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REVIEW

Using fatty acids as dietary tracers in seabird trophic ecology: theory, application and limitations

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Abstract Analysis of fatty acids (FAs) is an increasingly utilized tool in studies of trophic ecology in marine ecosystems. This powerful technique has proved useful in delineating spatial and temporal variability in diets, identifying the consumption of key species, and providing quantitative estimates of diet composition. Although consumer FA signatures are undeniably influenced by diet, they can also be affected by other factors including life-history stage, diet quality, and physiological state. Here, we review how FAs are assimilated, deposited, and metabolized in birds, and the implications of these processes on the various tissues commonly sampled for FA analyses. We then examine the assumptions underlying FA signature analysis when used in studies of seabird trophic ecology and propose a direction for future laboratory experiments that are needed to refine the approach. The correct interpretation of FA data relies on accounting for factors that alter predator FA metabolism and controlling for variability in the lipid content and FA composition of prey. Efforts should also be made to incorporate uncertainty associated with predator metabolism into models designed for quantitative diet estimation.

Keywords Fatty acid signature · Foraging ecology · Diet composition · Adipose tissue · QFASA

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Introduction

Seabirds are important consumers in marine ecosystems with annual prey consumption rivalling that of global fisheries landings (Brooke 2004). Understanding the role of seabirds in the structure and function of ecosystems requires detailed knowledge of diets, yet direct measures of prey consumption are extremely difficult to obtain, necessitating a reliance on indirect methods (Barrett et al. 2007). Conventional approaches, such as stomach content analysis, provide quantitative data on prey consumption but suffer from well-known, but difficult to avoid, biases associated with the retention of hard parts and the rapid digestion of soft-bodied organisms (Votier et al. 2003). Nestling diets can sometimes be observed directly, but are limited in temporal scope, and there is increasing evidence that they are often not reflective of adult diets (Williams et al. 2008a; Davies et al. 2009). Because of the inherent bias and limitations associated with conventional techniques, seabird ecologists are increasingly reliant on molecular trophic markers such as DNA-based fecal analysis (Deagle et al. 2007), stable isotope analysis (Hobson et al. 1994), and fatty acid (FA) analysis (Iverson et al. 2007). Use of FAs shows particular promise because of the development of a statistical model that allows quantitative estimation of diet (Iverson et al. 2004). Analysis of FAs can also be used to delineate spatial and/or temporal differences in diet (Wang et al. 2009a), to detect the consumption of a particular prey species (Raclot et al. 1998) or prey from a particular foraging guild (Käkelä et al. 2006; Budge et al. 2007), and to identify niche partitioning within (Williams et al. 2008a) and between species (Dahl et al. 2003).

FA signatures are used as dietary tracers most extensively, although not exclusively, in the marine environment

because of the diverse array of polyunsaturated FAs that originate in phytoplankton and are subsequently transferred up the food web. Monogastric predators are only capable of synthesizing certain FAs and, therefore, FA signatures of storage tissues largely reflect diet. Predator FA signatures will never directly match dietary FA composition because of de novo synthesis of FAs, FA modification, and selective catabolism and/or storage of FAs. However, the strong influence of diet on the FA composition of various tissues permits qualitative and quantitative dietary inferences to be made (reviewed in Budge et al. 2006). Quantitative FA signature analysis (QFASA; sensu Iverson et al. 2004) involves the use of a statistical model to determine the combination of prey FA signatures that comes closest to matching the predator FA signature, after accounting for the effects of FA metabolism. The QFASA model utilizes calibration coefficients (the ratio of a given FA in the consumer to that in the long-term diet) derived from controlled feeding experiments to account for predator metabolism.

A framework for the interpretation of FA data in seabirds comes from the extensive number of studies conducted in marine mammals. However, seabird ecologists need to be cognizant of differences between avian and mammalian FA metabolism to avoid potential pitfalls associated with the technique. This is especially true for obtaining species-level estimates of seabird diets using QFASA, where relatively small changes in the calibration coefficients and/or the subsets of FAs used in the model have sometimes produced quite different results (Nordstrom et al. 2009; Wang et al. 2009b). The primary assumptions underlying use of FAs in marine trophic ecology are that the effects of FA metabolism are predictable and, in the case of quantitative diet estimation, quantifiable. Some have questioned these underlying assumptions, suggesting that FA metabolism is too unpredictable to reliably use FAs as dietary tracers (Grahl-Nielsen 2009), although this assertion has been strongly repudiated (Thiemann et al. 2009). A large number of seabird studies that have utilized FA analyses involved validation with stomach content analyses or stable isotope data (e.g., Connan et al. 2007; Iverson et al. 2007; Käkelä et al. 2007; Karnovsky et al. 2008; Thiemann et al. 2008; Williams et al. 2008a). Nevertheless, changes in physiological state associated with periodic events including molt, incubation, migration, and nutritional stress may alter FA metabolism to some extent, and it is critical to consider alternative hypotheses when interpreting FA data. Additionally, ecologists must account for variability in the lipid content and FA signatures of potential prey species.

Use of FA signature analysis for studies of trophic interactions and diets in upper trophic level predators has been thoroughly reviewed by Budge et al. (2006) and Iverson (2009). The primary objective of this review/comment is to identify the assumptions underlying the technique and the conditions under which these assumptions are unlikely to be met. First, we briefly outline the structure and function of FAs and discuss how they are used as a tool in seabird ecology. Next, we outline how FAs are assimilated, deposited, and metabolized in birds and the implications of these processes on the FA composition of the various tissues commonly sampled for FA analyses. Finally, we discuss potential pitfalls associated with the technique and outline the experiments needed to further validate the approach.

