Chapter 12
Tracing Aquatic Food Webs Using Fatty Acids: From Qualitative Indicators to Quantitative Determination

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12.1 Introduction

Food web structure, predator–prey dynamics, foraging behavior, and consequences of these factors for individual growth, reproduction and survival are central to our understanding of ecosystem structure and functioning. Moreover, in the current context of understanding (and managing) ecosystems in the face of ongoing environmental change, important questions include: What are the critical prey of key consumers in relation to prey abundance, availability, and nutritional quality? What are the ecosystem processes responsible for food web production? And, how do these processes respond to changes in physical forcing? A fundamental requirement to understand any of these areas is an accurate assessment of trophic relationships and consumer diets. However, in aquatic, and especially marine ecosystems, such information is generally not easily or reliably obtained. In these systems, the relative inaccessibility of free-ranging organisms and the inability to directly observe species interactions make it difficult to accurately characterize diet. Traditional approaches, such as examining gut contents, have well-recognized biases in addition to representing only snapshots of recent meals and may therefore not be reliable indicators of long-term diet (Iverson et al. 2004). Thus, alternative approaches have been developed, which use various types of trophic markers. One of the most promising of these approaches is the use of lipids and fatty acids (FA) to study food web dynamics.

Lipids comprise a large group of chemically heterogeneous compounds, the majority of which include esters of FA as part of their structure. FA represent the "building blocks" of lipids and are the largest constituent of neutral lipids (NL), such as triacylglycerols (TAG) and wax esters (WE), as well as of the polar phospholipids (PL). All FA consist of carbon atom chains, which are most commonly even-numbered and straight, containing 14–24 carbons and 0–6 double bonds, with

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a methyl (CH_3) terminal at one end and an acid (carboxyl, COOH) group at the other. The array of FA present in nature is exceptionally complex with the possibility of routinely identifying 70 FA within a given organism (e.g., Table 12.1).

Three characteristics of FA and their storage patterns make them useful tracers of diets and marine food-web structure. First, organisms are able to biosynthesize, modify chain-length, and introduce double bonds in FA, but they are subject to biochemical limitations in these processes depending on the phylogenetic group and even species. Such limitations generally increase with increasing phylogenetic order, culminating in vertebrates (Cook 1996). Second, unlike other dietary nutrients (e.g., proteins and carbohydrates), which are completely broken down during digestion, FA are released from ingested lipid molecules during digestion, but are generally not degraded, and are taken up by tissues in their basic form. The important consequences of these restrictions within plants, bacteria, and animals, and the uptake of intact FA by consumer tissues, is that individual isomers as well as "families" of FA bioaccumulate through food chains, and they can be traced back to specific food web origins. Third, unlike most other nutrients, fat is stored in animal bodies in reservoirs. These often substantial stores can later be mobilized to provide fuel for short or long-term energy demands (e.g., Pond 1998). Thus, FA accumulate over

Table 12.1 Fatty acids (FA) routinely identified in marine organisms on a polar capillary column (e.g., DB-23, Agilent Technologies; 30 m × 0.25 mm ID), listed in order of elution (Iverson et al. 1997, 2002, 2004, 2006a)

12:0	16:1n-7	18:1n-9	20:1n-7	21:5n-3
13:0	7-*methyl* 16:0	18:1n-7	20:1n-5	22:4n-9
iso-14:0	16:1n-5	18:1n-5	20:2n-11/12	22:4n-6
14:0	16:2n-6	18:2Δ5,11	20:2n-9	22:5n-6
14:1n-9	*iso*-17:0	18:2n-7	20:2n-6	22:4n-3
14:1n-7	16:2n-4	18:2n-6	20:3n-6	22:5n-3
14:1n-5	16:3n-6	18:2n-4	20:4n-6	22:6n-3
iso-15:0	17:0	18:3n-6	20:3n-3	24:1n-9
anti-15:0	16:3n-4	18:3n-4	20:4n-3	
15:0	17:1	18:3n-3	20:5n-3	NMI FA
15:1n-8	16:3n-1	18:3n-1	22:1n-11	20:2Δ5,11
15:1n-6	16:4n-3	18:4n-3	22:1n-9	20:2Δ5,13
iso-16:0	16:4n-1	18:4n-1	22:1n-7	20:3Δ5,11,14
16:0	18:0	20:0	22:2n-11/12	22:2NMID (*unknown*)
16:1n-11	18:1n-13	20:1n-11	22:2n-9	22:2Δ7,13
16:1n-9	18:1n-11	20:1n-9	22:2n-6	22:2Δ7,15

FA are named as carbon number:number of double bonds and location (*n*-x) of the double bond nearest the terminal methyl group, where all additional double bonds are separated by a $–CH_2–$ group (i.e., "methylene-interrupted"). Non-methylene interrupted FA (NMI FA) are separated by more than one methylene group; these are generally very small peaks and require special attention in identifying, and thus are listed separately (Budge et al. 2007). Other FA that have been reported in aquatic or marine organisms include *iso*-4:0, 4:0, *iso*-5:0, 5:0, *iso*-10:0, *iso*-12:0 (Koopman et al. 1996, 2003, 2006) and minor or trace amounts of *iso*- and *anteiso*-isomers (methyl branch at the second and third carbon, respectively) of 13–18 carbon saturated FA, *trans*Δ6–16:1, 16:2n-7, 16:2n-1, 16:3n-4, 16:4n-4, 18:2n-3, 18:4n-6, 18:5n-3, 19:0, 22:3n-6 (e.g., Ackman 1980, 2002; Ackman et al. 1972; Budge et al. 2006)

time and represent an integration of dietary intake over days, weeks, or months, depending on the organism and its energy intake and storage rates.

Since the mid 1930s (Lovern 1935; Klem 1935), numerous studies have demonstrated the transfer of FA from prey to predator both at the base and apex of food webs (reviewed in Dalsgaard et al. 2003; Iverson et al. 2004; Budge et al. 2006). Until recently, FA have been used largely in a qualitative or semiquantitative way to infer aspects of food webs. However, recent advances involve the development of methods that use FA to quantitatively estimate diets of individual predators (Iverson et al. 2004, 2006b, 2007). The objectives of this chapter are: (1) to provide an overview of the biochemistry, metabolism, and key assumptions that are central to understanding how and why FA can be used as trophic tracers; (2) to discuss different qualitative and quantitative ways in which lipids can be used in food web and foraging ecology studies, and the methods necessary for those applications; and (3) to consider future areas to advance this research. This review focuses primarily on marine ecosystems but also refers to some freshwater and terrestrial systems.

12.2 Characteristics and Constraints on Lipid Biosynthesis, Digestion, and Deposition as They Relate to Tracing Trophic Relationships

12.2.1 De Novo Fatty Acid Biosynthesis

Important differences in FA biosynthesis among organisms allow the original source of some FA to be identified. General and specific principles and characteristics of FA biosynthesis have been described extensively in a number of reviews (e.g., Cook 1996; Gurr and Harwood 1991; Kattner and Hagen 1995; Vance and Vance 1996; Dalsgaard et al. 2003; see Chap. 9). However, some particularly relevant points include that de novo synthesis of FA occurs from 2-carbon precursors by sequential additions of 2-carbon units to a growing chain, which is released from the enzyme complex usually at 14–18 carbons. Additional 2-carbon units may also be added, generally up to 24 carbons. During this process, double bonds can be added (i.e., desaturation) by specific enzymes. Primary producers such as unicellular phytoplankton and seaweeds (macroalgae) typically produce FA ranging from 14 to 24 carbons with various degrees of unsaturation. Alga are essentially the only organisms that possess the enzymes necessary for producing long-chain polyunsaturated FA (PUFA), such as 20:5n-3 and 22:6n-3 (e.g., Sargent and Henderson 1995; Cook 1996). These FA occur throughout the marine food web in sequentially higher trophic levels, since animals (i.e., consumers) are not capable of inserting a double bond between the terminal methyl end and the n-9 carbon. Other unusual FA, such as 16:2n-4 and 16:4n-1, are produced only by certain algae and diatoms (Viso and Marty 1993; Dunstan et al. 1994).

