. 2004), consisting of about 8,325 breeding pairs during the 2003 breeding season (CBR 2003). The East Sand Island colony comprises approximately 25% of the total North

American population for the species (Wires and Cuthbert 2000). Caspian terns nesting in the Columbia River estuary are known to consume large numbers of juvenile salmonids (*Oncorhynchus* spp.) (Collis et al. 2002; Roby et al. 2002; Roby et al. 2003). Thirteen of the 20 evolutionarily significant units (ESUs) of salmonid from the Columbia River basin are listed as either threatened or endangered under the U.S. Endangered Species Act (NMFS 2002). In 1998, the estimated number of salmonid smolts consumed by Caspian terns nesting on Rice Island in the Columbia River estuary was between 9.1 and 15.7 million, or about 10-15% of all smolts reaching the estuary during out-migration (Roby et al. 2003). In an effort to reduce predation on juvenile salmonids by Caspian terns, wildlife managers relocated the Rice Island Caspian tern colony to an island closer to the ocean, East Sand Island, during the 1999-2001 breeding seasons. This move reduced the proportion of juvenile salmonids in the diet of Caspian terns to about half of what it was on Rice Island (Roby et al. 2002), due primarily to the increased availability of marine forage fish in proximity to East Sand Island. Despite decreased reliance on juvenile salmonids by terns nesting on East Sand Island, the consumption of millions of juvenile salmonids by piscivorous waterbirds in the lower Columbia River continues to be of concern to fisheries managers and others with a stake in the recovery of listed salmonid stocks.

Caspian terns transport whole prey in their bills to their mates and young on the breeding colony, unlike many other taxa of seabirds, so it is possible to identify fish prey to the taxonomic level of family with the aid of binoculars and spotting scopes. Ongoing studies have documented the diet composition of Caspian terns nesting in the Columbia River estuary since 1997, primarily due to concern over the consumption of listed

salmonids (Collis et al. 2002; Roby et al. 2002; Roby et al. 2003). Diet composition has been measured using a combination of (1) visual bill-load identification to obtain large samples of prey identified to the taxonomic level of family and (2) lethal collection of adult terns to obtain specimens of prey and more precisely identify bill-load fish and fish in stomach contents to the level of species.

Shifts in major fish prey types consumed by Caspian terns nesting at East Sand Island occur throughout the breeding season (Collis et al. 2002; Roby et al. 2002). For example, during the 2003 breeding season, the most prevalent prey types for Caspian terns on East Sand Island shifted from juvenile salmonids and smelts (Osmeridae) during April, May, and early June to northern anchovy (*Engraulis mordax)* and clupeids [Pacific herring (*Clupea pallasi*) and sardines (*Sardinops sagax*)] from mid-June through the end of July (CBR 2003). The extensive and reliable information collected on the diet composition of Caspian terns nesting in the Columbia River estuary throughout their breeding season presents an opportunity to test other potential nondestructive methods to assess diet composition, such as the fatty acid signature technique.

In this study, we test the utility of FAS technique to study the diets of piscivorous birds, specifically Caspian terns, both in captivity and in the wild. In chapter 2 we will determine FASs and FA-specific calibration coefficients for Caspian terns (which are necessary if diets are to be quantitatively modeled in future studies using QFASA) by conducting captive feeding trials with the birds on controlled diets. We will (1) compare the FASs of Caspian terns fed different controlled diets in captivity, (2) compare tern FASs with FASs of fish fed to terns, and (3) determine whether changes in the proportion of two fish species fed to captive terns can be detected in FASs of bird adipose tissue

after two weeks. In chapter 3 we investigate the performance of the FAS technique to study the diets of piscivorous birds in the wild. Specifically, we test whether the technique allows us to (1) distinguish FASs of fish prey species that are common in the diets of Caspian terns in the Columbia River estuary and (2) detect significant differences in adipose tissue FASs of wild Caspian terns between the early and late part of the breeding season, when the major dietary components are known to be different.

If it can be validated, the FAS method is an attractive alternative to more traditional diet studies. The FAS method can be used for dietary studies of species of concern; eliminates biases inherent in stomach contents analyses such as differential digestion of soft tissue and hard parts; and represents the integration of predator diets over time, as opposed to representing only the last meal. If the FAS technique can be used to determine fine-scale dietary changes of colonial-nesting piscivorous birds within and between breeding seasons, this could eliminate the need for lethal sampling to obtain information about the diet composition of these predators. In the Columbia River estuary, this could mean avoiding the collection of hundreds of Caspian terns and double-crested cormorants for stomach contents analysis each year in order to provide information to wildlife managers about impacts to juvenile salmonids listed under the U.S. Endangered Species Act.

CHAPTER 2

EVALUATING THE FATTY ACID SIGNATURE TECHNIQUE FOR STUDIES OF DIET COMPOSITION IN PISCIVOROUS BIRDS

Anne Mary Myers

ABSTRACT

We sought to evaluate a non-lethal method for investigating diets of piscivorous seabirds that might circumvent some of the inaccuracies, conservation issues, and ethical concerns of more traditional diet studies. We compared the fatty acid signatures of captive Caspian tern (*Hydroprogne caspia*) chicks raised on different diets of fish to those of their respective diets. We selected 2 fishes with large differences in fatty acid profiles, rainbow trout (*Oncorhynchus mykiss*) and Pacific herring (*Clupea pallasi*), for feeding trials. Chick adipose tissue sampled on day 20 of the feeding trial differed in levels of 6 of the 12 major dietary fatty acids between each of the 4 diet treatment groups. A subsequent change in diet treatment resulted in significant changes in chick fatty acid levels within 14 days. Fatty acid levels in chicks that were switched on day 20 from a mixed fish diet to a monotypic diet of either all-herring or all-trout were similar to those of chicks fed the respective monotypic diet throughout the 34-day treatment period. Five of the 12 major fatty acids in the diet were preferentially metabolized by tern chicks, while 6 were preferentially stored. The ratio of the level of a particular fatty acid in the consumer compared to the food (calibration coefficient) varied by fish type for 3 of the 12 major fatty acids. Also, calibration coefficients for 9 of these 12 fatty acids differed between chicks at day 20 (30 day-old chicks) vs. day 34 (44 day-old chicks) of the feeding trial, independent of diet treatment. Calibration coefficients for individual fatty acids in Caspian terns from this study were generally different from the comparable calibration coefficients in common murres (*Uria aalge*) from a separate study. Although fatty acid signatures of piscivorous birds clearly reflect the fatty acid composition of their

diet, the variation in fatty acid calibration coefficients associated with variation in seabird diet, age, and species seems highly problematic for modeling diet composition using fatty acid signatures of seabirds and their prey.

INTRODUCTION

Seabird diets can provide valuable information about the status and health of marine ecosystems, including abiotic factors that influence ocean conditions (Baird 1990) and the relative availability of forage fishes to seabirds (Hatch and Sanger 1992; Regehr and Montevecchi 1997) and other marine consumers. Prey availability for colonial nesting seabirds affects foraging trip duration, colony attendance, reproductive success, and, ultimately, demography of seabird populations (Cairns 1987). Measuring the composition of seabird diets can enhance the understanding of marine trophic structure at both the local and ecosystem scales, as well as aid in monitoring relative abundance of primary and secondary consumers, including fisheries stocks (Cairns 1992; Diamond and Devlin 2003).

Fatty acid signature analysis is a non-lethal technique recently developed to study diets of marine predators, and involves comparing fatty acid profiles of predators and their prey (Iverson 1993; Iverson et al. 2002; Iverson et al. 2004). Fatty acids are the primary constituent of most lipids. The quantitative profile of all fatty acids in fat reserves is referred to as the fatty acid signature (FAS) of the organism (Iverson 1993).

Fatty acid (FA) composition of seabird tissues is known to be affected by FAs assimilated from the diet (Iverson and Springer 2002; Dahl et al. 2003; Kakela et al.

2005). The analysis of FAs or fatty acid signatures (FASs) in predator tissues has been used to non-lethally study trophic level and spatial and temporal patterns in foraging behavior of free-ranging marine mammals and seabirds (Iverson 1993; Iverson et al. 1997; Raclot et al. 1998; Iverson and Springer 2002; Dahl et al. 2003). FASs of adipose tissue samples collected using biopsy techniques have also been used to verify the presence of fish and other prey types in the diets of mammals and birds (Johnson and West 1973; Rouvinen and Kiiskinen 1989; Wamberg et al. 1992; Colby et al. 1993; Pond et al. 1995; Raclot et al. 1998). Additionally, the composition of FAs found in certain fishes and invertebrates that serve as prey for seabirds and marine mammals has been used to accurately identify these prey organisms to the species level (Iverson et al. 1997; Budge et al. 2002; Iverson et al. 2002).

The FAS technique can potentially provide advantages over traditional methods for quantifying diet composition in piscivorous birds; first by eliminating the need to sacrifice subjects to acquire samples of stomach contents, and second by avoiding biases inherent in stomach contents analyses. The latter includes representation of only the most recently consumed prey in stomach contents and under-representation of prey with easily digested diagnostic parts (Jobling and Breiby 1986; Springer et al. 1996). Multivariate analysis of FA profiles has the potential to provide qualitative information on the composition of prey types in the diets of piscivorous waterbirds and other predators. Recently, a statistical model (QFASA) was developed that uses the FASs of predators to estimate the quantitative composition of prey in the predator's diet, based on a catalogue of prey types with known FASs (Iverson et al. 2004). Modeling is utilized because FAS patterns can be difficult to interpret superficially, especially when the number of potential

prey types is large, and when aspects of differential fatty acid metabolism in predators must be considered (Iverson et al. 2004).

In order to distinguish among prey types using complex QFASA models, each prey type must consistantly exhibit a FAS distinct from that of all other prey types consumed by the predator. Variation exists in FASs among individual prey within a prey type due to prey size, time of sampling, and diet (Budge et al. 2002; Iverson et al. 2002), which can confound distinguishing prey types. Also, prey with higher fat content will contribute proportionally more to a predator's FAS than prey with a lower fat content (Iverson et al. 2004). Additionally, differences in FASs among prey types must be reflected in the FAS of the predator's adipose tissue in a predictable way if differences in diet composition are to be detected.

Dietary fatty acids with carbon chain lengths ≥14 can be incorporated into fat stores with little modification to their structure. The accumulation of individual fatty acids in fat stores of birds, however, can be augmented by biosynthesis from other dietary constituents (proteins, carbohydrates). Also, selective metabolism of certain dietary fatty acids can result in lower levels of these fatty acids in adipose tissue of the predator compared with levels in the prey (Klasing 1998; Iverson and Springer 2002; Iverson et al. 2004). Accurately predicting diet composition using QFASA models is dependent on a consistent ratio of levels in predator fat stores to levels in the diet for each FA; these ratios were called "calibration coefficients" by Iverson et al. (2004). Calibration coefficients are used to account for preferential metabolism of certain fatty acids, as well as preferential deposition of other fatty acids by predators, which causes levels of each fatty acid to be either higher or lower relative to levels in the diet (Kirsch et al. 2000;

Iverson et al. 2004). Accurately predicting diet composition using QFASA models is dependent on a calibration coefficient for each fatty acid that is stable and does not vary with diet, nutritional status, or physiological state of the predator.

Predator tissues will not immediately reflect a shift in prey composition, and the length of time necessary for predator FASs to integrate dietary FAs varies depending on the predator tissue sampled. For example, a change in diet from demersal fishes to pelagic fishes could be detected in the FAS of blood plasma from captive herring gulls (*Larus argentatus*) within 5 days (Kakela et al. 2005). Significant differences in adipose tissue FASs of northern fulmars (*Fulmarus glacialis*) have been detected over a period of months at the same colony within a breeding season (Wang 2005). The minimum time required for seabird adipose tissue to reflect a change in diet, however, is unknown. This could vary depending on the physiological status of the individual (whether it is depositing or metabolizing fat reserves), the particular species of seabird studied (Iverson et al. 2004), and the magnitude of the change in diet.

The goal of this study was to evaluate the FAS technique, which might circumvent some of the problems of more traditional diet studies, as a method to determine diet composition of seabirds. More specifically, we sought to validate the use of FASs to accurately indicate diet composition in piscivorous waterbirds. We used captive feeding trials and comparisons of FASs of captive-reared Caspian tern (*Hydroprogne caspia*) chicks with that of their food. To do so, we determined the FASs and total lipid content of two fish species fed to tern chicks during feeding trials. We predicted that the FASs of fish would be significantly different between, but not within

fish species. We also determined the FASs of captive-reared tern chicks raised on diets of a single fish species vs. mixed fish species.

We predicted that FASs of terns fed a single fish species would closely reflect the average FAS of that fish species, and that tern FASs would differ between 2 diet treatment groups fed two different fish species, but not within diet treatment groups. We also wanted to investigate changes in FASs of tern adipose tissue following a change in diet, and determine whether a dietary shift could be detected in tern adipose tissue FASs within weeks. We hypothesized that FASs of tern adipose tissue would not differ between a treatment group fed a single fish species vs. a treatment group fed a mixed diet of two fish species and then switched to a diet of the single fish species. Lastly, we sought to calculate fatty acid-specific calibration coefficients to assess differential metabolism or storage of dietary fatty acids by Caspian terns, an essential precursor to modeling diet composition using QFASA.

METHODS

Captive-rearing

Forty Caspian tern hatchlings (1-3 days post-hatch) were collected on June 1, 2004 from the Caspian tern colony on East Sand Island, Oregon (46º15'45"N, 123º57'45"W). All hatchlings were collected during one incursion on the colony in order to minimize the impact of human disturbance on tern nesting success. One hatchling was removed from each of 40 nests that contained a minimum of 2 hatchlings or one

hatchling and one pipped egg. We employed this strategy in order to minimize the impact on overall productivity of the colony, because few nesting pairs successfully raise more than one young per nesting attempt.

Hatchlings were transported to a field laboratory in Warrenton, Oregon in well-ventilated coolers with cardboard dividers and chemical hand warmers to provide adequate heat, and were continuously monitored for signs of overheating. Upon arrival at the field laboratory, chicks were carefully examined for injuries or physical abnormalities and photographed individually. Chicks were aged based on body mass and wing length measurements of known-age chicks (D.E. Lyons, unpubl. data). Chicks were initially housed indoors in one of two 2-m diameter circular wading pools lined with sand substrate, 20 chicks per enclosure. We utilized heat lamps to warm the enclosures and provided shaded areas in each pool to allow chicks to behaviorally thermoregulate. Prior to initiation of diet treatments, chicks were transferred to individual 14-gallon plastic storage tubs in order to simplify the feeding regimen and preclude competition for food among chicks. All tubs were cleaned and sterilized daily with a solution of Nolvasan (2% chlorhexidine diacetate).

Feeding Trials

Thirty-two hatchlings were randomly assigned to one of 4 diet treatments, 8 hatchlings per treatment, for the feeding trials:

Treatment 1: 100% hatchery-reared juvenile rainbow trout (*Oncorhynchus mykiss*)

Treatment 2: 100% wild juvenile Pacific herring (*Clupea pallasi*)

Treatment 3: 2/3 juvenile rainbow trout and 1/3 juvenile herring (by mass), switched to 100% juvenile rainbow trout on day 20 of the feeding trial

Treatment 4: 2/3 juvenile herring and 1/3 juvenile rainbow trout (by mass), switched to 100% juvenile herring on day 20 of the feeding trial

Each chick was fed *ad libitum* its respective diet from 10 days post-hatch through the conclusion of the feeding trial 34 days later at age 44 days. Every effort was made to ensure that fish fed to tern chicks were from homogenous lots. Zoo-grade winter-caught Pacific herring were obtained from Xanadu Outfitters, Seattle, Washington. All herring were obtained from the same lot during the same fishery. Rainbow trout were tank-reared at the Trout Lodge Hatchery, Soap Lake, Washington and fed Bio-Oregon (Hammond, Oregon) BioMoist feed, a common feed for hatchery-reared salmonids in the Columbia River Basin, for 30 days prior to freezing for use in this study.

Hatchling Caspian tern chicks were not initially able to consume whole fish from their treatment diets due to size restrictions of the gape. Consequently, all study chicks were fed a starter diet of *ad libitum* Atlantic silversides (*Menidia menidia*) through day 5 post-hatch. Diets during the next 4 days, days 6-9 post-hatch, consisted of a mixture of about 50% silversides and 50% treatment diet by mass, so that treatment diets were phased in over a period of several days. Treatment diets were then initiated on day 10 post-hatch. Atlantic silversides for starter diets were obtained from a single lot of fish used to feed aquarium specimens at the Oregon Coast Aquarium, Newport, Oregon.