Fatty acid structure

FAs are a common component of some of the most abundant lipids (e.g., triglycerides, phospholipids, and wax esters) that occur in animals. Most naturally occurring FAs in upper-trophic level consumers consist of a straight, even-numbered and unbranched hydrocarbon chain that is 14–24 carbons in length with a carboxylic acid (–COOH) group at one end and a terminal methyl ($–CH_3$) group at the other. FAs can be either saturated (no double bonds) or unsaturated with one or more double-bonds–in polyunsaturated fatty acids each double bond is normally interrupted by a methylene ($–CH_2$) group. Some marine invertebrates, such as bivalve molluscs, are capable of synthesizing FAs where the double bonds are non-methylene interrupted (Joseph 1982). Fatty acids with <12 carbons, >24 carbons, and/or >6 double bonds occur only in trace amounts in upper-trophic level predators (Budge et al. 2006). Individual FAs are most commonly referred to using the following shorthand notation: A:Bn-X, where A indicates the number of carbon atoms, B is the number of double bonds and X indicates the position of the first double bond relative to the terminal methyl group (Fig. 1; IUPAC-IUB Commission of Biochemical Nomenclature 1967). Some FAs contain a methyl branch on the second or third carbon atom closest to the terminal methyl group and these FAs are referred to as "iso" and "anti-iso", respectively.

The vast majority of FAs found in plant and animal tissues are not in free-form but are instead linked by an ester bond to other molecules. FAs are most commonly stored as triglyceride, a molecule that consists of three FAs esterified to a glycerol backbone. Because they are chemically reduced and stored in a nearly anhydrous state, FAs are an excellent storage fuel yielding eight to ten times more energy than protein or carbohydrate on a wet mass basis (McWilliams et al. 2004). Many species of marine fish and zooplankton store fatty acids as wax ester, which consists of a fatty alcohol esterified to a fatty acid. Phospholipid, the structural component that makes up all

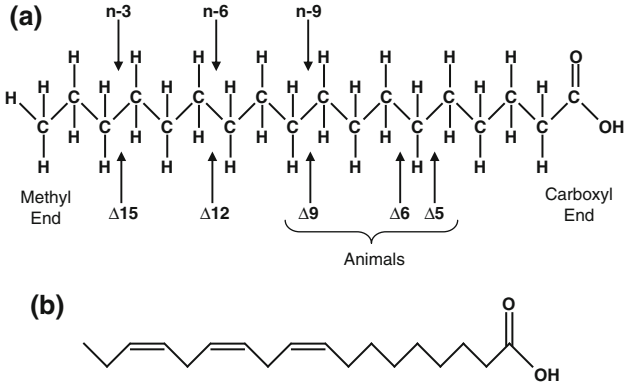

Fig. 1 Nomenclature and structure of FAs. FAs are referred to using shorthand notation of A:Bn-X, where A indicates the number of carbon atoms, B is the number of double bonds and X indicates the position of the first double bond relative to the terminal methyl group. **a** An example FA—18:0—with *downward facing arrows* under *n*-3, *n*-6, and *n*-9 indicating where the first position of unsaturation would be relative to the methyl end for individual families of polyunsaturated FAs. *Upward facing arrows* indicate the location where various desaturases (e.g., Δ*5*, Δ*6*, etc.) could introduce double bonds into the fatty acid. Vertebrates, including birds, lack desaturases needed for incorporation of double bonds beyond the Δ*9* position (relative to the carboxyl end) and are thus incapable of synthesizing *n*-3 and *n*-6 FAs de novo. In contrast, phytoplankton possess a more diverse array of desaturases (including Δ*12* and Δ*15*) and are, therefore, capable of synthesizing a diverse array of FAs (reviewed in Harwood and Guschina 2009). **b** An example FA—18:3*n*-3—with carbon and hydrogen atoms assumed. Note the methylene (CH_2) group separating each double bond. Figure adapted from Walzem (1996) and Budge et al. (2006)

cell membranes, consist of two FAs esterified to a glycerol molecule that also contains a polar head group. Due to their functional role in cell membranes, the FA composition of phospholipids is a predominantly regulated parameter, although membrane FA composition is sensitive to n-3 and n-6 polyunsaturated levels in the diet as these FAs cannot be synthesized de novo by vertebrates (Hurlbert et al. 2005). Non-esterified FAs occur in the plasma of birds bound to albumin following the hydrolysis of triglycerides mobilized from adipose tissue stores (McWilliams et al. 2004).

FA analysis—an ecological tool

Qualitative FA signature analysis

The most common use of FAs in marine trophic ecology involves qualitative assessment of dietary differences among groups based on a suite of FAs (e.g., a FA 'signature'). In seabird studies, such qualitative FA signature analysis has been used to identify spatial differences in diet (Wang et al. 2009a), to delineate temporal shifts in diets (Williams et al. 2008a), and to make inter-specific comparisons (Dahl et al. 2003; Käkelä et al. 2007). Qualitative FA signature analysis assumes differences in FA composition amongst groups reflect dietary differences although no quantitative information on diet composition is obtained. However, differences in FA signatures between groups must have a quantitative basis, and numerous multivariate statistical approaches have been utilized including discriminant function analysis, classification and regression trees, principal component analysis, and cluster analysis.

The main assumption underlying qualitative FA signature analysis is that differences in FA signatures are a function of diet rather than a reflection of differences in FA metabolism between or amongst groups. Due to the complexities of avian FA metabolism, this assumption may not always be met. Thus, as in any field, ecologists must be careful to scrutinize statistically significant results to determine if they are biologically meaningful. This is currently difficult, because few experimental studies have been conducted to determine the limits of inference that can be derived from FA field data.

Tracer FA approaches

A second use of FAs involves qualitative or semi-quantitative assessment of prey consumption based on one or several tracer FAs. For example, Raclot et al. (1998) determined that myctophid fishes were an important prey species for King Penguins (*Aptenodytes patagonicus*) based on the prevalence of several long-chain polyunsaturated FAs in adipose tissue. Ecological inferences made in this manner must be done cautiously, however, as the abundance of a particular FA in the predator will not necessarily match levels in prey due to non-random FA metabolism (e.g., Grahl-Nielson et al. 2003). Nevertheless, captive validation studies have shown that some FAs in seabird tissues can vary in a predictable manner relative to dietary FAs permitting dietary inferences in free-living seabirds (e.g., Käkelä et al. 2005, 2009a, b).