In contrast to primary producers, animals synthesize fewer and simpler FA. These tend to be restricted to 14:0, 16:0, and 18:0 saturated FA and their monounsaturated

isomers 14:1n-5, 16:1n-7, and 18:1n-9, respectively. These monounsaturates are produced by the Δ9 desaturase enzyme present in all animals, which inserts a double bond at the ninth carbon from the carboxyl end. Animals can elongate both endogenously and exogenously produced FA to some extent, but this is generally limited, and both de novo biosynthesis and elongation/desaturation of FA are inhibited by diets containing adequate or excess fat, and long-chain PUFA (Nelson 1992). However, some invertebrates tend to have greater capacities for biosynthesis and modification of FA than higher animals. Of the zooplankton, the best studied are the calanoid copepods, which have an unusual ability to produce large amounts of long-chain monounsaturated fatty alcohols (i.e., the WE of 20:1n-11, 20:1n-9, 22:1n-11, 22:1n-9) as part of their primary storage fats (Pascal and Ackman 1976; Sargent 1978; see Chap. 6). When found in higher trophic level organisms, these FA are thought to originate from copepods (Sargent and Henderson 1986). Although less is known about many other taxa of zooplankton, they appear to biosynthesize the relatively more common FA described above (Dalsgaard et al. 2003). Certain benthic bivalve mollusks and carnivorous gastropods produce unusual FA, in which the double bonds are separated by more than one methylene group (i.e., non-methylene interrupted, NMI FA) (Joseph 1982; Budge et al. 2007). Fishes, birds, and mammals have the greatest restrictions on FA biosynthesis and follow the typical animal pattern described above. Marine predators generally have very low carbohydrate diets, but amino acids from proteins consumed in excess of immediate energy and nutrient requirements can be broken down to enter the usual FA biosynthetic pathways. An important exception to these patterns is found in some odontocetes (toothed whales), which synthesize large amounts of very short branched-chain FA (*iso*-4:0, *iso*-5:0, *iso*-10:0, or *iso*-12:0) in their cranial fats and blubber, which have no relation to diet (Koopman et al. 2003, 2006).

12.2.2 Lipid Biosynthesis

FA in nature rarely exist in free form, and in both primary producers and consumers endogenously synthesized and exogenously derived FA are generally incorporated as part of a compound. Although there are a number of lipid classes, the ones of concern in the present context are TAG, WE, and PL (Budge et al. 2006). PL are found in structural components such as cell membranes. Since fairly specific FA compositions are required for proper membrane structure and function, FA in PL tend to be fairly specific (Chap. 10) and highly conserved relative to diet. Thus, although they can be influenced by dietary FA intake, FA in PL are not particularly useful as dietary tracers (see Sect. 12.3.1). FA are most commonly stored in NL, of which TAG are by far the most common storage form. TAG can be distributed as droplets throughout an animal's body or deposited as adipose tissue (i.e., the specific fat storage tissue of vertebrates). Adipose tissue is composed of specialized cells called adipocytes, which alternately increase or decrease in volume with fattening (deposition of TAG) or fasting (mobilization of TAG), respectively. WE are another

important storage form of fat in certain species of crustaceans (e.g., copepods and other zooplankton), fish (e.g., myctophids), and marine mammals (some odontocetes; Koopman 2007). In order for WE to appear in the storage lipids of an animal, that animal must synthesize them from dietary FA or from FA biosynthesized de novo, which includes the process of converting long-chain FA to their corresponding fatty alcohols (see Sect. 12.2.3).

12.2.3 Digestion, Modification, and Deposition of Dietary Lipids and Fatty Acids

The way in which lipids are digested by monogastric (i.e., non-ruminant) animals, and subsequently modified, has a significant impact on their utility as trophic tracers. Ingested TAG are hydrolyzed (ester bonds are broken) in the gut by lipases and esterases to its component FA, monoacylglycerol, and glycerol. If short-chain FA (<14 carbons) are ingested, they are transported to the liver and immediately oxidized (Brindley 1991). All other products are transported in the blood via various carriers (e.g., lipoproteins) to tissues where absorption takes place. At fat storage sites, FA are most commonly reesterified into TAG and sequestered. A modification of this process is found in animals that consume and that store WE, processes that are independent from one another within an organism (Budge et al. 2006). WE are prevalent in marine systems as food for higher trophic consumers where, upon ingestion, they are hydrolyzed in the gut to their component FA and fatty alcohol. Gut enzymes then oxidize the fatty alcohol to its corresponding FA (without modification of chain length and double bond positions) and then both FA enter the pool of FA available for transport and deposition (Sargent 1976; Budge and Iverson 2003). If the consumer's storage fat is in the form of TAG, then TAG are deposited regardless of whether the animal has a diet high in WE. However, according to phylogeny, some animals store their fat in part or whole as WE (see Sect. 12.2.2). In these animals, certain FA are reduced to their corresponding fatty alcohols after digestion, and these fatty alcohols are then incorporated, along with other ingested FA, into WE for storage (Sargent 1976; Sargent and Henderson 1986). Nevertheless, as with TAG digestion and deposition, FA chain lengths and double bond positions are generally conserved.

As a consequence of these digestive properties, dietary FA ≥ 14 carbons are generally deposited in animal tissue with no or minimal modification, and thus one can distinguish between FA that could be biosynthesized by the animal or those that most likely come from the diet. However, some (or even many) FA may be routed through the liver before deposition occurs, while others may be completely oxidized in tissues for immediate energy needs. Thus, although dietary FA consumed in excess of immediate energy requirements are deposited largely intact in storage reservoirs such as adipose tissue, there are several points during metabolism and transportation when there is the potential for FA to be modified by animals. For example, some marine invertebrates such as copepods (see Sect. 12.2.1) are generally thought to have a greater capacity to modify (elongate or desaturate) dietary FA

than higher animals. Freshwater *Daphnia* were shown to elongate/desaturate 18:3n-3 to 20:5n-3 (Schlechtriem et al. 2006). However even in these invertebrates, direct incorporation of dietary FA has been demonstrated (Sargent and Henderson 1986; Dalsgaard et al. 2003). In contrast, fishes have a more limited ability to modify FA, but may still be better able to modify some exogenous FA than birds and mammals. However, given that their natural marine diets contain high levels of essential long-chain n-3 and n-6 PUFA (e.g., Ackman 1980), overall modification of dietary FA in fish is probably limited relative to direct dietary deposition (e.g., Kirsch et al. 1998; see Sect. 12.3.2.1).

Birds and mammals have very limited abilities to modify exogenously consumed FA by elongation and desaturation. In these species, preformed dietary FA are less likely to enter typical lipid synthetic pathways and such processes are, in any case, inhibited by diets containing adequate or excess fat, as well as those high in long-chain PUFA (Nelson 1992). Additionally, desaturation of exogenously consumed FA may be confined primarily to the Δ9 desaturase enzyme acting on some saturated 16:0 (e.g., Budge et al. 2004). Thus, FA that have been elongated and desaturated within marine birds and mammals are unlikely to make a significant contribution to their adipose FA stores. A more important process however, especially in mammals, may be peroxisomal chain-shortening of some long-chain monounsaturated FA. Thus, some ingested 20:1 and especially 22:1 isomers are likely shortened primarily to their 18-carbon isomers (Norseth and Christophersen 1978; Osmundsen et al. 1979; Cooper et al. 2005, 2006), resulting in somewhat reduced and increased deposition of these FA relative to diet, respectively.

Finally, it is clear that the biochemical pathways that animals are *capable* of performing are not necessarily the same as their *propensity* for using these pathways. Studies of FA metabolism using foreign foods and/or feeding regimes that those organisms are not accustomed to may result in forced stimulation of compensatory biochemical pathways. Examples of such studies include aquaculture fish fed artificial feeds containing terrestrial plant oil FA, rats fed fish oil FA, or species "starved" on severe nutrient depleted diets. Under natural conditions, marine fishes are adapted to and require marine FA (Ackman 1980), and carnivorous marine mammals are likewise highly adapted to efficiently digesting and depositing lipids high in marine FA without modification (e.g., Iverson et al. 1995). Additionally, some seabirds and marine mammals are well adapted to periods of prolonged fasting during which they do not enter the terminal phases of starvation, but instead maintain homeostatic regulation (Castellini and Rea 1992; Mellish and Iverson 2001). A question often asked is what happens to the FA profile of a predator when mobilizing lipid stores rather than depositing them. The issue of differential mobilization is not fully understood; however, differential release of some FA during fasting has been reported (Groscolas 1990; Raclot 2003); this is in contrast to data from natural long-term fasting studies in several phocid and otariid pinniped pups and juveniles, which have shown no temporal change in overall FA composition (D. Noren, S.J. Iverson and J.E. Mellish unpublished data). Although further studies are needed, current evidence indicates that effects of short-term fasting are unlikely to have major impacts on overall FA composition. In summary, although biosynthesis

and modification of FA does occur, by far the greatest quantitative contribution to the fat stores of higher marine or aquatic predators arises from direct deposition of dietary FA (Ackman and Eaton 1966; Rouvinen and Kiiskinen 1989; Colby et al. 1993; Iverson 1993; Kirsch et al. 1998; Iverson et al. 1995, 2007).