During the 34-day feeding trial, fish of a pre-determined range of fork lengths were hand-fed to chicks *ad libitum* during 6 daily feedings at 3-hour intervals, beginning

at 06:00 and ending at 21:00 PDT. The diet of thawed fish was supplemented (in the first fish offered each day) with half of a Seatabs multiple vitamin (Pacific Research Labs, El Cajon, California). In addition, each fish was injected with water to prevent dehydration of chicks.

Because all tern chicks were fed Atlantic silversides for at least 6 days prior to the onset of feeding trials, a residual effect of this diet may have influenced FASs of tern chicks during and after feeding trials. Consequently, we fed a group of 7 tern chicks Atlantic silversides for 6 days after capture, after which they were sacrificed to measure FASs. The average FAS of these silverside-fed chicks was then used to assess the magnitude of a potential residual silverside effect on FASs of chicks in feeding trials.

Morphometrics

Body mass (± 1 g) of each chick was measured daily on an electronic balance, prior to first feeding. Approximately every fifth day, we measured wing chord length (± 1 mm) with a wing-chord ruler, and diagonal tarsus (± 0.1 mm), head-bill (± 0.1 mm), and exposed culmen (± 0.1 mm) with dial calipers.

Adipose Tissue Sampling

We sampled synsacral adipose tissue from chicks after 20 days on treatment diets and prior to altering the diets of chicks in treatment groups 3 and 4. We used sterile surgical procedures to biopsy adipose tissue (Enderson and Berger 1968). Synsacral fat from Caspian terns was assumed to be representative of overall body fat deposits (Iverson and Springer 2002). Ten minutes prior to the biopsy, 1mL of 2% lidocaine solution was

applied topically to the incision site as a local anesthetic. A small area of the synsacrum was wetted with 70% ethanol, plucked of feathers, and the exposed skin swabbed with Betadine. Feathers were taped away from the incision site with surgical tape. A small (< 1 cm) incision was made dorsal to the synsacrum (lower back anterior to the uropygial gland) with a sterile, stainless steel surgical blade. A minimum of 0.1 g of subcutaneous adipose tissue was removed with stainless steel surgical scissors and foreceps, placed in a Kimax tube with Teflon-lined cap, and the sample frozen at -20°C until analysis. The incision site was closed with Vetbond adhesive to promote prompt healing.

On day 34 of the feeding trial (44 days post-hatch; normal fledging age), all chicks were sacrificed by cervical dislocation. Synsacral and other subcutaneous fat depots were sampled for fatty acid analysis. The carcasses were then placed in plastic bags and immediately frozen at -20ºC for later analysis in the lab. Captive-reared terns were not released into the wild because of potential disease transmission to wild birds and because Caspian tern fledglings receive considerable post-fledging parental care, so chances of survival for a fledgling tern unaccompanied by a parent is essentially nil. All procedures and protocols used with live birds followed protocol #3042, approved by the Oregon State University Institutional Animal Care and Use Committee (USDA Research Facility Certificate #92-R-0005, OLAW Assurance #A3229-01).

Laboratory Methods

Dietary fish and Caspian tern fat samples were shipped frozen on dry ice to the Center of Marine Biotechnology in Baltimore, Maryland, where they were lyophilized (freeze-dried) and subsequently stored at –80°C. Each frozen fish was homogenized in a

Waring blender to prevent samples from coming into contact with plastics. Total lipids were extracted from 500-mg aliquots of fish and from fat samples using a modified Bligh and Dyer (1959) method. This method is one of the most frequently-used methods for determining total lipids in biological tissues (Smedes and Askland 1999; Iverson et al. 2001), and is commonly preferred over the Folch (1957) method because of a significantly lower solvent to sample ratio (Iverson et al. 2001). Although the Bligh and Dyer method may significantly under-represent total fat content of samples with greater than 2% fat content compared with the Folch method, no differences were detected in fatty acid composition between the two methods (Iverson et al. 2001; Iverson et al. 2002).

Total lipids were extracted using a CH_2CL_2/MeOH (1:1) mixture of at least 5 times the sample volume. Samples were manually sonicated by glass probe, and by 2 x 40-min. extractions in a sonicating water bath at 40°C. Particulates were removed from the sample by vacuum through a Whatmann glass filter. Distilled water was added to the sample to arrive at an overall ratio of CH_2CL_2:MeOH:H_2O (1:1:0.8). After mixing and phase separation, the organic (lower) layer was recovered, followed by 2 extractions with CH_2CL_2:MeOH (3:1), and dried under N_2 gas. Sample mass was noted in order to determine lipid content of fish samples and lipid concentration of the mixture. The neutral lipid residue was brought to 1 ml in CH_2CL_2/MeOH (1:1), flushed with N_2 gas, and stored at -4^{o}C in amber glass vials with Teflon-lined caps labeled with the lipid/solvent concentration.

Five hundred μg of the lipid extract was saponified/transesterified and used to generate fatty acid methyl esters (FAMES). Ten μg of C19:C21 internal standard (5 μg of each FA) and the lipid extract were added to glass tubes and dried under N_2.

Saponification of lipids was accomplished by adding 4 ml KOH-saturated MeOH and 0.5 ml dH_2O to each tube, which was then capped with a Teflon-lined screw cap under a stream of N_2 gas and incubated at 70^oC for 1 h. After cooling, 0.5 ml of dH_2O was added and neutral lipids were removed by extracting 3x with 1.5 ml hexane:ether (9:1). Charged lipids were then recovered by lowering the *p*H of the mixture remaining in the tubes to < 2 by drop-wise addition of concentrated HCl, repeating the extraction (3x) with hexane:ether (9:1), removing the upper phase in each extraction. Charged lipids were dried under N_2.

Methylation of charged lipids was accomplished by the addition of 1 ml 14% BF3:CH_3OH solution to each sample of dry free fatty acids, the tubes capped with Teflon-lined screw caps, and incubated at 40^oC for 2 h. After cooling, 1 ml dH_2O was added to each tube and FAMES were removed by extracting 3x with 1.5 ml hexane:ether (9:1). After mixing samples by vortexing, the organic phase containing FAMEs was recovered to new glass tubes, dried under N_2, resuspended in 500 µl hexane, transferred to screw-top GC sample vials, and stored at -80^oC until analysis.

Identification of FAMEs was accomplished by comparing gas chromatography retention data with authentic quantitative standards from NU-CHECK, Inc. (stds 3B, GLC-68D, GLC-17AA') and qualitative standards from Matreya (PUFA No. 1 and 2 – Marine Source). Quantitative standards, for which we were able to verify peak identities based on the manufacturer's designated % peak area, included the following fatty acids: 8:0, 10:0, 12:0, 14:0, 14:1, 16:0, 16:1, 17:0, 18:0, 18:1n-9, 18:1n-7, 18:2n-6, 18:3n-3, 20:0, 20:1n-9, 20:2n-6, 20:4n-6, 20:5n-3, 22:0, 22:1n-9, 22:6n-3, 24:0, and 24:1. Peaks in some samples, including 18:5n-3 and 22:1n-11, were also confirmed by GC-MS. We

used a Hewlett-Packard 6890 GC equipped with a 30 m x 0.25 mm I.D. capillary column with 0.25 mm film thickness (DB Wax, J & W Scientific, Folson, CA) and a flame ionization detector at 300^{o}C. The GC was run in 'constant flow rate' mode at 1.5 ml min^{-1} with hydrogen as the carrier gas. The column temperature profile was as follows: 50^{o}C for 0.5 min, hold at 195^{o}C for 15 min after ramping at 40 ^{o}C•min^{-1}, and hold at 220^{o}C for 7 min after ramping at 2 ^{o}C•min^{-1}. Total runtime was 38.13 min.

Chromatograms were individually corrected and reintegrated as required. Sample fatty acid concentrations are represented as mass percent of total fatty acids and are identified by abbreviation: carbon chain length, number of double bonds, and location of double bonds nearest the terminal methyl carbon.

Data Analysis and Statistical Methods

Although 40-50 individual fatty acids were quantified in Caspian tern adipose tissue and dietary fish samples, a subset of fatty acids were selected for analyses based on mass composition of the samples. Only fatty acids that comprised ≥ 1% of the total mass of fatty acids in adipose tissue of at least one of the tern diet treatment groups or in one of the dietary fish types were used in statistical analyses. Twelve fatty acids met this criterion for analyses: 14:0, 16:0, 16:1n-7, 16:4n-1, 18:0, 18:1n-9, 18:2n-6, 20:1n-9 and n-7 (combined), 20:1n-11, 20:5n-3, 22:1n-11, and 22:6n-3. (The fatty acid 22:5n-3 was also included in analyses of chicks fed only Atlantic silversides through day 7 post-hatch because this fatty acid constituted > 1% of the total mass of fatty acids in silversides.)

These 13 fatty acids represented the great majority of the mass of all fatty acids in each sample.

Differences between fatty acid profiles of dietary fish were evaluated by MANOVA. We used Welsh's 2-sample t-tests to test for differences in individual fatty acids between the two fish types, and used a Bonferroni correction factor to account for multiple comparisons between 12 fatty acids. Fixed-effects ANOVA was used to test for differences in lipid content of fish types. Differences between Caspian tern adipose tissue and dietary fish fatty acids were evaluated using Welsh's 2-sample t-tests with a Bonferroni correction factor for multiple comparisons. MANOVA was used to detect differences in the fatty acid profiles between 30 day-old chicks in the 4 diet treatment groups, followed by fixed-effects ANOVA and Tukey's pairwise comparisons with Bonferroni correction factor to identify fatty acids whose levels distinguished terns from each of the 4 diet treatment groups. Paired t-tests with a Bonferroni correction factor were used to compare adipose tissue samples of chicks fed a diet of 2 fish types and then switched to a diet of one fish type and re-sampled. Welsh's two-sample t-tests with a Bonferroni correction factor were used to evaluate the response of FASs to a change in diet. We compared FASs of 44 day-old chicks fed a mixed diet of 2 fish types for 20 days and switched to a diet of one fish type for 14 days with FASs of chicks fed a diet of one fish type for the entire 34-day treatment period. MANOVA was used to detect differences in FASs among all 4 diet treatment groups of 44 day-old chicks, followed by fixed-effects ANOVA and Tukey's pairwise comparisons with Bonferroni correction factor.

In order to calibrate fatty acid levels measured in Caspian tern adipose tissue with fatty acid levels in their food and account for differential metabolism or storage of fatty acids in chick adipose tissue, fatty acid-specific calibration coefficients were calculated for 30 and 44 day-old Caspian tern chicks raised on one of two single-fish species diets. Average coefficients were calculated by the following formula for each major fatty acid:

$$\frac{\textbf{fatty acid mass as \% of total mass of fatty acids in Caspian tern adipose tissue } (n_1 \ldots n_x)}{\textbf{average fatty acid mass as \% of total mass of fatty acids in dietary fish}}$$

If numerator or denominator values used to calculate calibration coefficients were consistently below the measurement detection threshold (0.01 g) of the GC equipment used in sample analysis, the value of the calibration coefficient for a particular fatty acid was spurious and therefore removed from statistical comparisons and analyses.

We performed Welsh's 2-sample t-tests with a Bonferroni correction to test the effect of 2 different single fish species diets on calibration coefficients at age 44 days, after 34 days on the respective diets. To test for age effects on fatty acid calibration coefficients, we performed paired t-tests with a Bonferroni correction to compare calibration coefficients between herring-fed chicks at ages 30- and 44-days, and between trout-fed chicks at ages 30- and 44-days.

We should note that typically univariate analyses for correlated outcomes, as the FAs certainly are, can result in serious Type I errors (Rencher 1995). Because the p-

values resulting from the univariate tests are very low, however, we take this as protection against these errors.

RESULTS

Dietary Fish Fatty Acids and Lipid Content

There were significant differences between the fatty acid profiles of the three species of fish used for captive-rearing: Atlantic silversides, rainbow trout, and Pacific herring; inter-specific variability was generally much higher than intra-specific variability ($p < 0.0038$, MANOVA). For the two fishes used in feeding trials (trout and herring), levels of all 12 major fatty acids were significantly different between species ($p < 0.0042$, Welsh's 2-sample t-tests; Table 2.1). Average lipid content (% wet mass) of the rainbow trout (9.2 ± 1.26, $n = 20$), Pacific herring (8.4 ± 3.32, $n = 20$), and Atlantic silversides (7.7 ± 2.13, $n = 19$) were not significantly different ($p = 0.1585$, fixed-effects ANOVA).

Caspian Tern Fatty Acid Profiles

Fatty acid profiles of adipose tissue from Caspian tern chicks fed diets consisting of a single species of fish (herring or trout) were generally different from those of their respective diets. Levels of 10 of 12 major fatty acids in trout-fed chicks and 11 of 12 fatty acids in herring-fed chicks were significantly different between chick adipose tissue and their diets, after 34 days on their respective treatment diets ($p < 0.0042$, Welsh's 2-sample t-tests, Tables 2.2 a & b). Levels of most individual fatty acids in tern adipose tissue were consistently either higher or lower compared with levels in food, irrespective

of fish species consumed (Tables 2.2 a & b). The exceptions were 18:2n-6 for both trout-fed and herring-fed terns, and 16:4n-1 for trout-fed terns. Levels of 16:4n-1, however, were much less than 1% of the total mass of fatty acids.

Fatty acid profiles of tern adipose tissue collected on day 20 of the feeding trials were significantly different among the four diet treatment groups ($p < 0.0001$, MANOVA; Table 2.3). Mean levels of six of 12 major fatty acids (18:0, 18:1n-9, 18:2n-6, 20:1n-9 and n-7, 20:1n-11, and 22:1n-11) in tern adipose tissue were significantly different among all four diet treatment groups ($p < 0.0006$, fixed-effects ANOVA and Tukey pairwise comparisons; Table 2.3; Figure 2.1). Additionally, levels of these six distinguishing fatty acids in terns occurred at progressively higher levels as herring or trout was more prevalent in the diet (Figure 2.1).

The levels of 5 of 12 major fatty acids changed significantly in terns that were switched from a mixed fish diet of 2/3 trout and 1/3 herring to an all-trout diet on day 20 of the feeding trial and re-sampled on day 34 (Table 2.4a). The levels of 11 of 12 major fatty acids changed significantly in terns that were switched from a mixed diet of 2/3 herring and 1/3 trout to an all-herring diet on day 20 of the feeding trial and re-sampled on day 34 ($p < 0.0042$, paired t-tests; Table 2.4b).

Fatty acid levels in terns that were switched from a mixed diet after 20 days to a single species diet of either trout or herring for 14 days were generally not significantly different from those of terns fed the respective diet for the entire 34-day duration of the feeding trial; the exceptions were 18:2n-6 and 22:1n-11 in trout-fed chicks ($p < 0.0001$ MANOVA; $p < 0.0006$, fixed effects ANOVA and Tukey pairwise comparisons, Table 2.5). The levels of 7 of 12 major fatty acids conformed to the pattern of similarities and

differences expected if diet during the last 14 days was the sole determinant of fatty acid levels in tern adipose tissue(Table 2.5).

Calibration Coefficients

Mean values of fatty acid calibration coefficients (% fatty acid mass in consumer / % fatty acid mass in diet) for the 12 major fatty acids ranged from 0.02 to 4.36 in 44 day-old chicks fed single-species diets throughout the 34 days of the feeding trials (Table 2.6). Calibration coefficients for five of the 12 major fatty acids were < 1.0, indicating that these fatty acids were preferentially metabolized after assimilation (Table 2.6). Conversely, calibration coefficients for 6 of the 12 major fatty acids were > 1.0, indicating that these fatty acids were preferentially deposited in adipose tissue after assimilation (Table 2.6). The calibration coefficients of one fatty acid (16:4n-1) were quite different between terns fed herring vs. terns fed trout; 16:4n-1 was preferentially metabolized by chicks fed a herring diet and preferentially stored by chicks fed a trout diet (Table 2.6).

Calibration coefficients for 9 of 12 fatty acids were not statistically different between terns fed the trout diet and those fed the herring diet. There was, however, a significant effect of diet on calibration coefficients for 3 fatty acids: 16:4n-1, 22:1n-11, and 22:6n-3. The calibration coefficients for these three fatty acids were significantly different between chicks fed all-herring vs. all-trout throughout the 34-day feeding trial ($p \leq 0.0014$, Welsh's 2-sample t-tests; Figure 2.2).

Calibration coefficients for chicks of different ages (30 days-old vs. 44 days-old) were significantly different for 9 of 12 fatty acids (14:0, 16:0, 16:1n-7, 16:4n-1, C20:1n-9

and n-7, 20:1n-11, 20:5n-3, 22:1n-11, 22:6n-3), even when diet was held constant ($p < 0.0042$, paired t-tests; Table 2.6). Of the 9 fatty acids whose calibration coefficients differed with age, three (16:4n-1, 22:1n-11, 22:6n-3) exhibited confounding effects of diet, as calibration coefficients for these fatty acids in 44 day-old chicks were also significantly different between trout-fed and herring-fed birds ($p < 0.0014$, Welsh's 2-sample t-tests; Figure 2.2).