The qualitative tracer technique is most useful when unique/unusual FAs occur in particular prey items. For example, non-methylene interrupted FAs synthesized by certain benthic molluscs have been traced to pinnipeds (Budge et al. 2007) and subsequently to polar bears (Thiemann et al. 2007). Additionally, anthropogenic food sources often contain *trans*-FAs which are inadvertently introduced during hydrogenation and the presence of these *trans*-FAs in mammalian predator tissues can be used to infer foraging on human refuse (Thiemann et al. 2008). In addition to anthropogenic sources, predators can obtain *trans*-FAs by consuming the fat of ruminants which contains *trans*-FAs due to bacterial hydrogenation of unsaturated fatty acids in the rumen. We are unaware of any studies measuring non-methylene interrupted or *trans*-FAs in free-living birds.

Quantitative diet estimation based of FAs

The most ambitious use of FAs as dietary tracers involves the use of a mixing model that selects the combination of FA signatures of potential prey that comes closest to matching the predator signature after accounting for FA metabolism using calibration coefficients. The QFASA model developed by Iverson et al. (2004) utilizes the empirically determined lipid content and composition of potential prey species to calculate their relative contribution to the diet on a biomass basis. Because many ecologists are unaware of the assumptions underlying the QFASA model, we discuss these assumptions and caveats of the technique in detail below (see "The QFASA model").

Seabird FA metabolism

Following the ingestion of a meal, ester bonds of triglycerides are hydrolyzed in the duodenum releasing FAs for subsequent uptake by intestinal mucosal cells. For many seabirds, wax esters represent the dominant dietary neutral lipid, and their hydrolysis in the duodenum produces one fatty acid and one fatty alcohol per wax ester molecule. The resultant fatty alcohol is subsequently oxidized to produce the corresponding fatty acid which joins the pool of fatty acids available for metabolism and/or subsequent deposition in triglyceride stores (Roby et al. 1986). In seabirds, the rate of hydrolysis for wax esters and triglycerides is essentially equivalent (Place 1992). Plasmalogens are a type of phospholipid commonly found in the marine environment, and they contain a vinyl-ether linked alkyl chain that is also converted to a FA during digestion (Budge and Iverson 2003). Therefore, when determining the FA composition of potential prey species, it is critical to include FAs that correspond to the fatty alcohols from wax esters and the vinyl ether linked akyl chain from plasmalogens.

Lipids are insoluble in blood plasma and other tissue fluids. This physical property necessitates that they be transported in the blood as components in lipoproteins. In mammals, ingested FAs are absorbed in the intestine, reassembled into triglycerides, and incorporated into chylomicrons which are subsequently released into the blood stream (Fig. 2). In birds, absorbed FAs are incorporated

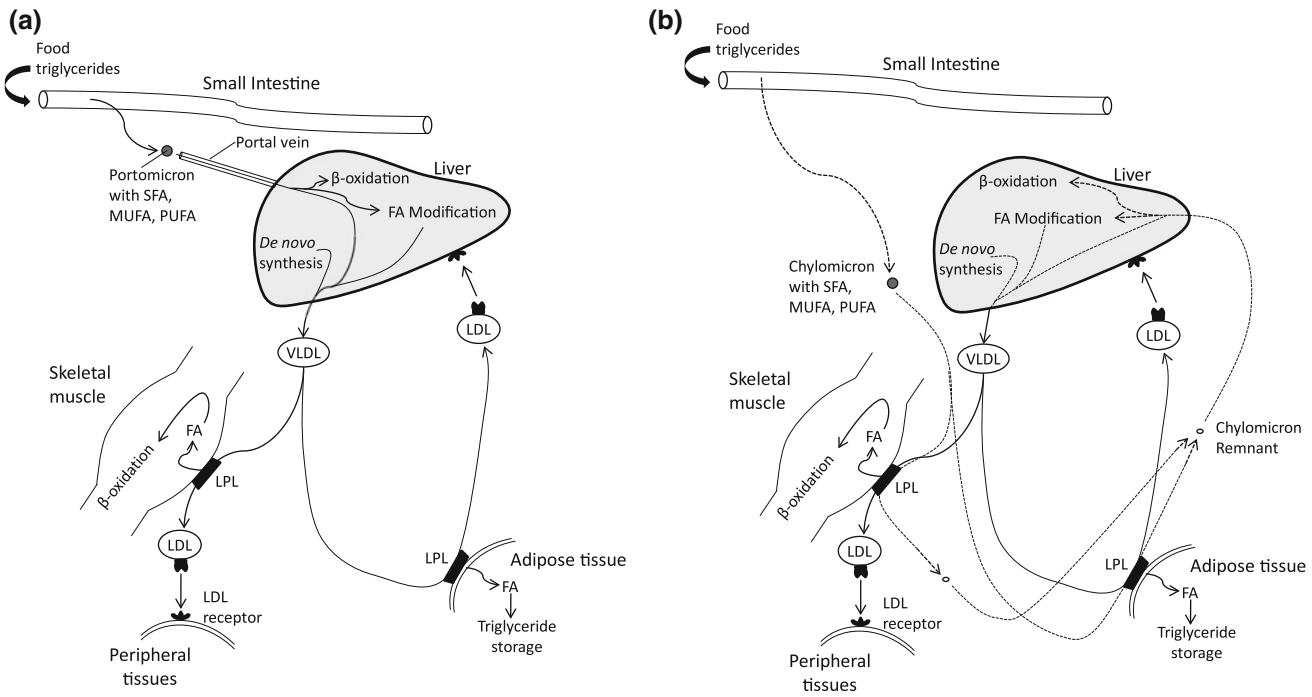

Fig. 2 Schematic diagram of the transport routes for FAs in **a** birds (adapted from Klasing 1998), and **b** mammals. In birds, ingested FAs, including saturated FAs (*SFA*), monounsaturated FAs (*MFA*), and polyunsaturated FAs (*PUFA*), are absorbed in the small intestine, packaged as triglycerides in portomicrons, and delivered to the liver via the portal vein. In the liver, dietary FAs may be selectively oxidized or packaged with de novo synthesized FAs into very-low-density lipoproteins (*VLDLs*) which are subsequently released into the general circulation. Dietary FAs may be modified via chain elongation, desaturation, and/or peroxisomal chain-shortening prior to incorporation into *VLDLs*. Lipoprotein lipase (*LPL*) hydrolyzes triglycerides from *VLDLs* resulting in free FAs that can be taken up by peripheral tissues. Triglyceride-depleted *VLDLs* circulate as low-density lipoproteins (*LDL*) which are rich in cholesterol and can be taken up by tissues via the *LDL* receptor. In mammals, chylomicrons are released into the general circulation delivering FAs directly to peripheral tissues. Remnant chylomicrons are subsequently taken up by the liver where select FAs can be modified prior to incorporation into *VLDLs* along with de novo synthesized FAs