12.3 Tracing Trophic Pathways Using Lipids and Fatty Acids

Fatty acids can be used to study trophic relationships and food webs in several ways to provide information about consumers and their diets. One approach assumes that consumers of similar phylogeny will also share similarities in their capacity to biosynthesize, digest, and modify dietary FA. Thus, finding differences or changes in FA composition allows inferences to be made about differences or changes in diets of predators, both within and between populations, without trying to specify what prey species are eaten. The second approach uses individual biomarkers to infer or possibly identify predator–prey relationships. These biomarkers tend to be relatively rare in nature, especially at higher trophic levels, but when found in consumers can indicate consumption of specific taxa at lower trophic levels. More recently, a third approach has been developed. This uses a statistical model, combined with coefficients to account for predator metabolism and a comprehensive prey FA database, to quantitatively estimate species composition of predator diets from their FA stores. All three approaches can provide valuable insight about consumer diets and foraging ecology that otherwise could not be obtained in complex aquatic ecosystems. Each of these approaches is considered next with reference to how they have been used and validated, and their limitations.

12.3.1 Tissue Sampling and Analysis

Methods for isolation, preparation, and analysis of lipids and FA have been extensively reviewed elsewhere (Christie 1982; Ackman 1986, 2002; Parrish 1999; Iverson et al. 2001a; Budge et al. 2006; and references therein). Likewise, a thorough discussion of appropriate tissue sampling and storage for FA analyses can be found in Budge et al. (2006); however, several points are specifically pertinent to trophic studies. First, not all tissues provide equal information on diet using FA. Second, different tissues are usually required depending on whether the organism is being examined as prey or predator (in some studies certain species may be both).

12.3.1.1 Predator Sampling

A metabolically active energy storage reservoir will be most readily influenced by dietary FA intake and therefore should be the tissue sampled. This reservoir will

experience rapid turnover as a result of dietary intake and fat mobilization during fasting, and will be the most reflective of trophic relationships. As stated previously, PL FA reflect biosynthetic pathways and conserved membrane structural requirements, whereas TAG and WE FA largely reflect stored dietary FA. Thus, skin, muscle (but see caveat below) or other structural tissues will contain more structural PL FA and should be avoided. In vertebrates, the appropriate lipid samples will usually come from adipose tissue storage sites (or blubber in the case of pinnipeds and cetaceans). Such adipose tissue is often conveniently found subcutaneously and thus can be sampled in both live mammals and birds using relatively noninvasive biopsy techniques. However, it is important to confirm that the fat storage sites have comparable FA composition at different locations in an animal. Depth through the tissue sampled appears to be of importance only when appropriately sampling blubber (see Budge et al. 2006). Otherwise, when a true fat storage site is sampled, and not structural adipose tissues (e.g., cushions in eye sockets, tailstocks of cetaceans, skin-associated blubber in marine mammals), FA composition is uniform across body sites in various species of pinnipeds, cetaceans, polar bears (*Ursus maritimus*), and seabirds (Koopman et al. 1996; Cooper 2004; Thiemann et al. 2006; Iverson et al. 2007). However, some fish store lipid as modified adipose or lipid pockets in their muscle (e.g., salmonids, mackerels, herring, *Clupea pallasi*) or liver (e.g., gadoids such as cod, *Gadus morhua*), whereas many invertebrates such as sea urchins and jellyfishes store lipid primarily in their gonads and digestive tracts. Additionally, muscle FA in such species, including those in the PL, may reflect longer term systemic differences in diet among populations. In zooplankton, lipid pockets may be more difficult to isolate and thus whole animals must be analyzed with the recognition that there will be substantial contribution of conserved PL FA to the FA patterns analyzed; a similar issue is encountered when sampling vertebrate blood to assess diet. One solution is to fractionate NL (TAG and WE) from PL in the extracted lipid for subsequent FA analyses. However, ideally, when blood is sampled, lipoproteins that specifically carry FA from the digestive tract (e.g., chylomicrons in mammals, portomicrons in birds) should be isolated from other lipoproteins (which reflect endogenous FA conservation) and analyzed to examine the most recent meal (e.g., Cooper et al. 2005). Finally, in marine and other aquatic mammals (especially carnivores, pinnipeds and cetaceans), milk is a useful tissue to sample as it sequesters recent preformed dietary FA (Iverson and Oftedal 1995) in income breeders (females that feed during lactation, e.g., Wamberg et al. 1992; Iverson 1993; Iverson et al. 2001b). In capital breeders (females that fast throughout lactation), milk fat output relies principally on uptake of FA mobilized from adipose tissue or blubber and thus will be most influenced by diet of individuals before lactation.

12.3.1.2 Prey Sampling

Most marine and aquatic predators, from invertebrates to the highest vertebrates, consume their prey whole, thus they consume all the NL and PL contained in the prey and its digestive tract. Hence, whole prey should be homogenized and analyzed, even

though including prey stomach contents may increase within-species variability in FA composition. One exception occurs if the goal is to directly link the producer of an individual FA biomarker to the consumer, in which case gut contents of the prey should be removed. Complications arise when a given organism is to be used as both a predator and prey. In this case, if the aim is to quantitatively estimate prey contribution to predator diet (see Sect. 12.3.3), sampling as both predator and prey needs to be considered. For example, to assess diets of both cod and seals, all potential prey of cod and seals must be sampled as prey (i.e., whole). However, for cod, the liver would be best isolated for direct assessment of its diet as predator (see Sect. 12.3.1.1), while the whole cod would have to be used as prey for seals. This can be solved by gravimetrically isolating the liver from the rest of the cod body, analyzing the two parts separately, and subsequently reconstructing the FA composition of the whole cod mathematically. The same would be true for determining the diet of a seal (i.e., sampling its blubber only), but analyzing the whole seal as prey for killer whales, *Orcinus orca*. However, for polar bears, which primarily consume only the blubber of seals, the same seal blubber sample can serve as both predator and prey sample (Iverson et al. 2006b).

12.3.2 Qualitative and Semiquantitative Approaches: Predators Alone and Tracers Which Infer Prey Type

12.3.2.1 Uses and Evidence

Qualitative evaluation of spatial or temporal variation in diets of predators can readily be studied by comparing profiles of FA present in consumer lipid depots. Such inferences are most informative in the context of knowing something about the FA characteristics of that ecosystem. This approach, and that of the individual FA biomarker, has been used successfully in a number of ecosystems. Much of the research studying trophic relationships, especially near the bottom of food webs, has used these two approaches. The biomarker approach can be extended by recognizing unusual levels of certain FA or of ratios among FA that can only be attributed to one or a few prey types and thus can indicate their likely importance in the diet. These methods are most successfully used in primary consumers and perhaps other lower trophic levels, as those FA originate at these lower trophic levels. In principle, this opportunity will be relatively rare in higher trophic level predators, as FA originating at the base of the food web become relatively ubiquitous throughout higher levels. Given that these biomarker approaches have been extensively reviewed (e.g., Napolitano 1999; Dalsgaard et al. 2003), they will only be summarized here, with emphasis on newer multivariate analyses of higher predator FA patterns and quantitative estimates of diet composition.

One of the earlier examples of individual FA markers, and confirmed more recently, was the finding that 16:2n-4 and 16:4n-1 are produced by only certain diatoms (Ackman and McLachlan 1977; Viso and Marty 1993; Dunstan et al. 1994). These

FA are metabolically inert in consumers and therefore their presence in the depot fats of some fish can be indicative of specific feeding habits (Ackman et al. 1975). In high latitudes, pennate diatoms, which dominate the sea ice flora, contain elevated amounts of 22:6n-3 and C_{18} PUFA compared with centric diatoms, which dominate the open water spring bloom flora (McConville 1985). This raises the possibility of investigating the relative contribution of these two groups of primary producers to their consumers (Cripps and Hill 1998), and possibly to food web production in the Arctic. The ability to trace diatom- versus flagellate-based food webs in juvenile cod was demonstrated by St. John and Lund (1996) using the ratio of 16:1n-7/16:0. Although other such "biomarkers" (e.g., 20:5n-3 and other PUFA) cannot be isolated to a specific primary producer, differing levels of these FA may characterize different producers. Calanoid copepods synthesize considerable amounts of isomers of 20:1 and 22:1, which they incorporate into their WE as fatty alcohols (see Sect. 12.2.1). Thus, these FA are not very useful for examining diet variations among copepods. Nevertheless, many studies have demonstrated the conservative incorporation of unaltered dietary FA into copepod WE, which can clearly be used as trophic tracers (Dalsgaard et al. 2003; see Chap. 6). Another example of a tracer was the discovery of *trans*Δ6–16:1 (*trans*-6-hexadecenoic acid) indicating jellyfish in the diet of both ocean sunfish (*Mola mola*) and the leatherback turtle (*Dermochelys coriacea coriacea*) (Ackman et al. 1972; Hooper et al. 1973).