We compared calibration coefficients from 7 day-old Caspian tern chicks fed silversides for the 6 days after collection from the wild, with calibration coefficients from chicks fed only herring or trout for 20 days and 34 days. We assumed that if there was a significant residual effect of pre-treatment diet (silversides) on calibration coefficients for chicks fed herring or trout for 20 days or 34 days, we would detect significantly different and progressively increasing or decreasing calibration coefficients between the 7-day-old chicks whose food intake was solely silversides and these older chicks. Calibration coefficients for 2 of 13 major fatty acids (20:5n-3 and 22:6n-3) met these criteria for both trout-fed birds and herring-fed birds, and one more major fatty acid (16:4n-1) met these criteria for herring-fed birds only ($p < 0.0013$, paired and Welsh's 2-sample t-tests; Figures 2.3 a & b). A potential residual effect of pre-treatment diet on calibration coefficients could not be ruled out in trout-fed birds for 16:4n-1, nor for 22:1n-11 in either trout-fed or herring-fed birds due to very low levels of these fatty acids in either the diet or the chicks, below the detection limits of the GC equipment. The trends in calibration coefficients for 20:1n-9 and n-7 were suggestive of effects from pre-treatment diet, but not conclusive because levels of this fatty acid were not significantly different between 7-day-old chicks fed silversides and 30-day-old chicks fed only herring or trout

($p < 0.0013$, paired and Welsh's 2-sample t-tests, Figures 2.3 a & b). Whereas 9 of 12 major fatty acids showed a significant effect of age on calibration coefficients (Table 2.6), only 3, and at most 6, of 12 fatty acids met our criteria for indicating a potential residual effect of pre-treatment chick diet on calibration coefficients (Figures 2.3 a & b).

DISCUSSION

Dietary Fatty Acids

A primary objective of this study was to assess the fatty acid signature technique for quantifying diet composition in piscivorous seabirds. One condition that must be met before differences in consumer fatty acid profiles can indicate differences in diet is a demonstrated difference in fatty acid profiles between diets. For our feeding experiment, we chose fish species that we assumed had very different FASs. As predicted, fatty acid profiles were markedly different between wild Pacific herring and hatchery-reared rainbow trout, the two fish species used in feeding trials. These two fish species differed in levels of all 12 major fatty acids, and intra-specific variability in fatty acid levels was much less than inter-specific differences.

Budge et al. (2002) also found that among-species variation in fatty acid composition was greater than within-species variation for forage fishes from the northwestern Atlantic. Inter-specific differences in fatty acid composition of the fishes used in our study were, however, likely greater than those of forage fishes consumed by piscivorous birds in the wild. The trout and herring used in our study consumed very

different diets; the former was raised on commercial feed in a hatchery and the latter in the wild in a coastal marine environment. Greater similarity in FASs among fishes from the same environments with similar food habits would result in greater difficulty distinguishing between diets based on FASs of adipose tissue from their predators. For example, Atlantic herring (*Clupea harengus*), mackerel (*Scomber scombrus*), and capelin (*Mallotus villosus*) had similar fatty acid signatures (Budge et al. 2002), and attempts to distinguish between single-species diets of these three fishes would be more difficult. Diets of wild seabirds are also more species-diverse than the diets we fed to subjects in the present study, further complicating inferences of diet composition from seabird FASs.

Comparison of Fatty Acid Levels in Food and Consumer

Levels of most individual fatty acids in tern adipose tissue were consistently higher or lower compared with levels in the diet, irrespective of the fish species consumed. This indicates that the Caspian tern chicks generally metabolized and stored individual dietary fatty acids in a predictable pattern, despite differing levels in the diet. One exception was 18:2n-6, which did not differ between food and consumer for either trout-fed or herring-fed birds. Another exception was 16:4n-1, which was preferentially metabolized by terns fed herring, but preferentially stored by terns fed trout, a diet low in 16:4n-1. With the exception of 16:4n-1, the 12 major dietary fatty acids differed between adipose tissue of trout-fed and herring-fed terns.

Six of 12 major dietary fatty acids allowed us to distinguish between tern chicks in each of the four diet treatment groups, based on their levels in tern adipose tissue. It was particularly impressive that these 6 fatty acids differed between terns fed different

proportions of the same two fish species. Because diets of wild seabirds nearly always consist of a greater diversity of prey species than were fed in this study, however, it will be more difficult to interpret the proportional contribution of each prey species to the diet based on FASs of free-ranging seabirds. Nevertheless, our results suggest that the FAS technique will detect shifts in diet between two predominant prey types with markedly different FASs.

Effects of Diet Shifts on Consumer Fatty Acids Levels

The FASs of Caspian tern chicks changed significantly within 14 days after they were switched from a mixed diet to a monotypic diet. Additionally, fatty acid levels in adipose tissue of these tern chicks were similar to fatty acid levels in chicks of the same age that were fed the respective single fish diet for a much longer period. These results suggest that fatty acids in adipose tissue of seabird chicks have high turnover rates and that fatty acid composition of seabirds can clearly reflect changes in the composition of food types in the diet. Major shifts in diet composition of free-ranging seabirds may be detectable in FASs from adipose tissue within weeks. This prediction assumes, however, that the FASs of major prey types differ significantly. Additionally, dietary shifts may be more difficult to detect as the number of prey types in the diet increases and dietary shifts are less abrupt.

Calibration Coefficients

In order to measure fatty acid calibration coefficients for a consumer it is necessary to restrict the diet of the consumer to a homogenous diet over an extended

period of time. This is the first study to measure fatty acid calibration coefficients of adipose tissue in Caspian terns which were fed homogenous diets for 34 days, and only the second study to measure calibration coefficients for a seabird (Iverson and Springer 2002). If the diets of Caspian terns and other seabirds are to be modeled using the Quantitative Fatty Acid Signature Analysis (QFASA) technique, calibration coefficients for each major dietary fatty acid must be known in order to understand how fatty acid levels in the diet translate into fatty acid levels in bird adipose tissue. The effects of differential metabolism, storage, and biosynthesis of fatty acids by the consumer can only be accounted for by comparing the levels of each fatty acid in the consumer with levels in the diet. If fatty acid calibration coefficients for a consumer vary with diet, age, or physiological status of the consumer, then predicting diet composition from the consumer's FAS will be subject to considerable uncertainty. For example, when Iverson et al. (2004) sought to determine seal diets by modeling using QFASA without specifying calibration coefficients, "estimates of the percent contribution to [fatty acid] signatures or to diets did not correspond to either known or expected diet contributions at any time."

In the present study, one fatty acid (16:4n-1) showed a particularly strong effect of diet on calibration coefficient; 16:4n-1 was preferentially metabolized in chicks fed a diet of herring and preferentially stored in chicks fed a diet of trout, where 16:4n-1 was scarce. Similarly, the calibration coefficients for 22:1n-11 and 22:6n-3 were considerably different between tern chicks on the two diets. Calibration coefficients of fatty acids that vary depending on the diet cannot be used to accurately model or estimate the proportional contribution of prey types to the diet.

Differences in FA calibration coefficients were also documented for 9 of 12 major fatty acids between 30-day old and 44-day old tern chicks fed the same treatment diets since 10 days of age. The FASs, and therefore the calibration coefficients, of the tern chicks may have been affected by the FA profile of the Atlantic silversides fed to chicks before treatment diets were initiated at age 10 days. Consequently, the age effect on FA calibration coefficients could conceivably result from the slow turnover of adipose tissue fatty acids that were deposited when tern chicks were consuming silversides, prior to the onset of the diet treatments. For example, estimates of the half-life of FAs in adipose tissue of juvenile domestic chickens ranged from 18 to 23 days (Foglia et al. 1994). Consequently, the significant differences in calibration coefficients between 30 day-old and 44 day-old tern chicks may have been due to the residual effects of fatty acids assimilated during pre-treatment diets, rather than age *per se*. Results from the present study, however, did not support such a low turnover rate of depot FAs in tern chicks. For the terns that were fed mixed diets early in the treatment period, the effects of the food type that was eliminated from the diet 14 days prior to sampling adipose tissue were barely detectable in the FASs of those samples.

We attempted an additional test of the hypothesis that pre-treatment diet affected post-treatment calibration coefficients. We compared calibration coefficients from 7 day-old Caspian tern chicks that were fed only silversides for 6 days with calibration coefficients from chicks fed diets of a single species of fish beginning at day 10 and sampled at day 30 and again at day 44. We assumed that if there were a significant residual effect of pre-treatment diet on calibration coefficients for chicks at day 30 and

day 44, we would detect significantly different and progressively increasing or decreasing calibration coefficients between the 7 day-old chicks fed silversides and the older chicks.

Our results indicate that age played a greater role than residual effects of pre-treatment diets in causing the differences in calibration coefficients between 30 day-old and 44 day-old tern chicks. Of the nine major fatty acids that exhibited age-related differences in calibration coefficients the differences were not consistent with a residual effect of pre-treatment diet for 5 of 9 fatty acids; the differences were consistent with the effect of pre-treatment diet for only 3 of 9 fatty acids (Figures 2.3a & b).

The calibration coefficients measured in this study on Caspian terns were significantly different from calibration coefficients of common murres (S.J. Iverson, unpubl. data) for 10 of 13 major dietary fatty acids; the exceptions were 16:4n-1, 18:1n-9, and 22:1n-11 ($p < 0.0019$, Bonferroni-corrected Welsh's 2-sample t-tests; Table 2.7). Of the 3 major fatty acids whose calibration coefficients did not differ between terns and murres, two fatty acids (16:4n-1 and 22:1n-11) had different calibration coefficients in terns depending on diet. Nevertheless, for 12 of 13 major dietary fatty acids in terns, calibration coefficients that indicated a metabolism bias (calibration coefficient <1) or a storage bias (calibration coefficient >1) showed the same bias in common murres. Calibration coefficients for 16:0, however, showed a metabolism bias in Caspian terns, but a storage bias in common murres (Table 2.6). These results indicate that calibration coefficients can vary among seabird species and appropriate feeding trials should be conducted on each seabird species to determine calibration coefficients for quantitatively modeling diets. It should also be noted that calculation of calibration coefficients

assumes that predators are random samplers of their environment, because the denominator is calculated based on a mean fatty acid level in sampled prey.

There were significant effects of diet and age on the calibration coefficients for 9 of the 12 major fatty acids in 44 day-old Caspian terns (i.e., 14:0, 16:0, 16:1n-7, 16:4n-1, 20:1n-9 & n-7, 20:1n-11, 20:5n-3, 22:1n-11, 22:6n-3). Use of these fatty acids to model diets of free-ranging Caspian terns is problematic, as they would not produce accurate estimates of the fatty acid composition of the diet. Of the 12 major fatty acids used to validate the FAS technique in this study, only three had consistent calibration coefficients regardless of age or diet, and the levels of these three fatty acids (18:0, 18:1n-9, 18:2n-6) also differed significantly among terns in the 4 diet treatment groups. Of these three fatty acids, only one (18:1n-9) had a calibration coefficient that did not differ between terns and murres.

Because of the variation in fatty acid calibration coefficients detected in this study, differences that were due at least in part to seabird diet, age, and species, we investigated whether the magnitude of variation in calibration coefficients associated with these variables was comparable to the magnitude of variation in tern FA levels due to differences in their diets. If differences in tern FA levels due to diet are much larger than differences due to variation in calibration coefficients, then the observed variation in calibration coefficients due to diet, age, and species would not preclude accurate modeling of seabird diets using the QFASA technique. For the 12 major fatty acids, the average magnitude of differences in tern FA levels due to diet was a factor of 3.5. This is somewhat greater than the average magnitude of differences in calibration coefficients for the 12 major fatty acids due to age (2.3x), diet (2.3x), and bird species (2.2x).

However, the magnitude of difference in fatty acid levels of wild Caspian terns sampled early and late in the breeding season averaged 1.9x for the 12 major fatty acids (Myers et al., in prep.), less than the average magnitude of differences in calibration coefficients due to age, diet, and bird species. Also, the diet composition of the wild Caspian terns differed considerably between the two sampling periods. This suggests that the variation in calibration coefficients observed in this study may be highly problematic for accurately modeling diets of free-ranging seabirds using the QFASA technique. A sensitivity analysis of the QFASA model to variation in calibration coefficients of the magnitude detected here should be conducted in order to assess the robustness of model output.

CONCLUSIONS

1. The two fish species used in these captive-feeding trials had distinctly different levels of all 12 major fatty acids; intra-specific variability in FA levels of these fishes was much less than inter-specific variability.

2. Caspian terns preferentially either metabolized or deposited 11 of 12 major dietary fatty acids in a consistent pattern, despite differing levels in the diet.

3. There were significant differences in levels of 6 of 12 major dietary fatty acids in adipose tissue of chicks fed 4 different diets for 20 days.

4. Fatty acid levels in tern adipose tissue changed significantly within 14 days of a change in diet; differences in fatty acid levels between terns experiencing a diet shift and terns fed the same diet throughout were barely discernable.

5. Because free-ranging seabirds forage on a greater number of prey types, and the spatial and temporal variability in prey consumed within a type is likely to be greater, we expect differences in fatty acid signatures of wild Caspian terns due to diet to be much more subtle than those of tern chicks in this study.

6. Calibration coefficients for 11 of 12 major fatty acids differed as a function of diet, age, and/or species of seabird. The demonstrated variability of calibration coefficients for most fatty acids appears to be a serious obstacle to accurately modeling diet composition in free-ranging seabirds using the Quantitative Fatty Acid Signature Analysis (QFASA) technique.

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Table 2.1. Mean percent of total fatty acid mass for 12 major fatty acids[1] in fishes fed to captive Caspian tern chicks.

Fatty acid	Rainbow Trout (n = 20)	Pacific Herring (n = 20)	p-value
C14:0	6.11 ± 1.354	9.06 ± 2.827	0.0002
C16:0	18.23 ± 1.894	14.84 ± 2.904	0.0001
C16:1n-7	10.32 ± 1.733	6.98 ± 1.816	<0.0001
C16:4n-1	0.11 ± 0.118	1.53 ± 0.846	<0.0001
C18:0	4.29 ± 0.490	1.76 ± 0.409	<0.0001
C18:1n-9	19.38 ± 2.248	9.66 ± 2.277	<0.0001
C18:2n-6	5.05 ± 0.644	1.31 ± 0.290	<0.0001
C20:1n-9, n-7	0.44 ± 0.306	3.00 ± 1.501	<0.0001
C20:1n-11	1.59 ± 0.262	4.60 ± 1.498	<0.0001
C20:5n-3	4.91 ± 0.854	7.80 ± 2.303	<0.0001
C22:1n-11	0.33 ± 0.344	8.85 ± 3.638	<0.0001
C22:6n-3	11.02 ± 1.236	7.06 ± 2.090	<0.0001

[1]Values are mean mass percent ± SD of fatty acids that comprised ≥ 1% average total fatty acid sample mass in one or both fish species. Fatty acid levels differed between the two fish species for all 12 fatty acids ($p < 0.0001$, MANOVA; $p < 0.0042$ Welsh's 2-sample t-tests).

Table 2.2a. Mean percent of total fatty acid mass for 12 major fatty acids[1] in trout and in adipose tissue of captive Caspian tern chicks fed only trout for 34 days.

Fatty acid[2]	Trout (n = 20)	Tern on trout diet (n = 7)
C14:0	6.11 ± 1.354	2.97 ± 0.275
C16:0	18.23 ± 1.894	13.42 ± 1.517
C16:1n-7	10.32 ± 1.733	5.41 ± 1.070
C16:4n-1	0.11 ± 0.118	0.14 ± 0.051
C18:0	4.29 ± 0.490	5.88 ± 0.624
C18:1n-9	19.38 ± 2.248	28.63 ± 3.761
C18:2n-6	5.05 ± 0.644	6.24 ± 0.747
C20:1n-9, n-7	0.44 ± 0.306	1.56 ± 0.299
C20:1n-11	1.59 ± 0.262	4.07 ± 0.680
C20:5n-3	4.91 ± 0.854	0.39 ± 0.189
C22:1n-11	0.33 ± 0.344	1.44 ± 0.313
C22:6n-3	11.02 ± 1.236	1.42 ± 0.485

[1]Values are mean mass percent ± SD of fatty acids that comprised ≥ 1% average total fatty acid sample mass in trout or in adipose tissue of terns.

[2]Fatty acids in bold type differ significantly between food and consumer ($p < 0.0042$, Welsh's 2-sample t-tests).

Table 2.2b. Mean percent of total fatty acid mass for 12 major fatty acids[1] in herring and captive Caspian tern chicks fed only herring for 34 days.