into large lipoproteins known as portomicrons that are secreted into the portal system and routed directly to the liver (Fig. 2). In the liver, dietary FAs are incorporated, along with FAs synthesized de novo and FAs modified via elongation and desaturation, into very low density lipoproteins (VLDL) prior to release into the general circulation for subsequent uptake by peripheral tissues (Jenni-Eiermann and Jenni 1998). In birds, FA synthesis in the liver is considered to be exceptionally high compared to most vertebrates (Hermier 1997; Stevens 1996). However, this view is likely an artefact of the overwhelming number of studies that have been conducted on poultry and other commercial species that consume diets rich in carbohydrates. In contrast, biosynthesis within the liver is low in Common Murres (*Uria aalge*) and most FAs synthesis occurs in adipose tissue (Herzberg and Rogerson 1990). Rates of FA biosynthesis in the liver are likely to be lower in avian predators because their diets are rich in lipids and essentially lack carbohydrates.

Within vertebrates, the vast majority of research on FA synthesis and modification pathways has occurred in mammals (reviewed in Miyazaki and Ntambi 2008). Similar to mammals, birds are capable of synthesizing FAs de novo and oxidizing them to mono- and di-unsaturated FAs up to the ninth carbon inward from the carboxyl end (Δ9; Walzem 1996). There is also some evidence that the types of FAs synthesized by birds are different from mammals; avian FAs synthesized de novo are characteristically mono-unsaturated due to an active Δ9—desaturase (Klasing 1998). Vertebrates lack the desaturases needed for incorporation of double bonds beyond the Δ9 position (relative to the carboxyl end) and therefore n-3 and n-6 FAs must come from their diet. However, dietary polyunsaturated FAs can be modified through desaturation (via Δ5 and Δ6 desaturases), chain-elongation, and peroxisomal chain-shortening (Fig. 3). The capacity of avian species other than galliformes (fowl) to elongate and desaturate FAs has not been determined. More importantly, the tendency for seabirds to modify FAs might be reduced due to their high-lipid diets that are rich in polyunsaturated FAs. For example, the tendency for desaturation decreases when dietary polyunsaturated FAs are abundant, as desaturases are strongly inhibited by their products (Ntambi 1999). Nevertheless, some modification of FAs does occur. Käkelä et al. (2009a) report that increased levels of 22:1*n*-11 in the diet of Herring Gulls (*Larus argentatus*) resulted in elevated plasma levels of its chain-shortened products (20:1n-11, 18:1n-11 and 16:1n-11). Similarly in mammals, Cooper et al. (2006) demonstrated that radio-labelled 22:1n-11 undergoes partial *β*-oxidation in Mink (*Mustela vison*) and Gray Seals (*Halichoerus grypus*) with 18:1 being the dominant product.

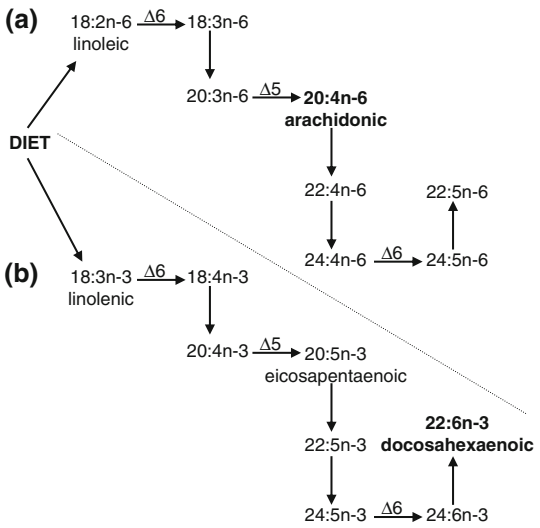

Fig. 3 Pathways for the modification of *n-3* and *n-6* dietary FAs (Sprecher et al. 1995) with dominant end products indicated in *bold*. The propensity for seabirds to elongate, desaturate, and shorten FAs has not been determined

Triglycerides within circulating VLDLs are hydrolyzed by lipoprotein lipases in peripheral tissues releasing free FAs and glycerol which diffuse into cells. During periods of negative energy balance, such as during fasts or high-intensity exercise, FAs are mobilized from adipose tissue stores to meet energy needs. During mobilization, hormone-sensitive lipase hydrolyzes triglycerides into glycerol and non-esterified FAs (free FAs) which are transported in the plasma bound to albumin. The pathways for triglyceride catabolism are highly conserved and birds appear to utilize similar pathways as other vertebrates (reviewed in Stevens 1996).

Tissue selection for FA analyses

FAs from a variety of seabird tissues can be used to make dietary inferences including adipose tissue, blood, stomach oil, and eggs. None of these tissues will have FA signatures that directly match dietary FA intake because of predator FA metabolism. Furthermore, different tissues will reflect different time periods of assimilation. A detailed review of the chemical methods for analyses of FAs can be found in Budge et al. (2006).

Adipose tissue

An adipose tissue biopsy protocol for seabirds has been developed that is rapid, non-lethal, and minimally invasive (Iverson et al. 2007). Adipose tissue functions as a storage organ for FAs and has an extremely large triglyceride content (Klasing 1998). Thus, membrane phospholipids,

which have a more highly regulated FA composition, will have little influence on the overall FA signature of adipose tissue.

Interpretation of FA data requires knowledge of the time period of assimilation, yet few studies have determined rates of FA turnover in the adipose tissue of birds. Foglia et al. (1994) found that marker FAs had a half-life of 18–23 days in growing Chickens (*Gallus domesticus*). In contrast, turnover of adipose tissue FAs in Tufted Puffin (*Fratercula cirrhata*) nestlings was nearly complete after 27 days on a controlled diet, although this rapid turnover partially reflects the accretion of fat stores during a period of rapid growth (Williams et al. 2009). Wang et al. (2009b) found that FA turnover in adult Spectacled (*Somateria fischeri*) and Steller's Eiders *Polysticta stelleri*) took somewhere between 29 and 69 days. Thus, it appears that adipose tissue in birds reflects diet integrated over a period of approximately 1–2 months, although clearly there is a need to quantitatively assess FA turnover rates in a variety of species under different dietary, physiological, and developmental conditions. Mass-specific metabolic rates, for example, will almost certainly affect rates of FA turnover.