As trophic levels increase, the ability to use a unique FA to trace feeding to a specific food type is reduced. Certainly, 20:1 and 22:1 FA isomers are very useful as copepod markers in the next-higher level consumers such as larval herring (Ackman 1980). This is also true for the zooplanktivorous fin whale (*Balaenoptera physalus*), and allowed differentiation between North Atlantic and Antarctic populations (Ackman and Eaton 1966). However, in higher trophic level predators (including larger adult herring), these FA taken alone do not make it possible to determine whether the predator consumed copepods directly or consumed copepod consumers. For example, the FA 20:1n-11 and 22:1n-11, arising from copepod WE, are the dominant FA in herring from Prince William Sound, Alaska. These predictably increased in concentration from zooplanktivorous juvenile herring to large piscivorous adults (Fig.12.1), consistent with known ontogenetic changes in herring diets (Iverson et al. 2002). Here, the increase in 20:1 and 22:1 isomers could not be attributed to greater copepod consumption, but to greater consumption of fish that ate copepods and therefore concentrated copepod signatures. Likewise, at higher trophic levels such as seals, it is generally not possible to distinguish between direct consumption of zooplankton or of herring when using only these FA. Thus, in principle, inferring diets directly from one or a few FA is a risky practice for higher trophic levels.

Nevertheless, as indicated above, certain FA or combinations of FA can still serve as useful ecosystem markers at upper trophic levels. For instance, while 22:1n-11 is the dominant isomer over 22:1n-9 in most marine ecosystems, 20:1n-9 is by far the dominant isomer over 20:1n-11 in the North Atlantic (e.g., Budge et al. 2002). However, several studies have shown the reverse in the Pacific, with 20:1n-11 dominating 20:1n-9 in fish and invertebrates (Saito and Murata 1998). On this basis, North Pacific and North Atlantic harbor seals (*Phoca vitulina*) consuming

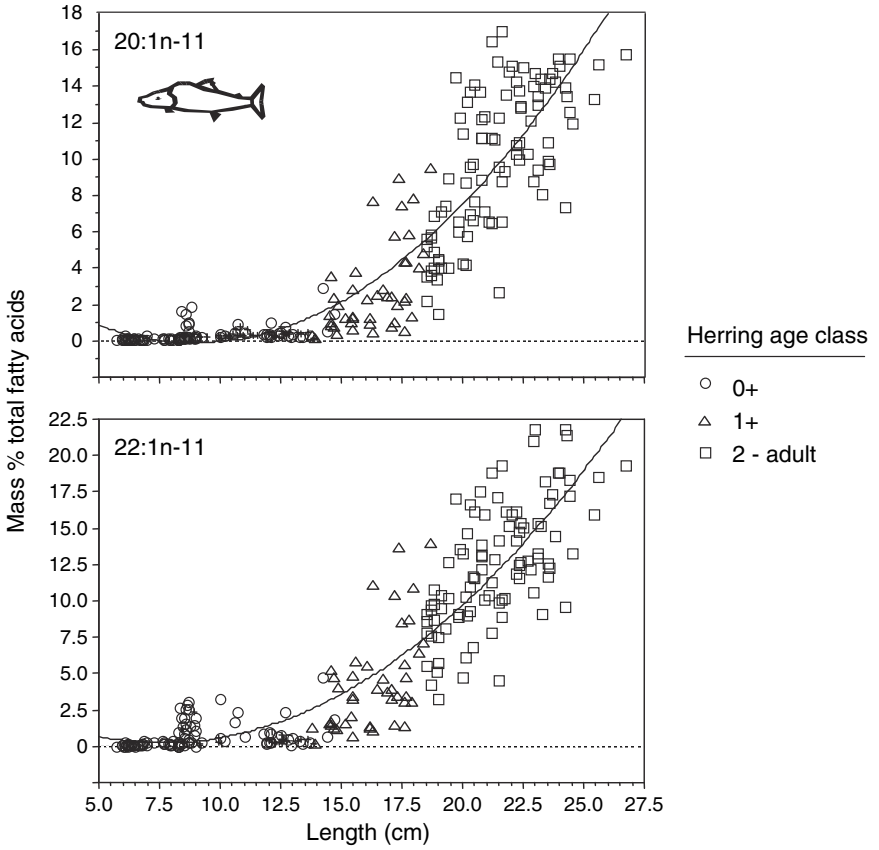

Fig. 12.1 Variation in two indicator FA in Pacific Herring (n = 300) from similar regions within Prince William Sound, Alaska, as a function of body length and age class. Data taken from Iverson et al. (2002)

these prey can be readily distinguished (Iverson et al. 1997). Similarly, Smith et al. (1996) used low levels of such marine-based FA, coupled with high levels of 18:2n-6, 18:3n-3, and 20:4n-6 (FA typical of primary producers in freshwater and terrestrial ecosystems, with low abundance in marine ecosystems), to distinguish freshwater from marine harbor seals. Based on the same principle, terrestrial foraging was confirmed in polar bears in late summer in Hudson Bay after measuring up to 7% 18:3n-3 in the milk of several females (which also had berry stains on their teeth; S.J. Iverson and I. Stirling unpublished data). Typically, levels of 18:3n-3 in marine ecosystems (seals or prey) are <0.5%, and have only been found at high levels in the milk of carnivores that have fed directly on plants (Iverson and Oftedal 1995; Iverson et al. 2001b).

Other types of biomarkers, or at least general trophic tracers, can include odd- and branched-chain (i.e., *iso* or *anteiso*) FA of 14–18 carbons, but these may or may not be useful depending on the phylogenetic order. These FA arise from bacteria and

may indicate detrital feeding in primary consumers (Dalsgaard et al. 2003). However, in higher trophic levels, trace quantities of these FA could also largely reflect bacteria present in the gut flora of the consumer itself, rendering conclusions based on these trophic markers less certain (Iverson et al. 2004). In contrast, a potentially important group of FA are the NMI FA, which are produced only by certain benthic mollusks and gastropods (see Sect. 12.2.1), leading Paradis and Ackman (1977) to suggest that NMI FA might be useful biomarkers in food web studies. Recent work used the different types or proportions of these NMI FA produced by different mollusk species to reveal niche separation between sympatric bearded seals (*Erignathus barbatus*) and walruses (*Odobenus rosmarus rosmarus*), which both specialize on benthic mollusks (Budge et al. 2007). Furthermore, differences in the proportions of specific NMI FA have been used to estimate the relative importance of bearded seals and walruses, amongst other marine mammal prey, in the diets of large adult male polar bears; and these findings were confirmed through quantitative diet analyses (see Sect. 12.3.3) using entirely independent FA and diet estimates (Thiemann et al. 2007).

Finally, an example of another type of biomarker, but at the whole lipid level, is the case of WE, although this is in principle limited to a few predators. Only certain species synthesize and store WE (see Sect. 12.2.2), but because WE are broken down during digestion in the predator and reassembled independently as TAG or WE (see Sect. 12.2.3), they will generally not be useful tracers. However, an exception is found in Procellariiform seabirds (e.g., albatrosses and petrels), which store a significant amount of dietary lipid in stomach oils, in addition to their adipose tissue. These stomach oils do not undergo metabolic processing and can therefore contain large amounts of WE reflecting recent prey ingestion; at the same time no WE are ever present in adipose tissue of the same individuals, which instead reflects deposition of both dietary FA and WE fatty alcohols over a longer integration period (Wang et al. 2007). Thus, the presence of WE in stomach oils in Procellariiforms can be used not only as confirmation of consumption of prey species that make WE, but also provides an opportunity to examine different time scales of dietary integration.

Evaluating differences in the full array of FA among predators at similar phylogenetic levels is a promising, qualitative, way to look at trophic interactions, especially at higher trophic levels. The term "signature," or profile, refers to the relative concentration of the full array of FA identified or a subset of those FA most indicative of dietary intake, rather than just one or a few biomarkers (Iverson 1993). Such arrays will likely be most informative at higher trophic levels. Support for this approach comes from a number of experimental studies on fish, birds, and mammals, and can be illustrated clearly by a few examples. When Atlantic cod were switched to diets consisting of a single prey, i.e., low-fat squid (*Illex illecebrosus*) or high-fat mackerel (*Scomber scombrus*), the whole body FA signatures of these cod clearly reflected the FA signature of their diets (Fig.12.2). Had only liver (the major site of lipid storage in cod) been sampled and analyzed, there would likely have been an even closer correspondence with diet (see Sect. 12.3.1.1). Such findings demonstrate that FA composition of whole fish will largely be defined by their general ecology and dietary habits. This raises the possibility of using FA to characterize foraging