Fatty acid[2]	Herring (n = 20)	Tern on herring diet (n = 8)
C14:0	9.06 ± 2.827	4.45 ± 0.805
C16:0	14.84 ± 2.904	10.02 ± 2.327
C16:1n-7	6.98 ± 1.816	2.40 ± 0.914
C16:4n-1	1.53 ± 0.846	0.21 ± 0.080
C18:0	1.76 ± 0.409	2.73 ± 0.176
C18:1n-9	9.66 ± 2.277	14.99 ± 2.506
C18:2n-6	1.31 ± 0.290	1.47 ± 0.233
C20:1n-9, n-7	3.00 ± 1.501	8.17 ± 1.401
C20:1n-11	4.60 ± 1.498	10.75 ± 1.534
C20:5n-3	7.80 ± 2.303	0.13 ± 0.091
C22:1n-11	8.85 ± 3.638	16.06 ± 2.613
C22:6n-3	7.06 ± 2.090	0.31 ± 0.221

[1]Values are mean mass percent ± SD of fatty acids that comprised ≥ 1% average total fatty acid sample mass in herring or in adipose tissue of terns.

[2]Fatty acids in bold type differ significantly between food and consumer ($p < 0.0042$, Welsh's 2-sample t-tests).

Table 2.3. Mean percent of total fatty acid mass for 12 major fatty acids[1] in adipose tissue from 30 day-old Caspian terns fed treatment diets for 20 days.

	Diet Treatments			
Fatty acid[2]	all trout (n = 7)	2/3 trout, 1/3 herring (n = 8)	2/3 herring, 1/3 trout (n = 8)	all herring (n = 8)
C14:0	4.65[a] ± 0.360	4.34 [a] ± 0.518	6.64 [b] ± 1.322	5.98 [b] ± 0.293
C16:0	20.26 [a] ± 0.900	17.19[ab] ± 1.626	17.69[ab] ± 1.863	16.02 [b] ± 1.197
C16:1n-7	10.00 [a] ± 0.655	7.42[bc] ± 0.976	7.48 [b] ± 1.263	5.95 [c] ± 0.566
C16:4n-1	0.08 [a] ± 0.137	0.24 [b] ± 0.044	0.57 [c] ± 0.161	0.44 [c] ± 0.035
C18:0	6.32 [a] ± 0.240	5.03 [b] ± 0.501	3.91[c] ± 0.237	2.97 [d] ± 0.189
C18:1n-9	30.49 [a] ± 1.297	25.07 [b] ± 2.213	19.68 [c] ± 1.894	16.17 [d] ± 1.399
C18:2n-6	6.96 [a] ± 0.276	5.24 [b] ± 0.500	3.02 [c] ± 0.362	1.68 [d] ± 0.106
C20:1n-9, n-7	0.80 [a] ± 0.103	1.87 [b] ± 0.263	3.90 [c] ± 0.355	4.85 [d] ± 0.407
C20:1n-11	2.23 [a] ± 0.268	3.63 [b] ± 0.419	6.00 [c] ± 0.388	7.20 [d] ± 0.678
C20:5n-3	0.97 [a] ± 0.532	1.12[ab] ± 0.251	1.72 [b] ± 0.650	1.51[ab] ± 0.463
C22:1n-11	0.28 [a] ± 0.272	3.32 [b] ± 0.456	7.84 [c] ± 0.703	10.02 [d] ± 0.949
C22:6n-3	3.34 [a] ± 0.855	2.73 [a] ± 0.531	2.22 [a] ± 0.760	1.93 [a] ± 0.560

[1]Values are mean mass percent ± SD of fatty acids that comprised ≥ 1% average total fatty acid sample mass in one or both fish species or in adipose tissue of terns. Fatty acid values that do not share a common superscript are significantly different from one another ($p < 0.0001$, MANOVA; $p < 0.0006$, fixed-effects ANOVA and Tukey pairwise comparisons).

[2]Average levels of fatty acids in bold type were significantly different between all 4 diet treatment groups.

Table 2.4a. Mean percent of total fatty acid mass for 12 major fatty acids[1] in adipose tissue from Caspian tern chicks after 20 days on a mixed diet of 2/3 trout, 1/3 herring compared with the same chicks after switching to a diet of all-trout for 14 days.

	20d, 2/3 trout diet (n = 8)			14d, all-trout diet (n = 7)		
C14:0	4.34	±	0.518	3.16	±	0.455
C16:0	17.19	±	1.626	12.62	±	1.704
C16:1n-7	7.42	±	0.976	4.32	±	0.785
C16:4n-1	0.24	±	0.044	0.11	±	0.017
C18:0	5.03	±	0.501	5.57	±	0.614
C18:1n-9	25.07	±	2.213	26.09	±	3.081
C18:2n-6	5.24	±	0.500	5.40	±	0.691
C20:1n-9, n-7	1.87	±	0.263	3.19	±	1.000
C20:1n-11	3.63	±	0.419	6.02	±	1.686
C20:5n-3	1.12	±	0.251	0.34	±	0.122
C22:1n-11	3.32	±	0.456	5.99	±	2.511
C22:6n-3	2.73	±	0.531	1.36	±	0.578

[1]Values are mean mass percent ± SD of fatty acids that comprised ≥ 1% average total fatty acid sample mass in one or both fish species or in adipose tissue of terns.

[2]Fatty acids in bold type differ significantly between terns before and after the change in diet ($p < 0.0042$, paired t-tests).

Table 2.4b. Mean percent of total fatty acid mass for 12 major fatty acids[1] in adipose tissue from Caspian tern chicks after 20 days on a mixed diet of 2/3 herring, 1/3 trout compared with the same chicks after switching to a diet of all-herring for 14 days.

Fatty acid[2]	20d, 2/3 herring diet (n = 8)			14d, all herring diet (n = 8)		
C14:0	6.64	±	1.322	4.25	±	0.749
C16:0	17.69	±	1.863	10.69	±	2.462
C16:1n-7	7.48	±	1.263	2.73	±	1.054
C16:4n-1	0.57	±	0.161	0.21	±	0.075
C18:0	3.91	±	0.237	3.25	±	0.282
C18:1n-9	19.68	±	1.894	16.96	±	2.176
C18:2n-6	3.02	±	0.362	2.19	±	0.340
C20:1n-9, n-7	3.90	±	0.355	7.18	±	1.943
C20:1n-11	6.00	±	0.388	9.58	±	1.734
C20:5n-3	1.72	±	0.650	0.19	±	0.135
C22:1n-11	7.84	±	0.703	14.11	±	3.983
C22:6n-3	2.22	±	0.760	0.41	±	0.126

[1]Values are mean mass percent ± SD of fatty acids that comprised ≥ 1% average total fatty acid sample mass in one or both fish species or in adipose tissue of terns.

[2]Fatty acids in bold type differ significantly between terns before and after the change in diet ($p < 0.0042$, paired t-tests).

Table 2.5. Mean percent of total fatty acid mass for 12 major fatty acids[1] in adipose tissue from 44 day-old Caspian terns fed treatment diets for 34 days.

	Diet Treatments			
Fatty acid[2]	all trout (n = 7)	2/3 trout, 1/3 herring switched to 100% trout at day 20 (n = 7)	2/3 herring, 1/3 trout switched to 100% herring at day 20 (n = 8)	all herring (n = 8)
C14:0	2.97 ^{a} ± 0.275	3.16 ^{a} ± 0.455	4.25 ^{b} ± 0.749	4.45 ^{b} ± 0.805
C16:0	13.42 ^{a} ± 1.517	12.62 ^{ab} ± 1.704	10.69 ^{ab} ± 2.462	10.02 ^{b} ± 2.327
C16:1n-7	5.41 ^{a} ± 1.070	4.32 ^{a} ± 0.785	2.73 ^{b} ± 1.054	2.40 ^{b} ± 0.914
C16:4n-1	0.14 ^{abc} ± 0.051	0.11 ^{a} ± 0.017	0.21 ^{bc} ± 0.075	0.21 ^{c} ± 0.080
C18:0	5.88 ^{a} ± 0.624	5.57 ^{a} ± 0.614	3.25 ^{b} ± 0.282	2.73 ^{b} ± 0.176
C18:1n-9	28.63 ^{a} ± 3.761	26.09 ^{a} ± 3.081	16.96 ^{b} ± 2.176	14.99 ^{b} ± 2.506
C18:2n-6	6.24 ^{a} ± 0.747	5.40 ^{b} ± 0.691	2.19 ^{c} ± 0.340	1.47 ^{c} ± 0.233
C20:1n-9, n-7	1.56 ^{a} ± 0.299	3.19 ^{a} ± 1.000	7.18 ^{b} ± 1.943	8.17 ^{b} ± 1.401
C20:1n-11	4.07 ^{a} ± 0.680	6.02 ^{a} ± 1.686	9.58 ^{b} ± 1.734	10.75 ^{b} ± 1.534
C20:5n-3	0.39 ^{a} ± 0.189	0.34 ^{ab} ± 0.122	0.19 ^{bc} ± 0.135	0.13 ^{c} ± 0.091
C22:1n-11	1.44 ^{a} ± 0.313	5.99 ^{b} ± 2.511	14.11 ^{c} ± 3.983	16.06 ^{c} ± 2.613
C22:6n-3	1.42 ^{a} ± 0.485	1.36 ^{a} ± 0.578	0.41 ^{b} ± 0.126	0.31 ^{b} ± 0.221

[1]Values are mean mass percent ± SD of fatty acids that comprised ≥ 1% average total fatty acid sample mass in one or both fish species or adipose tissue of terns. Fatty acid values that do not share a common superscript are significantly different from one another ($p < 0.0001$, MANOVA; $p < 0.0006$, fixed-effects ANOVA and Tukey pairwise comparisons).

[2]Fatty acids in bold type conform to the predicted pattern of differences among treatment groups if fatty acid levels were determined only by diet in the previous 14 days.

Table 2.6. Mean calibration coefficients[1] for individual fatty acids from adipose tissue of captive Caspian tern chicks fed treatment diets.

	30 day-old Caspian terns				**44 day-old Caspian terns**			
	trout diet 20d (n = 7)		herring diet 20d (n = 8)		trout diet 34d (n = 7)		herring diet 34d (n = 8)	
C14:0	0.76 ± 0.022		0.66 ± 0.011		0.49 ± 0.017		0.49 ± 0.031	
C16:0	1.11 ± 0.019		1.08 ± 0.029		0.74 ± 0.031		0.68 ± 0.055	
C16:1n-7	0.97 ± 0.024				0.52 ± 0.039		0.34 ± 0.046	
C16:4n-1			0.29 ± 0.008		1.27 ± 0.168		0.14 ± 0.018	
C18:0	1.47 ± 0.021		1.69 ± 0.038		1.37 ± 0.055		1.55 ± 0.035	
C18:1n-9	1.57 ± 0.025		1.67 ± 0.051		1.48 ± 0.073		1.55 ± 0.092	
C18:2n-6	1.38 ± 0.021		1.29 ± 0.029		1.24 ± 0.056		1.12 ± 0.063	
C20:1n-9, n-7	1.81 ± 0.089		1.62 ± 0.048		3.56 ± 0.257		2.73 ± 0.165	
C20:1n-11	1.41 ± 0.064		1.57 ± 0.052		2.57 ± 0.162		2.34 ± 0.118	
C20:5n-3	0.20 ± 0.041		0.19 ± 0.021		0.08 ± 0.015		0.02 ± 0.004	
C22:1n-11	0.86 ± 0.304		1.13 ± 0.038		4.36 ± 0.357		1.81 ± 0.104	
C22:6n-3	0.30 ± 0.029		0.27 ± 0.028		0.13 ± 0.017		0.04 ± 0.011	

[1]A calibration coefficient for a particular fatty acid is the biomass of that fatty acid as a percent of total fatty acid biomass in the consumer divided by the average fatty acid biomass as a percent of total fatty acid biomass in the diet. Values are mean calibration coefficients ± SE of 12 major fatty acids.

[2]Fatty acids listed in bold type had calibration coefficients that were significantly different between 30 day-old and 44 day-old chicks fed the same diet ($p < 0.0042$, paired t-tests).

Table 2.7. Mean calibration coefficients[1] for individual fatty acids from adipose tissue of Caspian terns (44 days-old) and common murres[2] (45 days-old) fed controlled diets in captivity.

	Calibration Coefficients			
	Caspian Tern			**Common Murre**
Fatty acid	trout diet (n = 7)	herring diet (n = 8)	both diets (n = 15)	silverside diet (n =13)
C14:0			0.49 [a] ± 0.018	0.96 [b] ± 0.017
C16:0			0.70 [a] ± 0.033	1.14 [b] ± 0.020
C16:1n-7			0.43 [a] ± 0.038	0.91 [b] ± 0.010
C16:4n-1	1.27 ± 0.168	0.14 ± 0.018		0.59 ± 0.043
C18:0			1.46 [a] ± 0.039	1.87 [b] ± 0.046
C18:1n-9			1.52 [a] ± 0.058	1.52 [a] ± 0.029
C18:2n-6			1.17 [a] ± 0.044	1.44 [b] ± 0.052
C20:1n-9, n-7			3.11 [a] ± 0.181	2.20 [b] ± 0.053
C20:1n-11			2.45 [a] ± 0.100	3.20 [b] ± 0.096
C20:5n-3			0.05 [a] ± 0.011	0.32 [b] ± 0.021
C22:1n-11	4.36 ± 0.357	1.81 ± 0.104		3.34 ± 0.180
C22:5n-3			0.40 [a] ± 0.079	0.82 [b] ± 0.033
C22:6n-3	0.13 ± 0.017	0.04 ± 0.011		0.45 ± 0.025

[1]A calibration coefficient for a particular fatty acid is the biomass of that fatty acid as a percent of total fatty acid biomass in the consumer divided by the fatty acid biomass as a percent of total fatty acid biomass in the diet.

[2]unpublished data provided by S. Iverson, Dalhousie University

[3]Values are mean calibration coefficients ± SE of the 13 major fatty acids used in analyses. Calibration coefficients that do not share a common superscript are significantly different between Caspian terns and common murres ($p < 0.0013$; Bonferroni-corrected Welsh's two-sample t-tests). Caspian tern calibration coefficients listed separately by diet were statistically different depending on diet treatment ($p \leq 0.0014$; Bonferroni-corrected Welsh's two-sample t-tests).

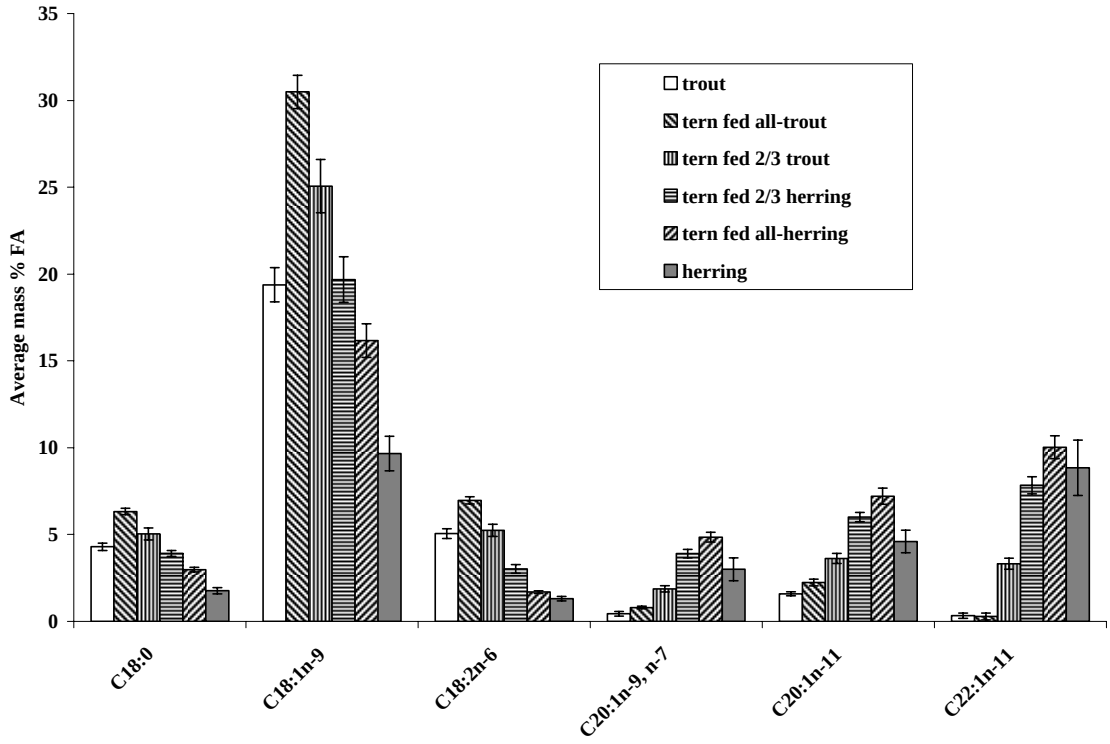

Figure 2.1. Mean fatty acid levels in adipose tissue of 30 day-old Caspian tern chicks after 20 days on their respective diets, with dietary fish fatty acid levels for reference. Only those fatty acids whose levels differed among all 4 diet treatment groups are shown ($p < 0.0006$, fixed-effects ANOVA and Tukey pairwise comparisons).