Whole blood and plasma

When the primary goal of FA analysis has been to make inferences regarding seabird foraging ecology, most studies have measured adipose tissue FAs. Nevertheless, blood plasma has also been used extensively because blood sampling is easy and less invasive compared to adipose tissue biopsies. Although plasma FA composition is reflective of diet, the caveats associated with this tissue are more numerous. Problems are inherent in the interpretation of plasma FA signatures because blood plasma is a transport tissue and, as such, the composition of the tissue is in a continual state of flux depending on physiological state. In mammals, the FA signature of chylomicrons isolated from blood plasma is more representative of dietary FAs than of plasma FA composition, although the resorptive state significantly affects the chylomicron FA signature (Cooper et al. 2005). Unfortunately, FAs from portomicrons in birds cannot be used in an analogous manner because they are routed through the liver where repackaging of triglycerides into VLDLs occurs prior to their release into general circulation (see Fig. 2). During this process, FAs synthesised de novo and those of dietary origin will both be incorporated into VLDLs, although the ratio of exogenous to endogenous FAs depends on the resorptive state (Hermier et al. 1984).

To circumnavigate these issues, seabird FA studies that have utilized blood samples have relied on the plasma FA signature rather than attempting to isolate only the components that are rich in triglycerides (e.g., Käkelä et al. 2006, 2007). Some have suggested that plasma and whole blood FAs are not useful for dietary inferences because FAs in these tissues are found predominantly in membrane phospholipids which are regulated to a much higher degree (Cooper et al. 2005; Budge et al. 2006). However, diet has been shown to have predictable effects on the FA signature of plasma and whole blood phospholipids in a variety of vertebrates including humans (Baylin and Campos 2006) and seabirds (Käkelä et al. 2005, 2009a). Nevertheless, the correlation between adipose tissue FA composition and diet is higher than between plasma or whole blood FA composition and diet (Baylin and Campos 2006; Käkelä et al. 2009b). Käkelä et al. (2005, 2009a) demonstrated that changes in dietary FAs result in predictable and quantifiable changes in plasma FA composition, but these studies utilized post-absorptive birds where the influence of triglycerides absorbed from the diet is expected to be minimal. Although the FA composition of plasma will typically be determined primarily by phospholipid FAs, birds in the resorptive state will have a plasma FA signature that is influenced by FAs in triglycerides. Neuman et al. (1997) report considerable intra- and inter-specific variation in the concentration of triglycerides in the plasma of free-living seabirds indicating FAs from neutral lipids will influence plasma FA composition to varying degrees.

Plasma fatty acid signatures might also be influenced by egg formation which requires the deposition of a large amount of yolk lipids during several days prior to ovulation. In the liver, dietary, modified, and de novo synthesized FAs are packaged into yolk-targeted VLDLs and, to a lesser extent, vitellogenins (proteins complexed with phospholipids and cholesterols), which are then secreted into the plasma for delivery to the ovary. During yolk formation in seabirds, vitellogenins and VLDLs can be an order of magnitude higher resulting in circulating levels that are, on average, two to three times higher during the egg laying period in free-living seabirds (Vanderkist et al. 2000). Therefore, during the egg-laying period, plasma FA signatures are potentially influenced by triglycerides from yolk-targeted VLDL and vitellogenins.

Eggs

Yolk lipids are rich in triglycerides and their FA composition reflects that of diet, particularly in the amount of polyunsaturated fatty acids (Noble et al. 1996; Leskanich and Noble 1997). However, the composition of fatty acids that are deposited in yolk is more tightly regulated compared to adipose tissue. As with other tissues, dietary inferences will be strongest when based on the FA composition of triglycerides rather than on that of phospholipids. For example, Surai et al. (2001) found high levels of

arachidonic acid (20:4n-6) in yolk phospholipids of Northern Gannets (*Morus bassanus*) and Great Skuas (*Stercorarius skua*) despite a marine diet with a low arachidonic acid content. Thus, although levels of n-3 and n-6 FAs in phospholipids will be influenced by diet, these levels will not necessarily closely match those of the dietary signature.

Only freshly laid eggs should be used for ecological inferences based on FA analyses, as certain yolk FAs, such as arachidonic acid (20:4*n*-6), are preferentially incorporated into the developing embryo (Groscolas et al. 2003). Additionally, it is critical to consider the nutritional state of the female during oocyte formation. For example, Emperor Penguins (*Aptenodytes forsteri*) fast during oogenesis, and egg FAs are derived primarily from mobilized adipose tissue, although distinct differences between the FA composition of adipose tissue and yolk are evident (Speake et al. 1999). In birds that continue to feed during the period of oocyte development, the correlation between the dietary FA signature and the yolk FA composition will depend on the degree to which FAs from storage tissues are mobilized to supply the developing egg. Few studies have determined to what degree seabirds rely on capital versus income to supply the egg, although recent stable isotope evidence suggests seaducks are predominantly income breeders (Hobson et al. 2005; Oppel 2008). Despite these limitations, eggs are easily collected in the field, and use of yolk FA analyses can provide insight into the foraging behaviour of free-living seabirds. However, studies that quantitatively relate the FA composition of yolk to the dietary FA signature are needed.