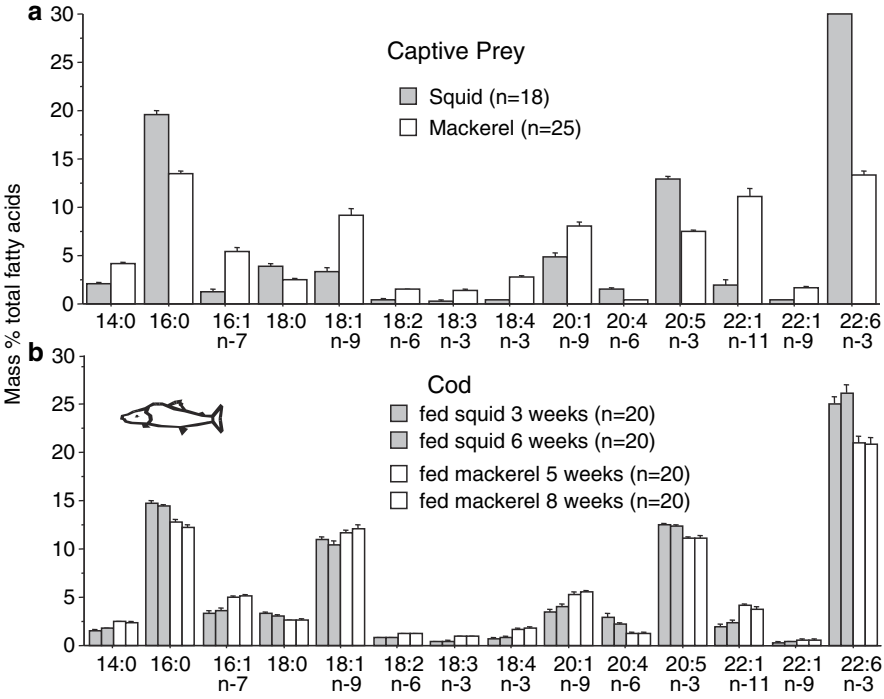

Fig. 12.2 Relative amounts (mass %) of selected abundant FA in (**a**) captive prey diets and (**b**) Atlantic cod dietary treatment groups. *Bars* are means and *vertical lines* are +1 SE. All cod were caught from the same net trawl and housed in seawater tanks at 2.8–4.0°C; all cod were fed other cod from the same lot for 4 weeks and then all were switched to the same diet treatment, but individuals were sequentially removed for whole body FA analysis. The first two *bars*: cod switched to diet of squid (2% lipid) for 3 and 6 weeks, respectively; second two *white bars*: remaining cod switched to diet of mackerel (16% lipid) for 5 and 8 weeks, respectively. Data taken from Kirsch et al. (1998)

differences within and between species and also to use their FA profiles in the evaluation of diet studies of higher predators (Budge et al. 2002; Iverson et al. 2002).

Similarly, in a study of two captive seabird species large differences in the overall FA signatures of two different prey types were reflected in synsacral adipose tissue biopsies of common murres (*Uria aalge*) and red-legged kittiwakes (*Rissa brevirostris*) (Fig. 12.3a). Although some absolute differences between diet and adipose tissue were apparent, the greatest influence on the FA composition of adipose tissue was the diet. Equally important, FA profiles of the two different species on the same diets were nearly identical and slight differences could be attributed to early feeding differences prior to the experiment. FA patterns in these same seabird species in the wild bear no resemblance to those of the captive birds (Fig.12.3b) and correspond to known foraging differences between murres and kittiwakes (Iverson et al. 2007). Thus, differences found in FA patterns of free-ranging individuals indicate differences in diet of those individuals.

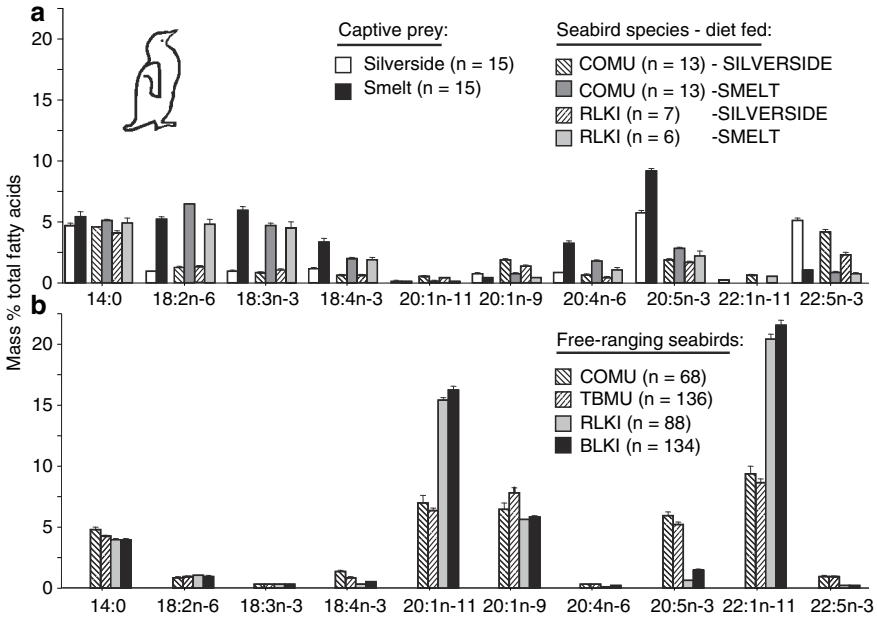

Fig. 12.3 (**a**) Selected abundant FA (mass %) with large overall variance illustrating characteristic differences in patterns among the two primary prey items and in the adipose tissue of captive common murre (COMU) and red-legged kittiwake (RLKI) chicks fed two prey types. *Bars* are means and *vertical lines* are +1 SE. For the diet trial, chicks were fed diets consisting of either silverside (*Menidia menidia*) or smelt (*Osmerus mordax*) from 15- to 42- or 45-day post-hatching. Prior to this, chicks had been fed either silverside (COMU) or a mixture of silverside and herring (RLKI) from 0- to 10- or 15-day post-hatching. (**b**) The same selected FA in free-ranging murres (COMU and thick-billed, TBMU, *Uria lomvia*) and kittiwakes (RLKI and black-legged, BLKI, *Rissa tridactyla*) in the Bering Sea. Data taken from Iverson et al. (2007)

Finally, in marine and aquatic mammals, a number of studies have documented the influence of dietary FA source on body FA composition (Iverson et al. 2004; Budge et al. 2006). An example of this is provided by another comparison of captive individuals to their free-ranging counterparts. The Hawaiian monk seal (*Monachus schauinslandi*) is one of only two pinnipeds to inhabit a tropical ecosystem. These systems contain organisms with FA profiles that are quite different from those of northern/temperate or polar ecosystems. For example, typical of tropical ecosystems (Dalsgaard et al. 2003) prey throughout the Northwestern Hawaiian Islands (NWHI) generally contain very low levels of long-chain monounsaturated isomers of 20:1 and 22:1, and relatively very high levels of long-chain n-6 PUFA. Comparison of blubber FA signatures of monk seals feeding in the NWHI versus captive monk seals maintained for an extended period on North Atlantic herring illustrates the dramatic and predictable influence that dietary FA have on lipid stores (Fig.12.4). Such comparisons of predator FA signatures alone have provided considerable insight into foraging ecology, diet shifts, and habitat segregation of many species.