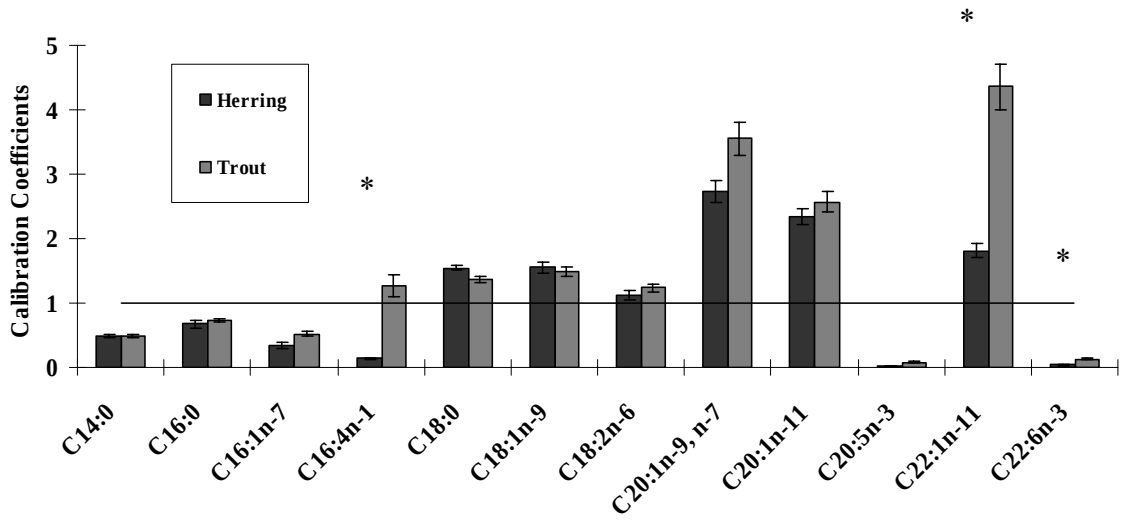

Figure 2.2. Calibration coefficients from 44 day-old Caspian tern chicks raised on single species diets of herring or trout for 34 days. Error bars are 95% confidence intervals. Fatty acids that show a statistically significant effect of diet treatment on calibration coefficients are denoted by an asterisk (*), ($p \leq 0.0014$, Welsh's 2-sample t-tests).

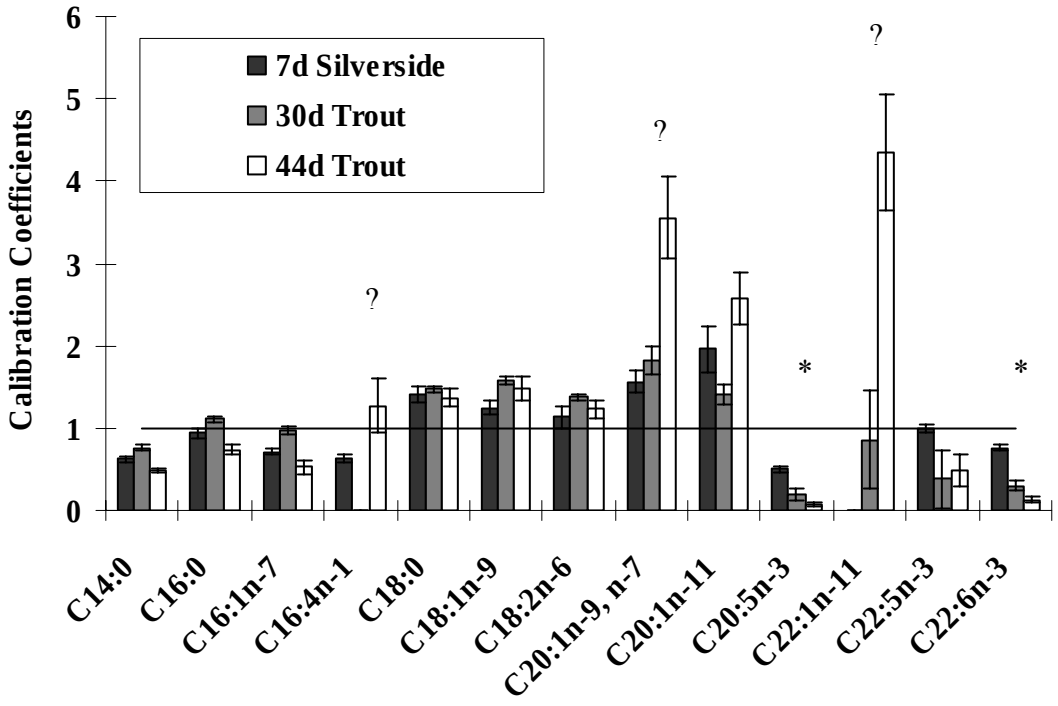

Figure 2.3a. Fatty acid calibration coefficients for silverside-fed Caspian tern chicks at 7 days post-hatch and trout-fed chicks at 30 days and 44 days post-hatch for 13 major fatty acids. Error bars are 95% confidence intervals. Fatty acids that potentially indicate a residual effect from early silverside diets on calibration coefficients are denoted by an asterisk (*). Fatty acids whose calibration coefficients are suggestive of a residual effect of silversides are denoted by a question mark (?).

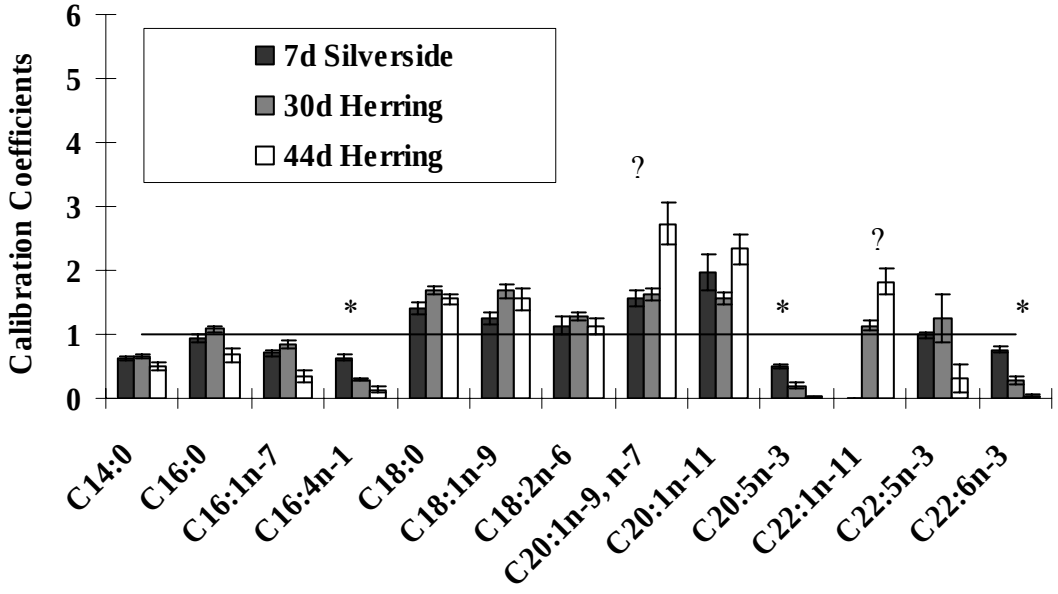

Figure 2.3b. Fatty acid calibration coefficients for silverside-fed Caspian tern chicks at 7 days post-hatch and herring-fed chicks at 30 days and 44 days post-hatch for 13 major fatty acids. Error bars are 95% confidence intervals. Fatty acids that potentially indicate a residual effect from early silverside diets on calibration coefficients are denoted by an asterisk (*). Fatty acids whose calibration coefficients are suggestive of a residual effect of silversides are denoted by a question mark (?).

CHAPTER 3

USING FATTY ACIDS TO INFER DIET COMPOSITION OF PISCIVOROUS BIRDS: FATTY ACID SIGNATURES OF CASPIAN TERNS AND THEIR PREY

Anne Mary Myers

ABSTRACT

We tested whether the fatty acid signature (FAS) technique can detect the presence of fishes of conservation concern in the diets of piscivorous waterbirds. We also investigated whether the FAS technique can be used to detect changes in diet composition of piscivorous waterbirds within a breeding season. There were significant differences in FASs of the predominant fish prey types in diets of Caspian terns (*Hydroprogne caspia*) during the breeding season: juvenile salmonids (*Oncorhynchus* spp.), including several threatened species; surf smelt (*Hypomesus pretiosus)*; and northern anchovy (*Engraulis mordax)*. The levels of only a few fatty acids, however, differed among the three species that comprised most of the salmonid portion of the diet (coho salmon [*O. kisutch*], yearling Chinook salmon [*O. tshawytscha*], and steelhead [*O. mykiss*]), probably due to the relative homogeneity of commercial foods fed to hatchery-reared smolts. Wild steelhead smolts, however, had significantly higher levels of highly unsaturated fatty acids (HUFAS) compared to their hatchery-reared counterparts. Declines in the proportion of wild steelhead in the diet, however, were not reflected in declines in HUFA levels in terns, apparently because other prey types in the diet (marine fishes) were also high in HUFAs. We detected significant changes in FA levels of nesting Caspian terns over the breeding season, which corresponded with observed shifts in prey composition at the nesting colony. These results suggest that the FAS technique can be used to qualitatively detect within-season changes in prey composition of piscivorous birds over periods of months. It may be possible to model the percentage of juvenile salmonids (as a generic prey type) in the diets of piscivorous birds using the FAS

technique. Our results indicate, however, that the percent composition of various wild and hatchery-reared salmonid species can not be estimated using the FAS technique.

INTRODUCTION

Fatty acid (FA) composition of seabird adipose tissue is known to be affected by the FA composition of the diet (Iverson and Springer 2002; Dahl et al. 2003; Kakela et al. 2005). The analysis of FA composition or fatty acid signatures (FAS) in predator tissues has been proposed as a non-lethal method to study trophic relationships, as well as spatial and temporal patterns in diet composition of free-ranging marine mammals and seabirds (Iverson 1993; Iverson et al. 1997; Raclot et al. 1998; Iverson and Springer 2002; Dahl et al. 2003). FASs have been used to verify the presence of particular fishes and other prey types in the diets of both mammals and birds (Johnson and West 1973; Rouvinen and Kiiskinen 1989; Wamberg et al. 1992; Colby et al. 1993; Pond et al. 1995; Raclot et al. 1998). Additionally, the FASs of certain fishes and invertebrates that serve as prey for seabirds and marine mammals have been used to distinguish these prey in the diet to the level of species (Iverson et al. 1997; Budge et al. 2002; Iverson et al. 2002).

The FAS technique can potentially provide advantages over traditional methods for quantifying diet composition in free-ranging piscivorous birds; first by eliminating the need to sacrifice subjects to secure samples of stomach contents, and second by avoiding biases inherent in stomach contents analyses. The latter includes representation of only the most recently consumed prey in stomach contents and under-representation of prey with easily digested diagnostic parts (Jobling and Breiby 1986; Springer et al. 1996).

Multivariate analysis of FA profiles has the potential to provide qualitative information on the composition of prey types in the diets of piscivorous waterbirds and other predators. Recently, a statistical model (QFASA) was published that uses the FAS of predators to estimate the quantitative composition of prey in the predator's diet, based on a catalogue of prey types with known FAS (Iverson et al. 2004).

Quantifying diet composition of piscivorous birds can provide resource managers with valuable information about the impact of avian predation on threatened and endangered salmonids (Collis et al. 2002; Roby et al. 2003), informing management decisions for restoration. Since 1997, on-going studies have documented the diet composition of Caspian terns (*Hydroprogne caspia*) nesting in the Columbia River estuary, primarily due to concern over the annual consumption of millions of juvenile salmonids (*Oncorhynchus* spp.) by this predator (Collis et al. 2002; Roby et al. 2002; Roby et al. 2003). A decision by managers to relocate the Caspian tern breeding colony at Rice Island to an island closer to the river mouth, East Sand Island, reduced by almost 50% the proportion of juvenile salmonids in the diet of Caspian terns, due primarily to the increased availability of marine forage fishes in proximity to East Sand Island (Roby et al. 2002). Despite decreased reliance on juvenile salmonids by terns nesting on East Sand Island, the annual consumption of millions of juvenile salmonids by piscivorous waterbirds in the lower Columbia River continues to be of concern to fisheries managers and others with a stake in the recovery of salmonid stocks listed under the U.S. Endangered Species Act.

Changes in the predominant types of fish consumed by Caspian terns nesting at East Sand Island occur during the breeding season (Collis et al. 2002; Roby et al. 2002).

Documentation of these dietary changes has revealed shifts in relative availability of various forage fishes in the Columbia River estuary, including the relative availability of various salmonid species. Detailed data on seasonal changes in diet composition also allow estimation of numbers of juvenile salmonids taken annually by Caspian terns, using bioenergetics modeling (Roby et al. 2003). Diet composition of terns at this breeding colony has been measured systematically throughout the breeding season using a combination of non-lethal and lethal techniques. Identification of fish in tern bill-loads is conducted from observation blinds at the breeding colony to obtain large samples of prey identified to the taxonomic level of family. Caspian terns that are collected using shotguns provide prey samples that can be identified to the taxonomic level of species, including various species of juvenile salmonids (Collis et al. 2002; Roby et al. 2002).

Predator FASs will not immediately reflect a shift in prey composition, and the length of time necessary for predator FAS to integrate dietary FAs varies depending on the predator tissue sampled. A change in diet from demersal fishes to pelagic fishes could be detected in the FAS of blood plasma from captive herring gulls within 5 days (Kakela et al. 2005). Myers et al. (in prep) demonstrated that an abrupt shift in proportion of two fish types in the diet could be detected in the FAS of adipose tissue from captive-reared Caspian terns within two weeks. It is unknown, however, whether a gradual dietary shift involving multiple prey types could be detected from the FASs of wild piscivorous birds measured over the breeding season.

The goal of this study was to evaluate the FAS technique for identifying fish prey types prevalent in the diet of wild Caspian terns. We investigated between- and within-species variability in FASs of 3 prey fish types that were most prevalent in the diets of

terns over the breeding season, and tested whether the FAS technique could distinguish these fish prey types in the diet. Particular attention was paid to the FASs of the 3 salmonid species that made up the bulk of the salmonid portion of the diet. We predicted that FASs of fish prey would be significantly different among prey types, but not within prey types. We also predicted that hatchery-reared and wild smolts within each salmonid species could be distinguished by FASs. If confirmed, the FAS technique might be used to determine the relative prevalence of wild and hatchery salmonids in Caspian tern diets.

We also sought to determine the potential of the FAS technique for detecting temporal shifts in diet composition of piscivorous waterbirds within a breeding season. Specifically, we investigated whether FASs in adipose tissue of Caspian terns breeding in the Columbia River estuary changed significantly in response to dietary shifts over the breeding season. We predicted that the FASs of wild Caspian terns would reflect temporal changes in the composition of prey in the diet during the breeding season.

METHODS

Tern Diet Composition

We measured the mean contribution of individual prey types to diets of Caspian terns nesting at East Sand Island, Oregon (46º15’45”N, 123º57’45”W) during two stages of the 2003 breeding season when samples of tern adipose tissue were collected for fatty acid analysis: incubation (23 April - 25 May) and chick-rearing (19 June - 25 July) (Table 3.1). Diet composition of Caspian terns nesting at the East Sand Island colony was

measured throughout the breeding season by visual identification of bill load fish transported to the colony by adult terns (Roby et al. 2002). Estimates of the relative contribution of the various salmonid species to the diet throughout the season were based on identification of specimens collected by shotgun for stomach content analyses (Roby et al. 2002).

Values for mean percent diet composition were calculated based on average daily diet composition observed at the breeding colony for the 7-day period prior to collection of tern tissue samples. We decided to base diet composition on the previous 7-day period because the FASs of captive terns fed treatment diets changed significantly within 14 days of a change in diet composition (Myers et al., in prep). Diet composition during the 14-day period prior to sample collection, however, was quite similar to diet composition during the 7-day period prior to sample collection. Values used to calculate average percent composition of prey for the two sampling periods were weighted based on the frequency of samples collected per sampling date, as a percent of all samples analyzed within a sampling period. The contribution of various prey types to diets of Caspian terns was calculated using three separate metrics: percent of total prey items, percent of total prey biomass, and percent of total lipid in diets. Percent of total prey items is based on tern bill loads visually identified during delivery to the nesting colony by breeding adults (see Collis et al. 2002 for detailed methodology). Percent of total prey biomass is based on bill load identifications and average mass of each prey type from collected bill load prey items. Percent of total lipid in the diet is based on percent biomass of each prey type and average percent lipid of fresh mass for each prey type (see Roby et al. 2003 for detailed methodology).