Stomach oil

Stomach oil, which occurs in procellariids (shearwaters, petrels, albatrosses) with the exception of diving petrels, can also be used as a source of FA for dietary inferences (Connan et al. 2005, 2007, 2008; Wang et al. 2007). Procellarids possess a unique gastro-intestinal anatomy that results in much slower gastric emptying of dietary lipids which results in a stomach oil remaining in the proventriculus of adults and chicks (Roby et al. 1989). Although stomach oil is unquestionably of dietary origin, the FA signature will not match that of dietary intake exactly because of the rapid gastric emptying of more polar lipid constituents (i.e., phospholipids; Place et al. 1989). One key advantage to the use of stomach oil is that it provides a pool of FAs that does not include FAs synthesized de novo or dietary FAs that have been subsequently modified. Additionally, waxes are present in stomach oil because wax ester bonds have not yet been hydrolyzed. Thus, measuring the lipid class profile of stomach oil samples can furnish additional information that is useful for dietary inferences as only some prey species contain wax esters (Connan et al. 2008). Stomach oil FA signatures are expected to be different from the FA signatures of adipose tissue because of de novo synthesis, FA modification, and selective metabolism and/or storage. Nevertheless, as Wang et al. (2007) point out, differences between stomach oil and adipose FA signatures in free-living birds may also be reflective of the very different time scales of FA incorporation. FAs in stomach oils are likely from prey ingested during the preceding several days whereas the half-life of FAs in adipose tissue is somewhere in the order of weeks to months, depending on the size and physiological state of the seabird in question.

Factors affecting predator FA metabolism

Diet composition

Lipid biosynthesis has not been studied extensively in seabirds, and the effects of changes in dietary lipid content on the rate of de novo FA synthesis are currently unresolved. Budge et al. (2006) contend that the propensity for de novo FA synthesis and for modification of FAs via chain elongation and desaturation (but not chain shortening) is likely inhibited in seabirds, because their diets are high-lipid and rich in polyunsaturated FAs. Consistent with this hypothesis, dietary fat inhibits de novo FA synthesis, at least in poultry (Laurin et al. 1985). Although seabirds typically consume lipid-rich species, they will rely on low-lipid prey when high quality forage species are less available (Wanless et al. 2005). In poultry, a reduction in the lipid content of the diet stimulates increased de novo synthesis when lipids are replaced with an equivalent caloric intake of carbohydrates. However, replacement of dietary lipids with proteins results in increased levels of insulin-like growth-factor-I which suppresses lipid biosynthesis and enhances muscle deposition (Rosebrough et al. 1999). Romano (2000) found that carcasses of growing Black-legged Kittiwakes (*Rissa tridactyla*) and Tufted Puffins fed low lipid diets (2.2% lipid) contained less total body fat and had a lower fat index (total body fat/lean dry body mass) compared to chicks fed an isocaloric high-fat diet (8.7% lipid). Thus, the capacity for up-regulation of lipogenesis in response to a low-lipid diet appears to be limited in seabirds, possibly due to the consumption of high-protein diets that essentially lack carbohydrates. However, studies measuring the effects of dietary lipid intake on the FA composition of seabird tissues have yet to be undertaken. In particular, it is currently unclear to what degree FA modification is affected by lipid content of the diet. Although carbohydrates are generally not consumed by seabirds, seaducks will consume carbohydrates in plant and seed material to varying extents

during the breeding season which might affect rates of lipid biosynthesis (Wang et al. 2009b).

Physiological state

Free-living birds routinely experience changes in nutritional state associated with different life-history stages including hyperphagia prior to migration, loss of body mass associated with reproduction, and periodic fasting associated with breeding, molt, or migration. However, the degree to which such changes in physiology alter FA metabolism in seabirds needs to be measured empirically. Selective mobilization of FAs from adipose tissue stores could potentially alter FA signatures during extended periods with no or low energy intake and/or high energy expenditure. Studies of fasting mammals indicate release of FAs from adipose tissue stores is a selective process related to FA chain length and the degree of unsaturation (reviewed in Raclot 2003). In general, short chain and more unsaturated FAs are more readily mobilized, although this is an oversimplification of a more complex pattern. To date, few studies have been conducted in birds, although Price et al. (2008) found a similar pattern of selectivity when FA mobilization was triggered in vitro in adipose tissue sampled from Ruffs (*Philomachus pugnax*) and White-crowned Sparrows (*Zonotrichia leucophrys*). Similarly, Mustonen et al. (2009) found that Pheasants (*Phasianus colchicus mongolicus*) subjected to a 4-day fast selectively mobilized short-chain FAs and Δ9-unsaturated FAs. Thus, selective mobilization of FAs from adipose tissue is likely an issue for seabirds during periodic fasts associated with incubation, molt, and migration. Unlike mammals, birds have an exceptionally high lipolytic rate and are capable of fuelling high intensity exercise (e.g., flight) exclusively using their lipid stores (McWilliams et al. 2004). Assuming FA mobilization is also selective under these conditions, a relatively rapid alteration in adipose tissue FA signatures might occur during energetically demanding periods such as chick-rearing or long-distance migration. However, measuring the characteristics of FA mobilization under such conditions presents a considerable challenge. Long-distance migration fuelled by adipose tissue stores will also greatly increase the turnover rate of adipose tissue, and the ability of birds to rapidly accumulate and deplete lipid stores needs to be considered when making inferences regarding the time-period of FA incorporation.

Age-specific FA metabolism

We are not aware of any seabird studies that have directly compared FA deposition characteristics in adults to nestlings, although there are a number of physiological differences that might affect FA metabolism. For example, Pageaux et al. (1992) found the FA composition of phospholipids and triglycerides in both plasma and oviduct were markedly modified during sexual maturation in Japanese Quail (*Coturnix coturnix japonica*). They speculate that this effect was attributable to estradiol, which stimulates hepatic Δ9 desaturase and inhibits the oviduct Δ6 desaturase. Procellariid nestlings are fed a lipid-rich stomach oil diet which potentially causes a reduction in rates of de novo FA synthesis. However, in many species, including some procellarids, nestlings deposit extremely large lipid reserves to survive a prolonged period of parental neglect (e.g., Ricklefs and Schew 1994). In other species, nestlings deposit lipid stores to survive a period during post-fledging during which they develop foraging skills (e.g., Pelecaniformes; Montevecchi et al. 1984). Presumably, fat accumulation during the nestling stage is accompanied by changes in FA metabolism. Additionally, during the late fledging period, free-living and captive seabirds often undergo a period of mass recession when chicks consume little food and lipid stores fuel energetic demands (Morbey et al. 1999; Sears and Hatch 2008). Thus, the timing of sampling within the nestling period might affect adipose tissue FA signatures assuming mobilization of FAs is selective.