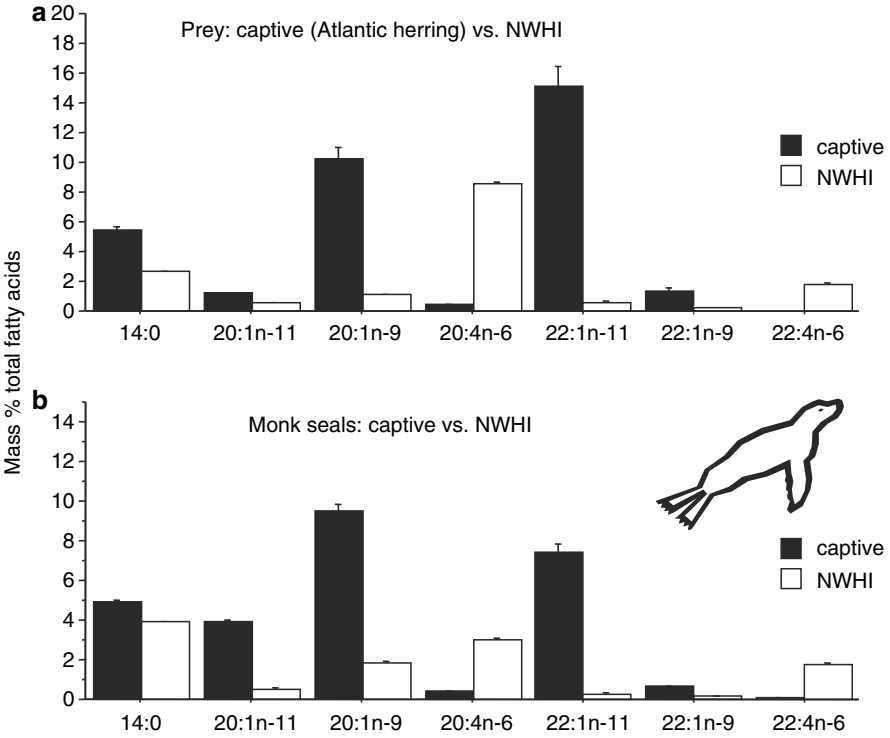

Fig. 12.4 Selected dietary FA (mean + 1 SE) in (**a**) the prey (herring) of captive monk seals in comparison to prey in the Northwestern Hawaiian Islands (NWHI) and (**b**) captive monk seals ($n = 10$) fed Atlantic herring in comparison to that of the blubber of free-ranging monk seals ($n = 157$) in the NWHI. Values for captive prey are the average of all herring analyzed ($n = 25$, from five different lots fed) and for wild prey are simply the average of all prey species previously analyzed in the NWHI data base ($n = 1{,}540$ individuals; S.J. Iverson and G. Antonelis, personal communication) for comparison purposes. The high levels of 14:0, 20:1n-11, 20:1n-9, 22:1n-11, and 22:1n-9 of Atlantic herring were reflected in captive seals, while much lower levels of these components and the high levels of n-6 PUFA in NWHI prey were reflected in wild seals. Data from Iverson et al. (2003)

12.3.2.2 Methods and Statistics

When evaluating different FA arrays, important issues include the choice of FA and subsequent data analyses. Finding a "significant" difference in levels of a specific FA among groups does not indicate whether this difference is biologically meaningful or whether the overall profile of FA differs between groups. Multivariate analyses, which also allow pattern recognition, are generally the most powerful as they use the maximum number of FA for differentiating predators and resolving trophic interactions (Budge et al. 2006). However, because there are generally a large number of FA identified (up to 70 in marine samples) in relation to sample size, there are restrictions on the number of FA that can be used (usually n-1 of group sample size) to meet statistical requirements. Thus, choices must be made as

to which FA to use. Clearly, not all FA provide information about diet for various consumers and these can usually be removed (see also Sect. 12.3.3). For instance, any FA with <14 carbons within a consumer will have arisen from de novo biosynthesis and has no relation to diet (see Sect. 12.2.3). In mammals, 14:1n-5 arises almost entirely from biosynthesis from precursors, as demonstrated by very elevated levels of this FA found in the blubber of newborns (Iverson et al. 1995) or in the structured outer blubber of small cetaceans (Koopman et al. 1996). Whether the isomers of 20:1 and 22:1 or odd and branched-chain FA are valuable will depend on the phylogenetic order of the consumer: the former will not be useful dietary markers in calanoid copepods and perhaps other zooplankton, while the latter should be used in primary consumers but not higher orders (see Sect. 12.3.2.1). The FA 22:5n-3 is often considered a potential intermediate between 20:5n-3 and 22:6n-3; however, this may require evaluation as it can also be highly indicative of diet (Fig.12.3a; Iverson et al. 2007). Other FA may be removed as they are not indicative of diet or reliably measured. Thus, when reducing the FA set for statistical reasons, a useful practice is to choose FA that are obvious dietary markers (e.g., Appendix A in Iverson et al. 2004), as well as those that are the most abundant and/or exhibit the greatest average variance across individuals. In higher order consumers, these can also include FA that could be biosynthesized, but are likely to be most heavily influenced by dietary intake (Fig.12.2). Since FA analyses generally report values as mass % of total FA, it may be necessary to renormalize the chosen FA over 100% and/or transform the data (e.g., taking the log ratio of all chosen FA over a reference FA) to meet requirements of normality (Budge et al. 2002; Iverson et al. 2002). Proportional data can also be arcsine square root transformed, i.e., = acrsine(sqrt(proportion)), to achieve normality.

Useful multivariate techniques for these types of analyses have been reviewed extensively by Budge et al. (2006). In brief, multivariate analysis of variance (MANOVA) tests whether the mean differences among groups, based on a linear combination of response variables, could have occurred by chance, and subsequently allows identification of the FA(s) contributing to the differences. Discriminant function analysis (DFA) is used to classify samples into groups, and to describe differences among those groups, by creating a series of uncorrelated linear relationships among the original FA variables. The plot of scores derived can effectively reveal relationships among and within sample groups. Similar to DFA, principal component analysis (PCA) can describe relationships among variables, as well as reduce large numbers of variables to fewer components that represent most of the variance in the data. Complementary techniques that have fewer restrictions on numbers of variables used in relation to sample size include hierarchical cluster analysis and classification and regression trees (CART), as well as analysis of similarity (ANOSIM, Clarke 1993). All of these techniques provide the investigator with effective methods to examine trophic relationships among predators, and also allow evaluation of the ability to differentiate the prey of a predator when subsequently considering quantitative methods for estimating diet (see Sect. 12.3.3). For example, DFA analysis of 13 species of fish and invertebrates in Prince William Sound characterized similarities and differences among these consumers

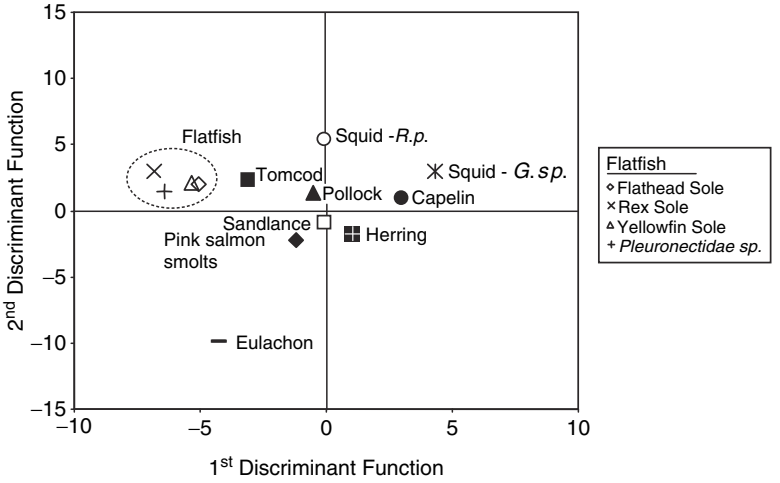

Fig. 12.5 Discriminant analysis performed on 13 common species of fish and invertebrates (each with $n \geq 17$, $n = 1{,}050$ total) in Prince William Sound, Alaska, using 17 of the most abundant FA, which together accounted for about 88% of total FA. The plot shows the group centroids (within group mean for each discriminant function) for the first and second (of 12 significant) discriminant functions, which accounted for 63% of total variance. This analysis classified individuals to species with 93.0% success rate when flatfish species were grouped as a whole. Herring (*see* Fig.12.1) were classified with 94.5% success. Reproduced from data in Iverson et al. (2002)

(Iverson et al. 2002). Overall, 93% of individuals were correctly classified to species by their FA composition. Flatfish species, which feed primarily on benthic infauna and epifauna, grouped closely together, and farther away from the more pelagic zooplanktivores and piscivores such as herring and pollock, clearly illustrating ecological differences (Fig.12.5). Despite the large FA variation in herring with ontogeny (Fig.12.1), 94.5% were still correctly classified, illustrating that between-species differences in FA signatures tend to be more pronounced than within-species differences as has been found elsewhere (Budge et al. 2002).

12.3.3 Quantitative Estimation of Predator Diets

The characteristics of FA biosynthesis, digestion, and deposition among organisms (see Sect. 12.2), coupled with the wide arrays of FA arising in marine food chains (Table 12.1) and the findings that characteristic FA signatures found in many prey types can be used to accurately classify individual species (e.g., Figs. 12.2a, 12.3a, 12.5) raises the possibility of using FA signatures to produce quantitative estimates of predator diets. This is perhaps the area of greatest current interest in using FA to elucidate food web relationships, especially for investigators working at higher trophic levels. Although statistical techniques described above (see Sect. 12.3.2) address important questions about patterns of FA composition among individuals

and populations, they cannot be used to determine species composition of the diet of higher predators. The number and patterns of FA within a predator, as well as among and within potential prey species, are extremely complex, and there will always be absolute differences between predator and prey FA signatures due to predator metabolism. Thus, it is neither possible to visually assess species composition of diets in higher predators in a quantitative manner, nor usually even in a qualitative manner. Quantitative FA signature analysis (QFASA) is a first generation statistical tool designed to quantitatively estimate predator diet using FA signatures (Iverson et al. 2004).