Prey Fish Collection

The following groups of fish, representing major prey types consumed by Caspian terns breeding at East Sand Island, Oregon during the 2003 breeding season, were analyzed for fatty acid composition:

1. Coho salmon (*Oncorhynchus kisutch*) smolts

2. Chinook salmon (*O. tshawytscha*) smolts

3. Steelhead (*O. mykiss*) smolts

4. Surf smelt (*Hypomesus pretiosus*)

5. Northern anchovy (*Engraulis mordax*)

Samples of each of the three salmonid species included specimens with the adipose fin removed and specimens with the adipose fin intact. Those with the adipose fin removed were assumed to have been raised in a hatchery; those with intact adipose fins may either have been raised in the wild or raised in a hatchery and not fin-clipped prior to release in the wild.

Specimens of juvenile salmonids were recovered from Caspian terns that were collected by shotgun while commuting to the large breeding colony on East Sand Island with fish in their bill during the 2003 breeding season. Surf smelt and northern anchovy were collected on June 10, 2003 by purse seine during a routine NOAA Fisheries forage

fish survey in the Columbia River estuary near East Sand Island. Fresh mass and fork length of each fish were measured before the samples were placed in individual bags, sealed, and temporarily stored for a few hours on ice until the specimens could be transported from the field location, frozen at -20°C for up to a week, and subsequently stored at -80°C until the samples were lyophilized. Fish prey were identified to the species level and confirmed by Paul Bentley, NOAA Fisheries, Hammond, Oregon.

Predator Adipose Tissue Sampling

Samples of subcutaneous adipose tissue were collected from the carcasses of adult Caspian terns shot for diet composition analyses during the 2003 breeding season. Subcutaneous fat samples of approximately 1 g were excised from the abdominal, synsacral, or furcular regions of carcasses with acetone-cleaned stainless steel instruments, placed in 16 x 125mm Kimax tubes with Teflon-lined caps, stored on ice for up to a few hours until transported to the field station, temporarily frozen at -20°C for up to a week, and subsequently stored at -80°C until samples were freeze-dried. The body region from which adipose tissue was sampled sometimes varied between individual birds in order to obtain samples not contaminated by steel shot or debris. Iverson and Springer (2002) found that mesenteric, breast, and subcutaneous synsacral fat sampled from individual red-legged kittiwakes (*Rissa brevirostris*), black-legged kittiwakes (*R. tridactyla*), common murres (*Uria aalge*), and thick-billed murres (*U. lomvia*) were similar within an individual. This supports our assumption that FASs from various fat storage depots in Caspian terns do not differ.

Samples of tern adipose tissue were selected for analysis from two sampling periods during the 2003 breeding season: incubation (23 April - 25 May) and chick-rearing (19 June - 25 July). Caspian tern diets were dominated by juvenile salmonids (42% of prey items) during the early tern sampling period, and by northern anchovies (43% of prey items) during the late sampling period (Table 3.1). Adipose tissue samples were selected for analysis with preference for samples taken from birds whose stomach contents contained the dominant fish prey type at the time of sampling.

Laboratory Methods

Lipid Extraction - Prey fish and Caspian tern adipose tissue samples were shipped frozen on dry ice to the Center of Marine Biotechnology, Baltimore, Maryland, for analysis in the laboratory of Professor A.R. Place. Fish and tern tissue samples were lyophilized (freeze-dried) and subsequently stored at –80°C. Care was taken not to allow samples to contact plastics; each frozen fish was homogenized in a Waring blender. Total lipids were extracted from 500-mg aliquots of fish and from tern fat samples ranging from 0.5 to 1.5 g using a modified Bligh and Dyer (1959) method, the most frequently-used method for determining total lipids in biological tissues (Smedes and Askland 1999; Iverson et al. 2001). The Bligh and Dyer method is preferred over the Folch (1957) method because of a significantly lower solvent to sample ratio (Iverson et al. 2001). Although the Bligh and Dyer method may significantly under-represent total fat content of samples with greater than 2% fat content compared with the Folch method, no differences were detected in fatty acid composition between the two methods (Iverson et

al. 2001; Iverson et al. 2002). Total lipids were extracted using a CH_2CL_2/MeOH (1:1) mixture of at least 5 times the sample volume. Samples were manually sonicated by glass probe, and extracted using 2 x 40-min. extractions in a sonicating water bath at 40°C. Particulates were removed from the sample by vacuum through a Whatmann glass filter. Distilled water was added to the sample to arrive at an overall ratio of 1:1:0.8 (CH_2CL_2:MeOH:H_2O). After mixing and phase separation, the organic (lower) layer was recovered, followed by 2 extractions with CH_2CL_2:MeOH (3:1), and dried under N_2 gas. Sample mass was noted in order to determine lipid content of fish samples and lipid concentration of the mixture. The neutral lipid residue was brought to 1 ml in CH_2CL_2/MeOH (1:1), flushed with N_2 gas, and stored at -4^oC in amber glass vials with Teflon-lined caps labeled with the lipid/solvent concentration.

Preparation of FAMES - Five hundred µg of the lipid extract was saponified/trans-esterified and used to generate fatty acid methyl esters (FAMES). Ten µg of C19:C21 internal standard (5 µg of each FA) and the lipid extract were added to glass tubes and dried under N_2. Saponification of lipids was accomplished by adding 4 ml KOH-saturated MeOH and 0.5 ml dH_2O to each tube, which was then capped with a Teflon-lined screw cap under a stream of N_2 gas and incubated at 70^oC for 1 h. After cooling, 0.5 ml of dH_2O was added and neutral lipids were removed by extracting 3x with 1.5 ml hexane:ether (9:1). Charged lipids were then recovered by lowering the *p*H of the mixture remaining in the tubes to < 2 by drop-wise addition of concentrated HCl, repeating the extraction (3x) with hexane:ether (9:1), and removing the upper phase in each extraction. Neutral lipids were stored at -4^oC for a separate study, and charged lipids were dried under N_2. Methylation of charged lipids was accomplished by the

addition of 1 ml 14% BF3:CH_3OH solution to each sample of dry free fatty acids, the tubes capped with Teflon-lined screw caps, and incubated at 40°C for 2 h. After cooling, 1 ml dH_2O was added to each tube and FAMES were removed by extracting 3x with 1.5 ml hexane:ether (9:1). After mixing samples by vortexing, the organic phase containing FAMEs was recovered to new glass tubes, dried under N_2, resuspended in 500 µl hexane, transferred to screw-top GC sample vials, and stored at -80°C until analysis.

Sample Analysis - Identification of FAMEs was accomplished by comparing gas chromatography retention data with authentic quantitative standards from NU-CHECK, Inc. (stds 3B, GLC-68D, GLC-17AA') and qualitative standards from Matreya (PUFA No. 1 and 2 – Marine Source). Quantitative standards, for which we were able to verify peak identities based on the manufacturer's designated % peak area, included 8:0, 10:0, 12:0, 14:0, 14:1, 16:0, 16:1, 17:0, 18:0, 18:1n-9, 18:1n-7, 18:2n-6, 18:3n-3, 20:0, 20:1n-9, 20:2n-6, 20:4n-6, 20:5n-3, 22:0, 22:1n-9, 22:6n-3, 24:0, and 24:1. In some samples, additional peaks, including 18:5n-3 and 22:1n-11, were also confirmed by GC-MS. We used a Hewlett-Packard 6890 GC equipped with a 30 m x 0.25 mm I.D. capillary column with 0.25 mm film thickness (DB Wax, J & W Scientific, Folson, CA) and a flame ionization detector at 300°C. The GC was run in 'constant flow rate' mode at 1.5 ml min^{-1} with hydrogen as the carrier gas. The column temperature profile was as follows: 50°C for 0.5 min, hold at 195°C for 15 min after ramping at 40°C•min-1, and hold at 220°C for 7 min after ramping at 2 °C•min-1. Total runtime was 38.13 min.

Chromatograms were individually corrected and reintegrated as required. Sample fatty acid concentrations are represented as mass percent of total fatty acids and are

identified by abbreviation: carbon chain length, number of double bonds, and location of the double bond nearest the terminal methyl carbon.

Data Analysis and Statistical Methods

Although 40-50 individual fatty acids were quantified in Caspian tern adipose tissue and in prey fish samples, a subset of fatty acids was selected for analyses based on their percent biomass in samples of total fatty acids. Only fatty acids that could be reliably identified and that comprised ≥ 1% of the total mass of fatty acids in at least one of the two samples of tern adipose tissue or one of the five prey fish types were used in statistical analyses. Thirteen fatty acids met these criteria: 14:0, 16:0, 16:1n-7, 16:4n-1, 18:0, 18:1n-9, 18:2n-6, 20:1n-9 and n-7 (combined), 20:1n-11, 20:5n-3, 22:1n-11, 22:5n-3, and 22:6n-3. These 13 fatty acids represented the great majority of the total mass of fatty acids in the samples.

Differences in FA profiles of tern adipose tissue between the two sampling periods were evaluated using MANOVA. This was followed by performing Welsh's 2-sample t-tests to detect differences in individual FAs between samples, with a Bonferroni correction factor to account for multiple comparisons. Differences in lipid content of the major prey fish types were evaluated using a fixed-effects ANOVA and Tukey's pairwise comparisons with a Bonferroni correction factor to account for multiple comparisons. FA profiles of salmonids known to be hatchery-reared (with a clipped adipose fin) were compared among salmonid species by MANOVA. We used Welsh's two-sample t-tests to evaluate whether hatchery-reared smolts vs. wild smolts could be distinguished among

coho salmon, yearling Chinook salmon, and steelhead by FASs, and used a Bonferroni correction factor to account for multiple-comparisons. If results of this analysis did not reveal significant differences between rearing types within a salmonid species, samples of the species were pooled, regardless of rearing type, for that prey type. FA profiles of the resultant major prey fish types (i.e., surf smelt, northern anchovy, coho salmon, yearling Chinook salmon, hatchery-reared steelhead, and wild steelhead) were evaluated by MANOVA, followed by fixed-effects ANOVA and Tukey's pairwise comparisons to detect differences in levels of individual FAs between groups, with a Bonferroni correction factor to account for multiple comparisons. We should note that typically univariate analyses for correlated outcomes, as the FAs certainly are, can result in serious Type I errors (Rencher 1995). Because the p-values resulting from the univariate tests are very low, however, we take this as protection against these errors.

RESULTS

Tern Diet Composition

The diet composition of Caspian terns nesting at East Sand Island, Oregon during the two tern sampling periods is listed in Table 3.1. Diet composition differed substantially between the early and late stages of the breeding season. The contribution of each prey type to Caspian tern diets varied somewhat depending on the method for measuring diet composition: the percent by frequency of total prey items in the diet, percent by contribution to total biomass of the diet, or percent by contribution to total

lipid in the diet (Table 3.1). Juvenile salmonids dominated the diet during the early sampling period in terms of percent of total prey items, percent of total biomass, and percent of total lipid intake. During the late sampling period, northern anchovy was the single most prevalent prey type, regardless of the method used to calculate diet composition.

Prey Fish Fatty Acid Levels and Lipid Content

FA profiles of hatchery-reared (clipped adipose fin) coho salmon, yearling Chinook salmon, and steelhead smolts did not differ significantly ($p = 0.5214$, MANOVA). We then compared the FA profiles of hatchery-reared smolts (clipped adipose fin) with their apparent wild conspecifics (intact adipose fin) for coho salmon, yearling Chinook salmon, and steelhead smolts. For steelhead, levels of 9 of 13 major FAs differed between adipose fin-clipped and non-adipose fin-clipped smolts ($p < 0.0038$, Welsh's 2-sample t-test, Table 3.2). There were no significant differences in FA levels between the hatchery-reared and apparent wild conspecifics for either coho salmon or yearling Chinook salmon ($p > 0.0038$, Welsh's 2-sample t-tests). Consequently, adipose fin-clipped and non-clipped smolts were combined in the coho salmon and yearling Chinook salmon samples for further analyses.

Average lipid content (% dry mass) varied among the five prey fish types, although lipid content was only significantly different between hatchery-reared steelhead smolts (high lipid content) vs. yearling Chinook salmon, wild steelhead, and smelt (relatively low lipid content) ($p < 0.005$, fixed-effects ANOVA and Tukey pairwise comparisons; Table 3.3). Coho salmon were excluded from the analysis due to high

variability in lipid content, which violated the equal variance assumption of ANOVA. The average lipid content of hatchery-reared steelhead was much greater and did not overlap with that of wild steelhead (Table 3.3), suggesting that all fish in the wild steelhead sample were likely raised in the wild. Average lipid content of coho and Chinook salmon with intact adipose fins were not different from those of hatchery-reared conspecifics (Table 3.3), suggesting that the samples of apparent wild smolts for these two species probably included both hatchery-reared and wild fish.

Differences in FASs of hatchery and wild steelhead were apparently due to differences in diet of the two groups. Both groups of juvenile steelhead had low within-group variation in FASs, compared with that of coho and chinook salmon, based on coefficients of variation (Table 3.4). Both coho salmon and yearling Chinook salmon tended to have higher variability in FA levels within samples of smolts with intact adipose fins compared with their hatchery-reared counterparts (based on coefficients of variation, Table 3.4). For Chinook salmon smolts in particular, samples of both hatchery-reared and apparent wild smolts had high coefficients of variation for FA levels (Table 3.4). This may explain the lack of a difference in FA levels between adipose fin-clipped and non-adipose fin-clipped smolts from these two species. The two marine forage fishes, anchovy and smelt, both had generally lower within-group variation in FA levels (Table 3.4).

There were differences among the FASs of the three primary prey fish types in the diets of Caspian terns during the 2003 nesting season: juvenile salmonids, surf smelt, and northern anchovy (Table 3.2). Salmonids were further subdivided into 4 fish prey types: coho salmon, yearling Chinook salmon, hatchery steelhead, and wild steelhead (see

above). Variation in FASs among prey types was much greater than variation within prey types ($p < 0.0001$, MANOVA). FASs of the two marine forage fishes, surf smelt and northern anchovy, were different based on levels of 4 of 13 major fatty acids. Smelt and anchovies each differed from all the predominantly freshwater juvenile salmonid prey types by at least 3 FAs ($p < 0.0003$, fixed-effects ANOVA and Tukey pairwise comparisons). Generally, the levels of more fatty acids were different between the marine fishes and salmonid smolts than between the two marine fishes ($p < 0.0003$, fixed-effects ANOVA and Tukey pairwise comparisons; Table 3.2).

We observed higher levels of n-3 highly-unsaturated FAs (HUFAs) (20:5n-3, 22:5n-3, 22:6n-3) in wild steelhead smolts compared with their hatchery-reared counterparts. Although other salmonid prey types were distinguishable based on FASs, it was generally only by a few FAs. Hatchery steelhead and yearling Chinook salmon, plus wild steelhead and Chinook salmon each differed in levels of only 2 fatty acids; coho salmon and Chinook salmon differed by only 1 FA (Table 3.2). FASs of coho salmon and hatchery-reared steelhead were not distinguishable. Of the salmonid prey types sampled, we observed the greatest differences in FASs between wild steelhead and the other three salmonid prey types (Table 3.2).

Fatty Acid Levels in Tern Adipose Tissue

FASs of Caspian tern adipose tissue changed significantly during the 2003 breeding season ($p < 0.0001$, MANOVA, Table 3.5). FA levels of nesting terns early in the breeding season (23 April – 25 May), when salmonids contributed 51.6% of total lipid intake, differed from those collected late in the season (19 June - 25 July) ($p <$

0.0038, Welsh's 2-sample t-tests, Table 3.5), when northern anchovy contributed 42.6% of total lipid intake (Table 3.1). Average levels of eight of the 13 major FAs (14:0, 16:4n-1, 18:1n-9, 18:2n-6, 20:1n-9 and n-7, 20:1n-11, 20:5n-3, and 22:1n-11) differed between early and late season sampling periods (Table 3.5). Changes in levels of six of these eight FAs corresponded with the difference in level of each of these FAs between the two major prey fish types (salmonid smolts vs. anchovy) consumed by terns during the respective periods of the breeding season (Tables 3.2 and 3.5). Fatty acids 18:1n-9, 18:2n-6, and 22:1n-11 changed the most from the early sampling period to the late period (Figure 3.5). Large differences in levels of the FAs 18:1n-9 and 18:2n-6 between terns collected during the early season vs. the late season corresponded with differences in levels of these FAs between salmon smolts and anchovy, the dietary items most prevalent in the diets of terns during these two periods. The substantial difference in levels of 22:1n-11 between terns from the two sampling periods, however, did not reflect differences in the levels of this FA between salmonids and anchovy, as levels of 22:1n-11 were similar in these two prey types.