In addition to predictable life-history stages that involve periodic fasting, seabird nestlings routinely experience acute variation in nutritional condition during growth depending on local food availability (e.g., Boersma and Parrish 1998; Gjerdurm et al. 2003). By feeding Tufted Puffin chicks variable amounts of the same prey species (same nutritional quality but different nutritional quantity), Williams et al. (2009) determined that variability in growth rates is reflected in adipose tissue FA signatures. Repeated biopsies of adipose tissue demonstrated that the observed differences were not a function of different rates of turnover. FA signatures in adults might also be affected by changes in nutritional state. In many seabirds, adults typically lose body mass during chick-rearing as lipid stores are depleted due to physiological stress associated with reproductive effort or as programmed loss in response to physiological demands (Niizuma et al. 2002; Williams et al. 2008b).

The QFASA model

The most ambitious use of FA signatures involves quantitative estimation of diet composition based on the FA signatures of the predator and of potential prey items. The QFASA model developed by Iverson et al. (2004) utilizes calibration coefficients to account for FA metabolism within the predator that will ultimately result in tissue FA

signatures that do not exactly match the dietary signature. Calibration coefficients are calculated as the ratio of FA deposited to FA ingested based on controlled feeding experiments and have thus far been developed for a handful of seabird species (Iverson et al. 2004, 2007; Williams et al. 2009; Wang et al. 2009b; Käkelä et al. 2009b). Calibration coefficients developed in seabird feeding trials have been generally similar across species although significant differences have been found, particularly in 20–22 carbon length saturated and monosaturated FAs. Such differences might be reflective of species-level variation in FA metabolism or could be a function of the different diet types used in these experiments.

The QFASA model assumes the ratio of deposited FA to ingested FA is independent of the FA signature of the diet. Although this is an appealing concept due to its mathematical simplicity, it may not be sufficiently complex to account for predator metabolism. For example, such ratios will not account for modification of FAs via chain elongation, desaturation, and peroxisomal chain-shortening. Additionally, for a FA that originates from both exogenous (diet) and endogenous (de novo synthesis) sources, it seems counterintuitive that the ratio of FA in adipose tissue to FA in diet would remain constant across all diets. Rather, as the level of a particular FA in the diet increases, the relative input from endogenous synthesis should decrease, resulting in a lower calibration coefficient. Interestingly, the calibration coefficients estimated for the FAs that are thought to be the predominate end-products of biosynthesis in birds (16:0, 16:1n-7, and 18:1n-9) have generally been quite similar across studies although Spectacled and Steller's Eiders fed a diet that included relatively high levels of carbohydrates had higher calibration coefficients for 18:1n-9 compared to other seabirds (Wang et al. 2009b). In general, the highest variability in calibration coefficients has occurred in saturated and monounsaturated 20–22 carbon length FAs. For example, Common Murres fed diets that had exceptionally low levels of 20–22 carbon length monounsaturated FAs (<0.2%) had calibration coefficients for these FAs that were twice as high as those found in Tufted Puffins fed diets rich in these fatty acids ($\sim$15%; Iverson et al. 2007; Williams et al. 2009). Thus, if small amounts of these particular FAs originate from biosynthesis, it could have a large effect on calibration coefficients when these FAs only occur as trace amounts in the diet. However, given the numerous differences between these studies, it is impossible to determine conclusively the underlying cause of the observed difference in calibration coefficients.

Although the QFASA model accounts for lipid content of the prey when estimating the amount of biomass consumed, it assumes that predator FA metabolism is unaffected by dietary lipid intake. However, in cases where a species is known to consume a particularly lipid rich diet during one life-history stage, novel calibration coefficients for that stage could also be measured in feeding trials. As previously mentioned, changes in nutritional state during growth also affect FA metabolism which will result in different calibration coefficients. For example, the calibration coefficients of Tufted Puffins fed low calorie and high calorie diets were significantly different, but highly correlated (Williams et al. 2009). The effect of such differences on diet estimation using the QFASA model is currently unclear. Wang et al. (2009b) found generally similar estimates of diet when different sets of calibration coefficients were used suggesting the model is somewhat robust to such variation. However, diets estimated in that study involved prey types with very distinct FA signatures and error rates are likely to be greater when prey with similar ecological niches (and therefore more similar FA signatures) are modeled. This highlights one of the limitations associated with using controlled feeding trials to validate diet estimation using QFASA: diets used in such experiments generally have very little intra-specific variation (because prey are from a single source or batch) and often have high levels of inter-specific variation. Ideally, the effects of changes in physiological state on calibration coefficients should be measured under controlled conditions but variability in diet estimation using those coefficients should be determined using realistic prey FA libraries.

The QFASA model does not use all FAs detected in predator and prey tissues. Instead, subsets of FAs that are thought to be most representative of diet are utilized (Iverson et al. 2004). Such subsets may include only the FAs that are thought to be of dietary origin, or may include FAs that can be synthesised de novo and/or arise through the modification of other FAs. Because there is uncertainty over which subset of FAs is best for modeling diets, validation studies have attempted to select the subset that optimizes accuracy of diet estimation (see details in Iverson et al. 2004; Nordstrom et al. 2009; Wang et al. 2009b). Interestingly, the "optimum" subset of FAs differed in each of these studies, although it is difficult to determine why such differences occurred given the variation in experimental conditions across studies. Wang et al. (2009b) found that subsets that included only FAs derived primarily from the diet (i.e., reduced dietary subsets) outperformed subsets that included FAs that can also arise from biosynthesis (i.e., extended dietary subsets). They speculate that the reduced subsets may have outperformed extended dietary subsets because experimental diets in their study included high levels of carbohydrate which would likely stimulate increased levels of de novo FA synthesis.

Although variability in FA subsets and calibration coefficients are cause for concern, QFASA diet estimates

were remarkably similar to consumption estimates based on video data in free-living Harbour Seals (*Phoca vitulina*; Iverson et al. 2004) and based on stomach content analysis in seabirds (Iverson et al. 2007). Thus, variability in predator FA metabolism either does not substantially affect diet estimates based on QFASA or at least does not always do so. Experiments with captive seabirds to date have proved invaluable in providing a better understanding of the relationship between dietary FA intake and the FA composition of select tissues. Nevertheless, it is critical to test the FA subsets and calibration coefficients derived from such experiments in different animals fed realistic diets to measure the misclassification rates for diet estimates derived from the QFASA model. Ultimately, uncertainty should be incorporated into modeling efforts, as has recently been done with stable isotope mixing models (see Moore and Semmens 2008).