The basic approach of QFASA is to determine the weighted mixture of prey species FA signatures that most closely resembles that of the predator's FA stores to thereby infer its diet. Details of the initial QFASA model are provided by Iverson et al. (2004) and are further discussed by Budge et al. (2006). Briefly, assuming appropriate sampling and analysis of predator and prey lipids (see Sect. 12.3.1), the model proceeds by applying weighting factors (calibration coefficients) to individual predator FA to account for the effects of predator metabolism on FA deposition in lipid stores. These are empirically determined from controlled long-term feeding trials. It then takes the average FA signature of each prey species (or within-species group) and estimates the mixture of prey signatures that comes closest to matching that of the predator's FA stores by minimizing the statistical distance (e.g., Kulback-Liebler) between that prey species mixture and the weighted predator FA profile. Finally, this proportional mixture is weighted by the proximate fat content (i.e., relative FA contribution) of each prey species to estimate the proportions in the predator's diet. Each of these steps requires careful consideration.

Perhaps the most important issue when using FA quantitatively is accounting for predator metabolism. Even in predators that consume high-fat diets, there will be physiological affects on FA deposition. Thus the FA composition of the predator will never exactly match that of their prey. At present, predator effects on FA deposition are accounted for using calibration coefficients, which are simple ratios for each FA in the predator fat divided by that FA in the diet. The principle is that if a predator had consumed a constant diet over a very long term, the FA signature of its lipid stores would resemble this diet as much as possible, and any differences would be attributable to metabolic processing of individual FA. These "coefficients" can be derived from studies where a predator has been fed a constant diet for a prolonged period, with the hope that this is sufficient time to have completely turned over all of its stored FA. Unfortunately, we do not yet know what the total FA turnover time is for most predators. Even so, data have been obtained from studies on captive pinnipeds, mink (*Mustela vison*), and seabirds (Fig.12.6). The key points from these data are that the calibration coefficients are, in general, similar across bird and mammal species and diets. This confirms the dominance of dietary FA deposition, as well as postulated secondary impacts from biosynthesis and modification (see Sect. 12.2). Second, the application of these coefficients in the QFASA model has been shown to be critical to accurate diet estimates (Iverson et al. 2004). To date, calibration coefficients have not been estimated or evaluated in fish or invertebrates, but could potentially be done from controlled studies such as those summarized in

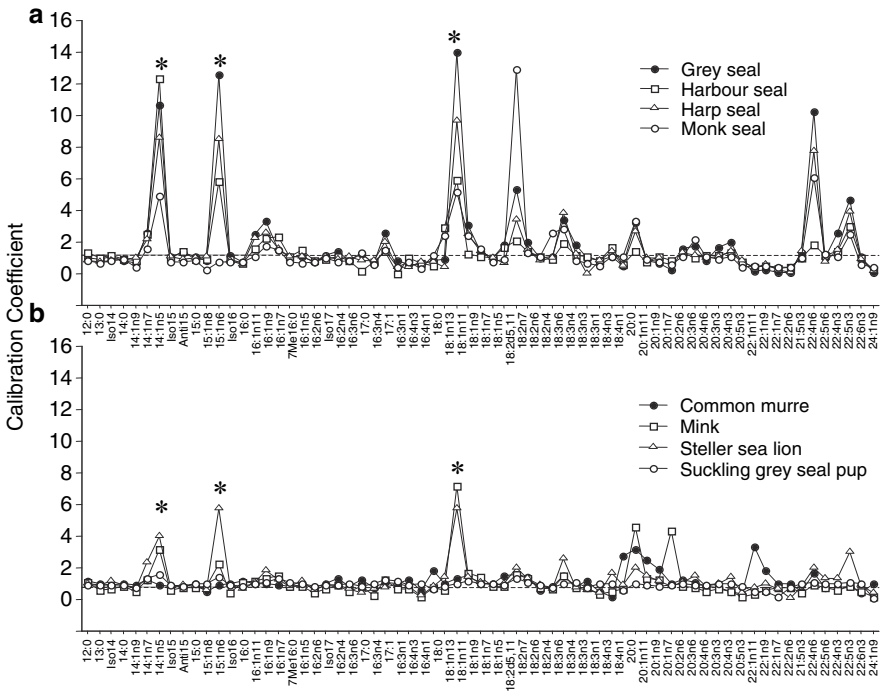

Fig. 12.6 Calibration coefficients (CCs, means of 5–16 individuals each) calculated from controlled experiments on (**a**) four species of phocid seals and (**b**) one otariid species, mink, and common murres all fed fish diets (3–10% fat), and in suckling grey seal pups consuming their mother's milk (60% fat). CCs were calculated according to Iverson et al. (2004) as: % of each FA in each predator/average %FA in diet, but without using trimmed means. The 1:1 line denotes the deviation of a given FA in the predator from that consumed in diet. *Asterisks* indicate examples of FA with large deviations from 1:1 but which occur at only minor or trace amounts in marine lipids, with contributions from biosynthesis, and routinely not used in QFASA modeling. Data taken from Iverson et al. (2004); Tollit et al. (2006), Iverson et al. (2007); and Nordstrom et al. (2008)

Chap. 6. There may be other ways to quantitatively incorporate metabolism effects on consumer FA deposition and such research should be encouraged.

There are a number of other important issues that should be recognized to successfully use QFASA (see Iverson et al. 2004 and Budge et al. 2006 for detailed discussion). These include building a representative and comprehensive prey FA database for each predator and sampling all prey sufficiently to allow quantitative evaluation of within and between-species variability to confirm the ability to reliably differentiate prey species in the model. The choice of the FA used is also critical to model outcomes and will likely also depend upon the reliability of calibration coefficients determined for specific FA. Ways to incorporate within-species variability in prey FA and fat content in estimates, as well as ways to measure goodness of fit, are still being developed. Optimization of model outputs can be assessed when there is corroborating evidence available (i.e., studies of captive animals or some free-ranging animals where other supporting diet information exists), but this is

often not possible. Understanding the detection limits of prey is important, especially in cases where there is interest in prey species rather than predators. Finally, the ability to apply QFASA to lower trophic levels has not yet been explored.

Despite issues concerning its further advancement, QFASA has now been successfully validated for a variety of predators including pinnipeds, mink, and seabirds (Iverson et al. 2004, 2007; Cooper 2004; Cooper et al. 2005; Nordstrom et al. 2008). Since most validation studies are performed within the constraints of captive animal experiments, tested diets are usually composed of only one or a few prey species, potentially limiting conclusions drawn. However, two studies have allowed evaluation of QFASA in free-ranging animals in their natural habitat consuming a wide array of prey. Results from a captive validation study of seabirds were extended to the study of 235 free-ranging murres and kittiwakes by comparing QFASA diet estimates to those derived from stomach contents in the same individuals. Although one would not expect results from the two analyses to be identical, mainly because the two methods integrate diet over different time scales, both methods indicated the same dominant prey (Fig.12.7a, b) and characterized well-established differences in the known diets of murres and kittiwakes in the Bering Sea. In the Northwest Atlantic, free-ranging harbor seals, fitted with head-mounted video-cameras, allowed estimates of prey capture, which were then compared with

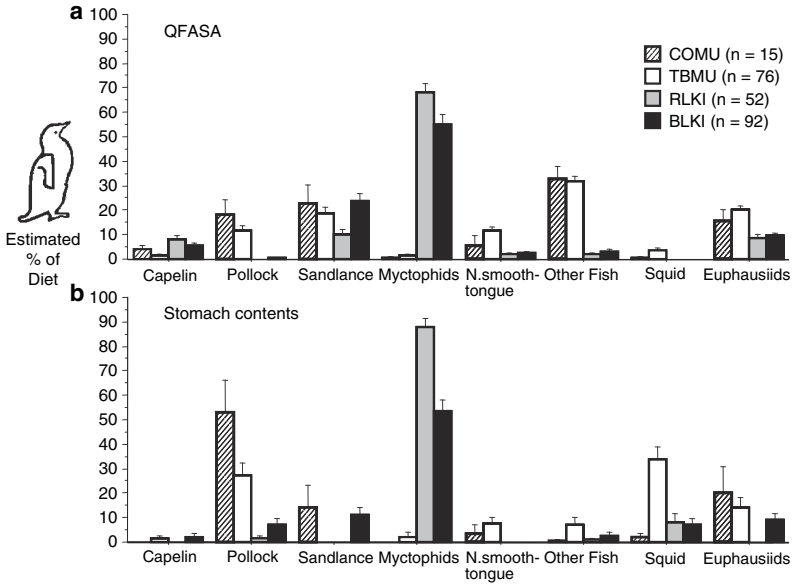

Fig. 12.7 Diet estimates of free-ranging murres (COMU, TBMU, *see* Fig.12.3) and kittiwakes (RLKI, BLKI) (n = 235) in the Bering Sea using (**a**) QFASA (modeled on 161 prey representing 15 species) in comparison with (**b**) stomach contents analysis in the same individuals. *Bars* are means of each species estimated in diets and vertical lines are +1 SE. Reproduced from data in Iverson et al. (2007)