DISCUSSION

Fatty Acid Signatures of Major Prey Fish Types

There were significant differences in FASs among the three fish prey types that were most prevalent in the diet of Caspian terns during the 2003 nesting season (juvenile salmonids, northern anchovy, and surf smelt). The levels of few FAs differed among the

three species of juvenile salmonids that comprised the bulk of this prey type, however, and none differed between hatchery steelhead and coho salmon smolts. There were, nevertheless, distinct differences in levels of highly-unsaturated FAs (HUFAs) (20:5n-3, 22:5n-3, and 22:6n-3) between wild steelhead and all other salmonid types. Observed differences between the 3 major categories of prey fish were expected, as between-species variation in FASs is generally greater than within-species variation in fish prey assemblages (Budge et al. 2002; Iverson et al. 2002). Thus, it may be possible to model the percentage of juvenile salmonids (as a generic prey type) in the diets of piscivorous birds using the FAS technique.

FASs were not different among the three species of juvenile salmonids (coho salmon, yearling Chinook salmon, and steelhead) for those smolts raised in hatcheries. Inability to detect species differences for hatchery-reared fish using the FAS technique is likely due to similarity in commercial feeds fed to hatchery-reared fish. There is, however, some variation in commercial feeds, their nutritional content, and the sources of marine oil in feeds used to raise different species of hatchery-reared juvenile salmonids in the Columbia River Basin (ODFW 2003). Because it is estimated that over 60% of juvenile salmonids that survive to the estuary are raised in hatcheries (NOAA 2006), distinguishing salmonid species in the diet using the FAS technique is problematic. Our results indicate, therefore, that the percent composition in the diet of the different salmonid species and rearing types can not be determined using the FAS technique.

We did not detect significant differences in FASs between smolts that had an intact adipose fin (apparently wild) and those that did not (hatchery-reared) for either coho salmon or yearling Chinook salmon. These results likely reflect the presence of

hatchery-reared smolts in the samples of smolts with intact adipose fins. Not all hatchery-reared smolts have their adipose fins removed prior to release (FPC 2003). For hatchery-reared coho, the proportion that have their adipose fin removed is only 67% (FPC 2003), and the overall percentage of wild smolts in the run is estimated to be less than 10% (NOAA 2006).

Both coho salmon and, especially, yearling Chinook salmon had relatively high variability in FA levels within samples of fish that had intact adipose fins (Table 3.4). Also, average lipid content in apparent wild coho and yearling Chinook smolts overlapped lipid content values of their hatchery-reared counterparts (Table 3.3); lipid content of hatchery-reared fish would be expected to be consistently higher due to the high lipid content of commercial fish feeds (e.g., fish feed formularies BioOregon, Warrenton, OR), as compared with a diet of aquatic insects obtained in the wild. For yearling Chinook salmon smolts, both the hatchery-reared and apparent wild samples had a particularly high coefficient of variation associated with FA levels (Table 3.4). This may be because juvenile yearling Chinook are especially difficult to successfully feed in hatcheries and a variety of feeds may be used (Ann Gannam, USFWS Abernathy Fish Technology Center, pers. comm.).

We detected a major difference in FASs between two rearing types of juvenile steelhead, those that had their adipose fin clipped (hatchery-reared) and those that did not (apparent wild fish). These results are probably due to differences in diets and physiological condition between hatchery-reared and wild fish. For example, the lipid content of hatchery-reared steelhead was consistently much higher than that of wild steelhead (Table 3.3). Most of the unclipped juvenile steelhead that reach the Columbia

River estuary are of wild origin because approximately 89% of hatchery steelhead released in 2003 were reportedly adipose fin-clipped (FPC 2003).

We observed higher levels of n-3 HUFAs (20:5n-3, 22:5n-3, 22:6n-3) in wild steelhead smolts compared with their hatchery-reared counterparts. This finding is consistent with other studies. For example, Sheridan et al. (1985) and Li and Yamada reported that tissue FASs of steelhead and masu salmon (*Oncorhynchus masou*) changed from relatively low levels of HUFAs prior to the parr-smolt transformation to enrichment with long-chain poly-unsaturated fatty acids (PUFAs) in preparation for the transition from freshwater to saltwater, even in the absence of dietary change. However, Atlantic salmon parr fed diets containing fish oil (which is a common component of hatchery feed for Pacific salmonids) experienced an inhibited natural increase in hepatic fatty acid desaturase activity, resulting in lower tissue levels of HUFAs compared with parr fed diets that contained vegetable oil (Bell et al. 1997). This suggests that hatchery fish fed diets high in marine fish oils will not pass higher levels of HUFAs on to their predators. Our results suggest that the lower levels of HUFAs in hatchery-reared steelhead can be used to distinguish rearing status in steelhead smolts. Additionally, we observed significantly higher levels of 18:2n-6 in hatchery-raised steelhead compared to their wild counterparts, consistent with Ackman and Takeuchi's (1986) finding of increased levels of this FA in hatchery-reared vs. wild Atlantic salmon smolts.

Tern Fatty Acid Signatures

We detected significant changes in FA levels from adipose tissue of Caspian terns during the 2003 breeding season (over a period of 1-3 months), corresponding with shifts

in diet composition. The majority of fatty acids (6 of 8) whose levels changed significantly in terns over the breeding season were lower or higher consistent with FA levels in the major prey types consumed as the breeding season progressed (from primarily salmonids to primarily anchovy). Consequently, it is reasonable to infer that the seasonal shift in FASs of terns reflected a shift in fatty acid composition of the diet. Substantially higher levels of the other two FAs (20:1n-7 & n-9 and 22:1n-11) in terns late in the breeding season were not expected based on levels available in the three major prey types; anchovy did not contain higher levels of these two fatty acids compared to salmonids or smelt. Higher levels of these two FAs may have been due to another dietary component, possibly clupeids, whose prevalence in tern diets was higher during the late sampling period. Pacific herring (*Clupea pallasi*), a clupeid commonly consumed by terns in the Columbia River estuary, that were caught in British Columbia had much higher levels of both 20:1n-7 & n-9 and 22:1n-11 than were detected in the smelt, anchovy, and salmonid smolts analyzed in this study (Myers et al., in prep.).

Although our results suggest that the higher levels of HUFAs (20:5n-3, 22:5n-3, 22:6n-3) in wild steelhead can be used to distinguish wild from hatchery-reared steelhead smolts, differenes in HUFA levels in terns between the two sampling periods did not reflect the differences in the prevalence of steelhead smolts in the diet between the two periods (Table 3.1). Lower HUFA levels in terns would be expected late in the breeding season, when wild steelhead were nearly absent from the diet, yet HUFA levels were similar or higher in terns collected during the late sampling period (Table 3.5). Apparently, other dietary components (e.g., clupeids, surfperch) that are more prevalent in the diet late in the breeding season enhance the HUFA content of the diet, masking the

decline in intake of HUFAs from lower consumption of wild steelhead. Consequently, HUFAs cannot be used to track the proportion of wild steelhead smolts in the diets of Caspian terns nesting in the Columbia River estuary.

Myers et al. (in prep.) demonstrated that diet changes in captive-reared Caspian terns were reflected in changes in FASs within two weeks. FASs of adipose tissue from wild, free-ranging terns are unlikely to reflect diet changes so clearly. First, free-ranging Caspian terns consume a greater diversity of prey types than did the captive terns, which were fed only two very different food types (wild caught juvenile herring and hatchery-reared rainbow trout). Second, forage fishes consumed by piscivorous birds exhibit natural variation in fat content and FAS due to differences in diet, size, and sampling location (Anthony et al. 2000; Budge et al. 2002; Iverson et al. 2002); the two types of fish fed to captive terns were homogenous with respect to diet, size, and collection location. Finally, Caspian terns nesting in the Columbia River estuary did not experience an abrupt and uniform shift in proportion of prey types in the diet; rather, the dietary shift occurred gradually as the relative availability of fish in the vicinity of the breeding colony changed (CBR 2003). Forage fish availability in the Columbia River estuary is dynamic; it is affected by changing distributions of fish assemblages, which in turn are influenced by large seasonal variation in river discharge and salinity, plus seasonal changes in the abundance and species richness of fish prey (Bottom and Jones 1990). Forage fish availability to seabirds is also influenced by the reproductive cycles of marine and anadromous fish species, the timing of fish migrations, and differential use of various habitats and invertebrate prey by species assemblages within the Columbia River estuary (Bottom and Jones 1990).

In order to accurately estimate the total consumption of juvenile salmonids by Caspian terns in the Columbia River estuary, changes in diet composition over 2-week periods are key input to bioenergetic models. Our results suggest that the FAS technique alone cannot yield precise estimates of salmonid smolt consumption by Caspian terns and cannot provide the level of detailed information on shifts in diet composition that occur within the breeding season. These data are sought by fisheries and wildlife managers to inform management decisions for avian predators on salmonid smolts from throughout the Columbia River Basin.

CONCLUSIONS

1. We detected differences in FASs among the 3 fish prey types most prevalent in the diets of Caspian terns during the breeding season, suggesting that the FASs of these fish types are sufficiently different to qualitatively monitor predator diets with enough precision to differentiate broad prey types.

2. Estimating the percentage of juvenile salmonids (as a generic prey type) in the diet of piscivorous birds may be possible using the FAS technique. Accurate estimates of the contribution of various salmonid species and rearing types to the diets of avian predators, however, are not possible using the FAS technique. This is partly due to similarity in FASs of hatchery-reared salmonids, which comprise the majority of salmonids consumed by Caspian terns nesting in the Columbia River estuary.

3. Higher levels of HUFAs characterized wild steelhead smolts compared with their hatchery-reared counterparts; further studies will be required to determine whether HUFA levels differ significantly between hatchery and wild rearing types for other species of juvenile salmonids.

4. Because the FAS technique cannot provide sufficiently detailed information on diet composition and the seasonal shifts in diet that occur within the breeding season of Caspian terns, particularly the prevalence of various species of juvenile salmonids in the diet, the technique cannot be used to calculate precise estimates of the consumption of salmonid smolts by terns during the breeding season.

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Table 3.1. Diet composition[a] of adult Caspian terns nesting on East Sand Island, Oregon at two stages during the 2003 breeding season.

Metric	Time period	Smelt	All Salmonids	Anchovy	Clupeid	Sand Lance	Sculpin	Surfperch	Non-Salmonid (Unid)	*Steelhead*	*Sub-yearling Chinook*	*Yearling Chinook*	*Coho*
% total prey items[b]	4/23-5/25	25.6%	43.1%	6.3%	14.3%	5.2%	1.3%	3.1%	1.1%	10.9%	1.8%	13.4%	16.9%
	6/19-7/25	4.8%	17.1%	43.8%	20.9%	1.4%	2.9%	8.9%	0.1%	0.2%	7.1%	3.1%	6.6%
% biomass[c]	4/23-5/25	20.1%	56.7%	4.5%	13.7%	0.9%	1.0%	2.3%	0.9%	17.7%	0.8%	19.1%	19.1%
	6/19-7/25	4.5%	19.0%	41.2%	24.1%	0.3%	2.6%	8.1%	0.1%	0.4%	3.8%	5.4%	9.3%
% lipid intake[d]	4/23-5/25	22.2%	51.6%	5.0%	16.9%	1.0%	0.6%	1.8%	1.0%	15.8%	0.6%	13.9%	21.3%
	6/19-7/25	4.8%	16.5%	42.6%	28.2%	0.3%	1.6%	5.9%	0.1%	0.4%	2.6%	3.7%	9.7%

[a] Values for mean percent diet composition were calculated based on average daily diet composition observed at the breeding colony for the 7 day period prior to collection of tern tissue samples. Values used to calculate average percent composition of prey for the two sampling periods were weighted based on the frequency of samples collected per sampling date, as a percent of all samples analyzed within a sampling period.

[b] Percent of identifiable prey items based on bill loads visually identified during delivery to the nesting colony by breeding adults.

[c] Percent of total prey biomass based on bill load identifications and average mass of each prey type from collected bill load prey items.

[d] Percent of total lipid intake based on percent biomass of each prey type and average lipid content for each prey type.

Table 3.2. Mean values for fatty acid composition of prey fish for Caspian terns nesting in the Columbia River estuary during 2003, sampled near East Sand Island, Oregon. Values are mean mass percent ± SD of fatty acids used in statistical analyses. Fatty acid values that do not share a common superscript are significantly different ($p < 0.0003$, fixed effects ANOVA and Tukey pairwise comparisons).

	Coho (n = 10)	Chinook (n = 8)	Wild Steelhead (n = 5)
C14:0	2.64 ^{a} ± 0.724	2.53 ^{a} ± 1.499	0.74 ^{b} ± 0.320
C16:0	13.96 ^{a} ± 1.388	17.35 ^{bc} ± 2.607	15.87 ^{bc} ± 0.940
C16:1n-7	4.46 ^{ab} ± 1.177	5.03 ^{ab} ± 4.672	2.15 ^{a} ± 0.579
C16:4n-1	0.22 ^{ab} ± 0.138	0.13 ^{ac} ± 0.115	0.00 ^{a} ± 0.000
C18:0	4.98 ^{ab} ± 0.832	5.41 ^{a} ± 0.909	5.62 ^{a} ± 0.203
C18:1n-9	26.41 ^{a} ± 5.999	19.79 ^{ab} ± 10.550	10.99 ^{abc} ± 1.841
C18:2n-6	6.57 ^{ab} ± 2.597	4.94 ^{bc} ± 3.272	2.75 ^{cd} ± 0.849
C20:1n-9, n-7	1.60 ^{ab} ± 0.709	0.91 ^{ab} ± 0.830	0.38 ^{a} ± 0.092
C20:1n-11	0.21 ^{a} ± 0.021	0.13 ^{ab} ± 0.088	0.16 ^{b} ± 0.025
C20:5n-3	4.11 ^{a} ± 1.812	5.88 ^{ab} ± 3.650	9.43 ^{b} ± 1.860
C22:1n-11	0.86 ^{a} ± 0.540	0.35 ^{ab} ± 0.453	0.00 ^{b} ± 0.000
C22:5n-3	2.36 ^{ab} ± 1.162	1.88 ^{a} ± 1.122	3.69 ^{b} ± 0.614
C22:6n-3	12.20 ^{a} ± 5.159	20.76 ^{abc} ± 12.536	30.33 ^{bc} ± 4.565

	Hatchery Steelhead (n = 5)	Surf Smelt (n = 5)	Northern Anchovy (n = 5)
C14:0	4.05 ^{a} ± 1.072	3.21 ^{a} ± 1.143	8.73 ^{c} ± 0.562
C16:0	14.99 ^{ab} ± 1.763	18.10 ^{bc} ± 0.390	18.33 ^{c} ± 1.400
C16:1n-7	6.34 ^{abc} ± 1.302	7.25 ^{bc} ± 2.647	9.81 ^{c} ± 0.513
C16:4n-1	0.36 ^{bc} ± 0.120	1.22 ^{d} ± 0.111	2.36 ^{e} ± 0.338
C18:0	4.15 ^{bc} ± 0.242	4.83 ^{ab} ± 0.328	3.34 ^{c} ± 0.293
C18:1n-9	22.61 ^{a} ± 5.524	9.72 ^{bc} ± 1.399	5.41 ^{c} ± 0.283
C18:2n-6	8.62 ^{a} ± 0.965	1.38 ^{cd} ± 0.335	1.01 ^{d} ± 0.111
C20:1n-9, n-7	1.98 ^{b} ± 0.516	1.22 ^{ab} ± 0.456	0.86 ^{ab} ± 0.890
C20:1n-11	0.19 ^{a} ± 0.020	1.05 ^{c} ± 0.338	0.75 ^{c} ± 0.417
C20:5n-3	4.83 ^{a} ± 1.225	15.01 ^{c} ± 1.195	16.73 ^{c} ± 1.066
C22:1n-11	0.82 ^{ab} ± 0.547	0.98 ^{a} ± 0.569	0.70 ^{ab} ± 0.310
C22:5n-3	1.83 ^{a} ± 0.473	1.58 ^{a} ± 0.182	1.86 ^{a} ± 0.306
C22:6n-3	13.78 ^{a} ± 1.010	29.48 ^{c} ± 5.669	12.46 ^{a} ± 2.011

Table 3.3. Mean lipid content (% dry mass) of major fish prey types for Caspian terns nesting in the Columbia River estuary. Values that do not share a common superscript are significantly different ($p < 0.005$, fixed-effects ANOVA and Tukey pairwise comparisons). Coho salmon were not included in the analysis because high variability in lipid content violated the equal variance assumption of ANOVA.