Variability in prey lipid content and FA signatures

A critical assumption underlying the use of FA as dietary tracers in marine trophic ecology is that changes in the FA composition of predator tissue reflect shifts in prey consumption. However, prey species might also exhibit spatial and/or temporal variability in lipid content or in whole body FA signatures reflecting dietary shifts or changes at the base of the food web (phytoplankton). Lipid content of some forage fishes varies with size, month, sex, reproductive status, and location (Anthony et al. 2000; Payne et al. 1999). The degree to which changes in lipid content are accompanied by changes in FA composition is currently unknown. In many species, decreases in lipid content are likely accompanied by a change in the phospholipid to triglyceride ratio which would result in altered FA signatures. Iverson et al. (2002) found that FA signatures accurately characterized species within Prince William Sound, Alaska, although lipid content and FA signatures differed among size classes and seasons. Additionally, Budge et al. (2002) found significant geographic variation in FA signatures across the Scotian Shelf, Georges Bank, and the Gulf of St. Lawrence in the Atlantic. In both of these studies intra-specific variability in prey species was lower than inter-specific variation. Nevertheless, making qualitative dietary inferences based on FA signatures requires an understanding of the degree to which prey signatures vary in space and time.

Most of the long chain polyunsaturated FAs found in marine ecosystems originate in phytoplankton and, therefore, changes in oceanographic conditions that alter the composition of the phytoplankton community will also affect the FA signatures of organisms at all trophic levels. Climate-driven changes in oceanography affect primary production, the timing of blooms, the mix of phytoplankton species, and their broad-scale distribution (Francis et al. 1998). These changes will consequently affect the concentration and distribution of FAs available to primary consumers and upper trophic level organisms. For example, the FA composition of particulate matter changes over the course of a bloom due to species succession of phytoplankton (Hayakawa et al. 1996). Fatty acids of the dominant phytoplankton in blooms can subsequently be traced to primary consumers (Napolitano et al. 1997). Thus, changes in ocean climate conditions will simultaneously affect the FA composition of the food web base as well as trigger reorganization of community structure at higher trophic levels. Inferring dietary differences based on FAs between different ocean climate regimes is therefore ill-advised in the absence of a concurrent prey FA signature database.

Conclusions and a call for controlled feeding experiments

FA signatures in a variety of tissues are undoubtedly influenced by dietary FA intake, and we argue that they can provide valuable insight into seabird diets. Rates of de novo synthesis are likely suppressed to a large extent because seabird diets are usually high lipid and contain little carbohydrate. Modification of FAs via chain elongation and desaturation might also be reduced to some extent due the availability of polyunsaturated FAs in the marine environment. However, changes in prey quality or nutritional state will have some effect on the FA signatures of seabirds, independent of changes in dietary FA composition. Only by measuring the size of such effects in carefully controlled experiments can we ensure that FA data is correctly interpreted. We do not wish to perpetuate the misconception that variability in predator FA metabolism precludes the use of FAs as dietary tracers. Instead, we argue that more work is needed to better elucidate the limits of inference that can be made based on FA data. In this review, we have outlined many of the factors that are likely to influence FA metabolism including changes in nutritional state, proximate composition of the diet, and ontogenic effects. Although FAs have been used much more extensively in marine mammals, seabirds are ideal organisms to better understand how such factors can influence FA metabolism because FA turnover in birds is much more rapid due to their high metabolic rates. Additionally, seabird adipose tissue likely lacks the vertical stratification and thermoregulatory specialized outer layers found in marine mammals (e.g., Strandberg et al. 2008).

Analysis of FAs is a powerful tool in marine trophic ecology and can provide precise (species level) estimates

of diet using the QFASA method. However, precise estimates are only useful if they are accurate, and more controlled feeding experiments are needed to measure variability in calibration coefficients and to determine the factors underlying variable FA metabolism. Once such variability has been measured, efforts should be made to refine modeling approaches to better incorporate uncertainty into quantitative diet estimates based on FA signatures. Measuring seabird diets remains a daunting task and no one technique is infallible. Therefore, we strongly advocate the continued use of multiple metrics when making dietary inferences.

Zusammenfassung

Die Nutzung von Fettsäuren als Nahrungsindikatoren in der Nahrungsökologie von Seevögeln: Theorie, Anwendung und Grenzen

Die Analyse von Fettsäuren (FAs) ist ein zunehmend genutztes Werkzeug in Untersuchungen der Nahrungsökologie in marinen Ökosystemen. Diese leistungsfähige Technik has sich als nützlich erwiesen, um die räumliche und zeitliche Variabilität in der Ernährung zu beschreiben, den Verzehr von Schlüsselarten zu identifizieren und quantitative Schätzungen der Nahrungszusammensetzung zu liefern. Obwohl die FA-Signaturen von Konsumenten unbestreitbar von der Nahrung beeinflusst werden, können sie auch von anderen Faktoren beeinflusst werden, einschließlich life history-Stadium, Nahrungsqualität und physiologischem Zustand. Hier besprechen wir, wie FAs von Vögeln aufgenommen, abgelagert und verstoffwechselt werden, und wie sich diese Prozesse auf die verschiedenen Gewebe auswirken, die für FA-Analysen häufig beprobt werden. Dann untersuchen wir die Annahmen, die FA-Signatur-Analysen zugrunde liegen, wenn sie für Untersuchungen der Nahrungsökologie von Seevögeln verwendet werden, und schlagen eine Richtung für zukünftige Laborexperimente vor, die nötig sind, um diesen Ansatz zu verfeinern. Die korrekte Interpretation von FA-Daten stützt sich darauf, dass Faktoren berücksichtigt werden, die den FA-Stoffwechsel von Prädatoren verändern, und dass die Variabilität im Lipidgehalt und die FA-Zusammensetzung von Beute in Betracht gezogen werden. Man sollte sich außerdem bemühen, Unsicherheiten hinsichtlich des Stoffwechsels von Prädatoren in Modelle einzuschließen, die zur quantitativen Bewertung der Ernährung konstruiert werden.

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