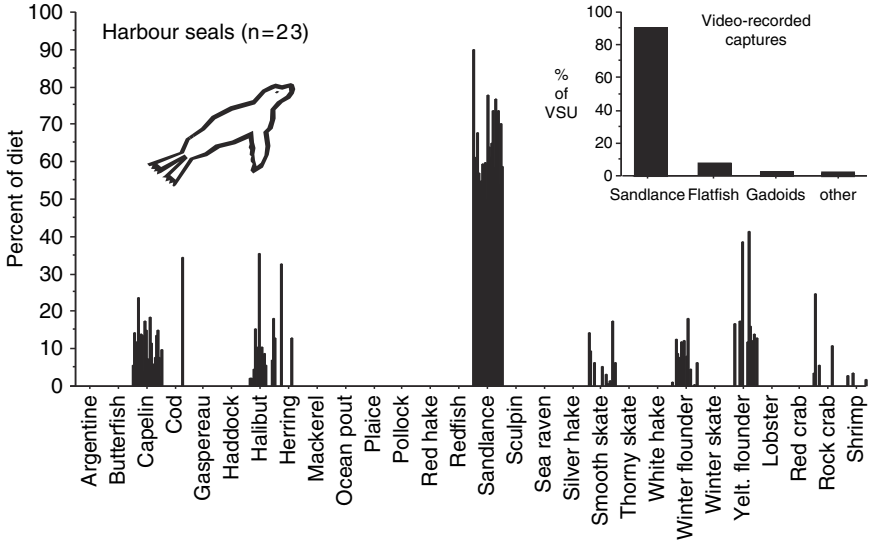

Fig. 12.8 Individual QFASA diet estimates of the contribution of prey species to diets of 23 free-ranging adult male harbour seals, at Sable Island, Nova Scotia, deployed with an animal-borne video system ("Crittercam") and filmed during natural feeding events. Seal signatures were modeled using a Scotian Shelf prey database of 954 prey representing 28 species. *Inset*: prey types consumed in video recordings of these seals, expressed as percent of all 10-min video-sampling units (VSU) that filmed prey captures and which contained identifiable items (n = 223). Reproduced from data in Iverson et al. (2004)

the diet estimates in the same individuals obtained using QFASA (Fig. 12.8). QFASA diet estimates were similar to those recorded on video and were also consistent with major prey identified in gastric lavage data and fecal analyses (Iverson et al. 2004). On the basis of these studies, QFASA is now being used to address broader ecosystem-scale processes (Bowen et al. 2006; Iverson et al. 2006b, 2007; Beck et al. 2007). The current conclusions drawn from such quantitative studies are that QFASA is a potentially powerful tool in ecological research, which has provided new insight into the foraging patterns and ecology of free-ranging predators that would otherwise not be discernable. Nevertheless, QFASA must be used with due diligence and investigators should understand that there are many issues that remain to be resolved, or need to be further investigated, to improve its reliability.

12.4 Summary, Conclusions, and the Future

In their very thorough review, Dalsgaard et al. (2003) noted that FA can only presently be used qualitatively or semiquantitatively and concluded that the state of the art for using FA as trophic tracers was essentially at the same level described 30 years ago by Sargent (1976): "*At the present state of our knowledge it would appear*

that fatty acid analyses represent a rather blunt tool in defining food chain inter-relationships. Until further knowledge is accumulated it would appear best to apply fatty acid analyses as a corroborative method to support prey–predator relationships already indicated on independent grounds, such as analyses of stomach contents". In just a few short years since this review, the field of FA signature analysis has clearly advanced. With the advent of QFASA at higher trophic levels, we are now successfully using FA to understand some otherwise entirely intractable systems and ecosystem processes. Using QFASA, we are beginning to understand demographic (i.e., sex, age) and individual sources of diet variability in an abundant marine predator, the grey seal (*Halichoerus grypus*), and how it uses the North Atlantic ocean relative to prey abundance and distribution (Bowen et al. 2006; Beck et al. 2007). We have studied polar bear foraging at continental scales (i.e., across the entire Canadian Arctic), providing new insight into individual and population responses to changes in prey distribution and climate (Iverson et al. 2006b). We have also linked seabird foraging to productivity and prey abundance throughout the Bering Sea (Iverson et al. 2007). In the tropical NWHIs, we are gaining insights into the key prey of the critically endangered Hawaiian monk seal (Iverson et al. 2006a). Nevertheless, there remains much work before we understand the full potential and limitations of QFASA. Although we have advanced considerably and are somewhat beyond the "first-cut" stage, the potential to use QFASA (or other statistical models or methods) to investigate trophic ecology will continue to be an area for fruitful research.

Understanding metabolism effects on FA stored in consumers (which for simplicity is referred to as "calibration") is extremely important in using FA to study food webs using any of the methods outlined above, and in QFASA, is also likely largely tied into choosing the appropriate FA set for modeling. QFASA was obviously designed for upper trophic level endothermic vertebrates, but so far little is known about how this can be applied to ectothermic fish and reptiles, and even less about its potential application to primary consumers such as zooplankton. Certainly one way to advance this knowledge would be to conduct more rigorously controlled long-term studies to estimate calibration in a wide variety of consumers, including whether the principle of calibration coefficients could be used in lower orders. Even at the highest trophic levels, coefficients have only been evaluated in one species of seabird and none have yet been evaluated for cetaceans, polar bears, or sea otters.

A potentially powerful trophic indicator, which should be further studied and calibrated is mammalian milk. Given the metabolic dominance of the lactating mammary gland, ingested FA are directed first into milk over other tissues (Iverson 1993). For example, even though consumption of small amounts of very low-fat terrestrial vegetation could not be detected in polar bear adipose tissue using either FA or stable isotope analyses (Hobson and Stirling 1997), it was detected in milk FA (see Sect. 12.3.2.1). However, QFASA has not yet been adapted to analyses of milk.

Additionally, within higher-predator species, the influence of fat content of the diet on quantitative calibration has not yet been addressed but should be important, as higher fat diets are presumably associated with greater fat deposition and lower

biosynthesis or modification of FA by predators. Similarly, the effects on predator FA deposition of consumption of prey with widely differing fat contents requires further evaluation. Future studies should also examine whether the FA composition of the control diet used to determine calibration affects the eventual modeling with prey of similar versus widely differing FA compositions. There also needs to be a better understanding of the quantitative effects of fasting and mobilization on the composition of predator FA stores, especially in species that routinely fast as part of their life history. Finally, while it has been demonstrated that QFASA estimates correctly reflect major components of diet, the ability to identify trace components may be limited. Care is also clearly required in interpreting results from prey species with similar FA signatures, which may cause false positives in estimates (e.g., Iverson et al. 2004; Budge et al. 2006; Nordstrom et al. 2008).

In theory, if all the above requirements for using QFASA (see also Sect. 12.3.3) were met for each consumer in a given ecosystem, one could model diets of each consumer/predator at each subsequently higher trophic level. Iteration backwards might allow estimates of ecosystem processes responsible for food web production and budgets. Whether this could ever be realized remains to be established, but almost certainly could not be accomplished using FA and QFASA alone. Additional tools for better understanding trophic transfer of FA in general and the performance of QFASA in particular could make use of advanced and targeted techniques, such as using radio or stable isotope-labeled FA to trace pathways from diet to deposition (Budge et al. 2004; Cooper 2004; Cooper et al. 2006). These types of methods should provide even better insight at all trophic levels but are fairly cost-intensive. Of course, combining FA and QFASA with other types of corroborative evidence will always be useful and aid in further validation of using FA as accurate trophic tracers. One particularly important area that should continue to be explored is combining FA with stable isotope analyses of tissues or of individual FA (e.g., Kharlamenko et al. 2001; Hebert et al. 2006). Recently, the convergence of diet estimates derived from FA and stable isotopes was illustrated by the correlation between estimates of the trophic position of free-ranging grey seals from QFASA and $\delta^{15}N$ values (Tucker et al. 2008). Additionally, the stable carbon isotopic composition of diatom FA has been used to investigate the contribution of ice algae to higher trophic levels and shows promise in tracing carbon flow through an Arctic marine food web (Budge et al. 2008).

In conclusion, while there are many areas of research to pursue and questions to address, it is clear that FA can be used as powerful trophic tracers of carbon flow, food webs, predator–prey interactions, and even ecosystem structure and dynamics within marine and other aquatic ecosystems. FA are particularly useful in that they are relatively easily measured, sensitive to change and responsive to change in a predictable manner, and are integrative. The fact that techniques have advanced to the point of estimating overall species composition of diets of individual predators, at time scales relevant to the ecological processes affecting survival, is exciting. Coupled with other information, FA can provide valuable insights about the ecology of individual species and ecosystem structure and functioning.

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