Prey Type	Mean % Lipid Content of Dry Mass	Range	SD	N
Surf Smelt	5.39 [a]	2.3-8.9	2.75	5
Northern Anchovy	7.85 [ab]	4.9-11.9	2.57	5
Chinook Salmon	5.70 [a]	1.2-11.1	3.19	8
Coho Salmon	13.47	2.5-26.9	7.44	10
Hatchery Steelhead	10.49 [b]	7.8-15.2	2.99	5
Wild Steelhead (apparent)	3.51 [a]	2.5-4.4	0.77	5

Table 3.4. Coefficients of variation (standard deviation/mean x 100) for levels of 13 major fatty acids in primary prey types for Caspian terns in the Columbia River estuary.

	Smelt	Anchovy	Chinook (hatchery)	Chinook (wild)	Coho (hatchery)	Coho (wild)	Steelhead (hatchery)	Steelhead (wild)
C14:0	35.6%	6.4%	43.8%	71.1%	20.0%	36.1%	26.5%	43.0%
C16:0	2.2%	7.6%	17.1%	8.7%	3.6%	14.4%	11.8%	5.9%
C16:1n-7	36.5%	5.2%	51.2%	99.8%	21.9%	31.2%	20.5%	26.9%
C16:4n-1	9.1%	14.3%	95.1%	93.2%	40.0%	91.5%	33.2%	
C18:0	6.8%	8.8%	19.3%	0.3%	10.3%	21.3%	5.8%	3.6%
C18:1n-9	14.4%	5.2%	42.3%	82.4%	11.9%	26.6%	24.4%	16.7%
C18:2n-6	24.2%	11.0%	54.8%	94.6%	23.3%	53.4%	11.2%	30.9%
C20:1n-9, n-7	37.2%	103.8%	92.1%	108.7%	13.1%	62.3%	26.1%	24.2%
C20:1n-11	32.3%	55.6%	92.2%	27.6%	5.5%	11.1%	10.5%	15.4%
C20:5n-3	8.0%	6.4%	46.3%	63.5%	32.8%	37.1%	25.4%	19.7%
C22:1n-11	58.3%	44.1%	120.1%	173.2%	9.3%	80.3%	66.3%	
C22:5n-3	11.6%	16.4%	29.6%	59.3%	29.9%	53.3%	25.8%	16.6%
C22:6n-3	19.2%	16.1%	54.6%	82.7%	54.0%	25.7%	7.3%	15.1%

Table 3.5. Mean values for fatty acid composition of adipose tissue from adult Caspian terns sampled at East Sand Island, Oregon at two stages during the 2003 breeding season. Values are mean mass percent (± SD) of major dietary fatty acids used in statistical analyses. Fatty acids listed in bold type were significantly different for terns sampled early in the nesting season compared with terns sampled late in the nesting season ($p < 0.0001$, MANOVA; $p \leq 0.0038$, Welsh's 2-sample t-tests with Bonferroni correction).

	April 23-May 25 (n = 14)			June 19-July 25 (n = 8)		
C14:0	2.37	±	0.340	3.02	±	0.437
C16:0	20.84	±	1.196	21.20	±	1.750
C16:1n-7	5.38	±	0.776	6.48	±	2.269
C16:4n-1	0.69	±	0.133	1.20	±	0.198
C18:0	10.13	±	1.469	10.84	±	1.212
C18:1n-9	30.57	±	2.260	20.55	±	2.977
C18:2n-6	7.80	±	2.217	1.74	±	0.605
C20:1n-9, n-7	1.44	±	0.820	3.04	±	1.094
C20:1n-11	3.11	±	0.817	4.56	±	0.795
C20:5n-3	2.42	±	1.130	4.56	±	0.511
C22:1n-11	2.60	±	1.274	9.05	±	3.843
C22:5n-3	2.12	±	0.357	2.46	±	0.341
C22:6n-3	6.98	±	1.388	8.10	±	1.576

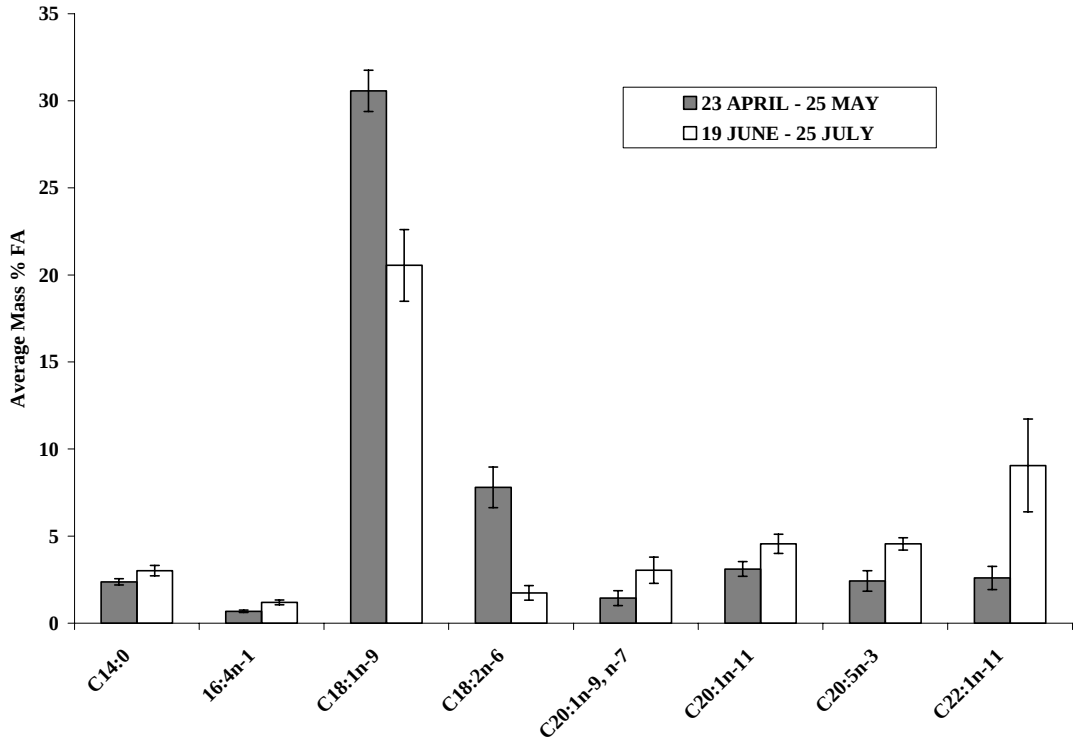

Figure 3.1. Mean levels of eight fatty acids in adipose tissue from adult Caspian terns sampled during two periods in the 2003 breeding season at East Sand Island, Oregon. Error bars are 95% confidence intervals. Levels of these eight fatty acids differed between the early season sampling period (23 April-25 May) and the late season sampling period (19 June-25 July) ($p \leq 0.0038$, Welsh's 2-sample t-tests with Bonferroni correction).

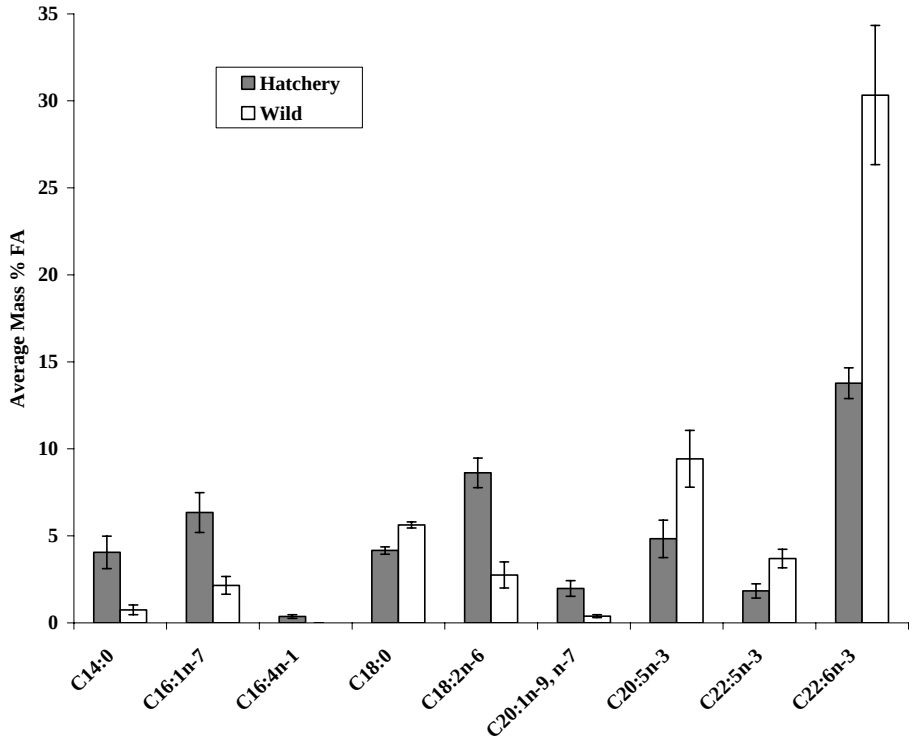

Figure 3.2. Mean levels of nine fatty acids that differed between hatchery-raised and wild steelhead smolts collected in the Columbia River estuary ($p < 0.0038$, Welsh's 2-sample t-tests with Bonferroni correction). Error bars are 95% confidence intervals.

CHAPTER 4

SUMMARY AND SYNOPSIS

Anne Mary Myers

This research was designed to evaluate of the Fatty Acid Signature (FAS) technique for studying the diets of piscivorous birds, specifically Caspian terns. The major objectives were: (1) to compare the FASs of Caspian terns fed different controlled diets in captivity; (2) to compare tern FASs with FASs of fish fed to terns; (3) to calculate fatty acid-specific calibration coefficients for Caspian terns, and (4) to investigate the performance of the FAS technique for determining the diet composition of piscivorous birds in the wild. Also, we sought to determine whether the FAS technique can distinguish fish species that are prevalent in the diets of Caspian terns nesting in the Columbia River estuary. Finally, we looked for differences in adipose tissue FASs of wild Caspian terns between the early and late stages of the breeding season, when differences in major dietary components were observed.

In Chapter 2, our results showed that FASs of piscivorous birds reflect the FASs of the diet. After 20 days, Caspian terns fed monotypic diets or mixed diets of two fish types displayed different FASs. When the diets were changed, adipose tissue FASs in terns reflected the dietary shift within two weeks. However, fatty acid-specific calibration coefficients (fatty acid level in the consumer divided by FA level in food) varied with diet, age, and species of piscivorous bird for some major fatty acids in the diet. This calls into question the accuracy of quantitative diet composition of predators determined using the QFASA technique, because calibration coefficients are necessary inputs to obtain reliable estimates of diet using QFASA models.

In Chapter 3, we demonstrated that three major prey types (salmonids, smelt, anchovy) consumed by Caspian terns breeding in the Columbia River estuary had different FASs. Also, the FASs of terns changed from early to late in the breeding

season, reflecting shifts in the proportions of these major prey types in tern diets. The FAS technique is limited in its ability to determine the diet composition of wild Caspian terns, however, because FASs were not different among some key prey types. The FASs of several species of juvenile salmonids, prey types of special concern, exhibited little variation, especially between salmonid species raised in hatcheries, which comprise the majority of smolts consumed by Caspian terns in the estuary. Nevertheless, we demonstrated that levels of highly-unsaturated fatty acids (HUFAs) are higher in wild steelhead compared with hatchery-reared steelhead. HUFA levels in terns, however, did not reflect the changing prevalence of wild steelhead in the diet, likely because other prey, such as marine forage fish, contributed HUFAs to the diet. Thus, HUFAs do not appear to be a useful indicator of wild steelhead in the diet of these birds.

Before undertaking a study of predator diets using FASs, researchers should be aware of potential limitations of the method. Adequate sampling of all potential prey items needs to be conducted concurrent with predator tissue sampling. Prey types of interest must have distinctly different FASs in order to be detected in the diet of predators based on the preator's FAS. However, what constitutes a distinctly different FAS is not easy to define. If the levels of only one fatty acid (FA) differ significantly between prey types, does that constitute a sufficient difference to allow inferences about a predator's diet composition? The more diverse and temporally variable the diet composition of a predator, the more difficult it will be to interpret its FAS.

Whether FASs of prey species of special concern are distinct from FASs of other prey types cannot be determined until FASs of all major potential prey types have been measured. Analysis of FASs is complex, time-consuming, and costly. Building an

adequate library of prey FASs could be a lengthy process, depending on the predator-prey system under study. There is no guarantee that the FAS of a prey type will remain consistent between seasons, years, and locations because food sources for prey species can vary, requiring the investigator to determine the level of temporal and spatial variation in FASs within prey types. It is highly questionable whether the FAS technique can detect short-term dietary shifts in predators with complex diets whose composition varies continuously. The FAS technique may be useful for documenting longer-term or major within-season changes in diet of piscivorous birds consuming prey with distinct FASs.

We did not attempt to validate the QFASA technique in this study, both because it was beyond the scope of this project, and because we were not able to obtain the model for our use. If the QFASA technique can be validated, it could be used to help estimate the impacts of birds foraging on prey species of special interest or conservation concern. In the Columbia River estuary alone, this could mean avoiding the collection of hundreds of piscivorous birds each year for stomach contents analysis. Additionally, the results of this study could be used to help document changes in diet composition of Caspian terns at sites where monitoring diet composition by collecting individuals for stomach contents analysis is not feasible or desirable. Once validated, the QFASA method could also facilitate detecting prey of conservation concern in the diets of birds at particular nesting sites. Additionally, the FASs technique could be used to study the diets of piscivorous birds with foraging strategies that do not allow for easy observation of prey items, such as birds that transport chick meals in their stomachs (e.g., cormorants). Double-crested cormorants are known to consume significant numbers of salmonid smolts in the

Columbia River Basin (Collis et al. 2002), where nearly all salmonids are of conservation concern.

The FAS technique could prove useful for providing a general idea of diet composition at breeding colonies visited only once per nesting season, due either to resource constraints or concern over disturbance of birds at nesting colonies. For example, it is common practice for researchers and wildlife managers to enter a bird colony on an annual basis to band fledglings for studies of survival and demography. Fat biopsies could be collected from nearly fledged birds concurrent with routine banding activities, without additional disturbance to the breeding colony or much additional handling time. Analysis of fat samples from fledglings could be used to make inferences about diet composition during previous weeks, or to detect the presence of particular prey types of interest (1) if adequate sampling of potential prey items was conducted concurrently and (2) provided the prey types of interest have distinct FASs. In this scenario, the FAS method could be useful for detecting inter-annual variation in the prey composition of bird diets.

Future Directions

Further studies will be needed to determine whether the variation in fatty acid-specific calibration coefficients associated with diet, age, and species of bird detected in this study impairs the accuracy of diet composition estimates using QFASA models. This could be accomplished by conducting a sensitivity analysis of QFASA to determine whether the models are robust to the magnitude of variation in calibration coefficients observed in this study. This validation seems imperative to ensure that the models are

correctly predicting diet composition of predators based on their FASs. Also, additional studies are needed to identify other factors that may contribute to variation in calibration coefficients, such as physiological or nutritional state.

An additional step in evaluating the QFASA technique could be to model the diets of the Caspian terns reared on controlled diets from this study. Because the FASs and diet composition of birds is known, and all prey FASs are known, it is possible to test how accurately models predict diet composition of birds with monotypic diets of fish as well as birds with mixed diets of two fish species. This would test how well models document diet composition of piscivorous birds with consistent diets of only a few prey types, when the prey have very different FASs. Additionally, our data could be used to evaluate two-source linear mixing models for accuracy in determining contribution of prey source FASs to bird predator FASs, as this method has been used previously to estimate diet composition of fish (Turner and Rooker 2005).

The next step in using FASs to study the diets of Caspian terns in the Columbia River estuary would be to complete the library of prey FASs by analyzing additional prey types that constitute > 5% of the lipid consumption of these birds. A study of the inter-annual variation in FASs of each prey type should also be undertaken. Once a more complete library of prey FASs is compiled, QFASA models, if available, could be run on FASs of wild birds at different times during the breeding season and model results compared with diet estimates from the more traditional methods that are currently used to provide information on tern diet composition to wildlife and fisheries resource managers. These further studies would be needed to validate the FAS technique; and attempting to validate the QFASA technique is warranted. However, given the similarity in FASs of

key prey types in the diets of Caspian terns, in particular salmonids, and the potential complications that variable fatty acid calibration coefficients could bring to modeling diet composition, investing further resources in attempting to validate the QFASA technique for studying diet composition of Caspian terns in the Columbia River estuary seems inappropriate.

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