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Similarity between predator and prey fatty acid profiles is tissue dependent in Greenland sharks (*Somniosus microcephalus*): Implications for diet reconstruction

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ABSTRACT

Fatty acid (FA) analysis is increasingly being applied to study the feeding ecology of sharks. However, very little knowledge exists regarding how sharks alter dietary FAs prior to incorporation into their tissues, or which tissue provides the most accurate representation of diet. To provide insight into these questions, we compared FAs of muscle, liver and blood (plasma) of 18 individuals of a large elasmobranch, the Greenland shark *Somniosus microcephalus* (Bloch and Schneider, 1801), to FA profiles of several known prey. Greenland sharks fed predominantly on Greenland halibut (*Reinhardtius hippoglossoides*, Walbaum 1792) and ringed seal (*Pusa hispida*, Schreber, 1775) based on stomach contents. Shark muscle FA profiles were the most similar to prey FA profiles, both in relative proportions (e.g. muscle 18:1n−7 = 7.1 ± 1.0%, ringed seal blubber = 7.2 ± 1.2%) and on a μg mg^{-1} basis (e.g. shark muscle 22:1n−11 = 47.9 ± 12.0 μg mg^{-1} dw, Greenland halibut muscle = 59.9 ± 18.5 μg mg^{-1} dw), indicating direct incorporation of most FAs from the diet. Shark blood plasma FAs also corresponded to prey FAs, and were more similar to shark muscle than liver, which supports the suggestion that muscle FAs were of dietary origin. Shark liver had the most variable FA profiles among individuals and retained higher amounts of long-chain monounsaturated FAs (e.g. 20:1n−9) than were observed in prey. As a consequence, shark liver FAs differed the most from known prey like ringed seal. Our results indicate that the FA profiles of shark tissues will not always match those of dominant prey items, and highlight the use of non-lethal tissues, like muscle and plasma, for studying shark diet using FA analysis. Further work is required to unravel what mechanisms underlie the observed differences in FA profiles and shark–prey FA relationships among different shark species, but data presented here will aid future researchers in more accurately applying FAs to study the diet of large, mobile sharks.

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1. Introduction

Sharks are important predators in marine and brackish waters, affecting prey populations both directly (i.e. predation) and indirectly (e.g. by generating predator avoidance behavior) (Ferretti et al., 2010). However, a complete understanding of how sharks affect energy flow through food webs requires a detailed knowledge of their feeding ecology (Myers et al., 2007). Fatty acids (FA), which serve a multitude of biochemical functions in animals (e.g. cell membrane constituents, precursors to eicosanoids, energy source, Tocher, 2003), are a promising tool for investigating the diet of marine predators like large sharks (Schaufler et al., 2005) that are difficult to study via traditional stomach content analysis. For example, the 'essential' FAs, arachidonic acid (ARA, 20:4n−6), eicosapentaenoic acid (EPA, 20:5n−3) and docosahexaenoic acid (DHA, 22:6n−3), can often be used as dietary tracers because they cannot be biosynthesized by most marine consumers in amounts sufficient to meet their needs (Parrish, 2009). Although some FAs (e.g. 16:0 and 18:0) can be biosynthesized by fishes, while others can be modified via chain shortening (e.g. 20:1n−9 to 18:1n−9) or elongation (e.g. 16:1n7 to 18:1n−7) (Tocher, 2003), it is generally accepted that the tissue FA profile of a consumer largely reflects FAs retained from the diet (Iverson, 2009).

Fatty acids have only recently been applied to investigate the diet of elasmobranchs (Pethybridge et al., 2011; Schaufler et al., 2005; Semeniuk et al., 2007; Wai et al., 2011). However, the current lack of data regarding how sharks alter dietary FAs prior to tissue incorporation is a major concern with this application. Predators are widely acknowledged to modify dietary FAs to meet their needs, which could lead to divergence between predator and prey profiles (Iverson, 2009). Thus, the question becomes, how much, on a tissue-specific basis, will a predator modify dietary FAs and other lipids? Based on the comprehensive work of Pethybridge et al. (2010), who identified inter-tissue differences in the lipid class and FA profiles of

Abbreviations: FA, fatty acid; dw, dry weight; ww, wet weight.

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16 deep water Chondrichthyans from Australian waters, shark liver is high in storage molecules (e.g. triacylglycerol, TAG) and MUFA, whereas muscle is high in phospholipids and polyunsaturated fatty acids (PUFA). Subsequently, Chondrichthyan muscle can be more similar to PUFA-rich prey, and liver to MUFA-rich prey (Pethybridge et al., 2011). Based on these results, it is clear that elasmobranchs are selectively incorporating dietary FAs into different tissues.

It is still unclear, however, to what extent shark tissue FA profiles differ from FA profiles of dominant prey, or which tissue provides the most accurate information regarding diet. Captive feeding studies will help unravel the origin of FAs in shark tissues, although these studies will most likely be restricted to young and/or small sharks. Because differences in locomotory mode and phylogeny contribute to differences in lipid classes and FA profiles among shark species (Pethybridge et al., 2010), researchers wishing to apply FAs to study the ecology of large sharks in the wild would benefit from a directed assessment of how FAs differ between tissues of a large, mobile shark and those of known prey.

Here, we investigated within- and among-tissue variability in FA profiles of muscle, liver and blood plasma of 18 individual Greenland sharks sampled from Cumberland Sound, Nunavut, Canada, and investigated the degree that shark tissue FA profiles differed from those of known prey items (based on stomach contents). We sought to identify which tissues are the most useful for diet studies, and which tissues are the most altered by these sharks. Because elasmobranch liver functions as the major site of: 1) FA catabolism (i.e. beta oxidation), 2) ketone body biosynthesis, and 3) buoyancy regulation (achieved through retention of lipids) (Ballantyne, 1997), liver FAs are predicted to be the most modified by the sharks and to differ the most from prey profiles. Plasma, on the other hand, functions in transporting dietary FAs to other elasmobranch tissues via lipoproteins (e.g. chylomicrons) (Ballantyne, 1997), and plasma FA are predicted to be the most similar to prey profiles. Elasmobranch muscle lacks the enzymes necessary to catabolize FA, and typically has low lipid levels (Ballantyne, 1997). However, neutral, storage lipids, like TAG, are still present in shark muscle (Sargent et al., 1973), and we predict that muscle FA will provide some information about shark diet. The data presented here should help future researchers more accurately apply FA to reconstruct the dietary history of large sharks.

2. Materials and methods

2.1. Sampling of Greenland sharks

Greenland sharks were sampled for the present study from Cumberland Sound, Nunavut, Canada. The Greenland shark is one of only two sharks known to regularly inhabit polar, ice-covered waters that reach temperatures <0 °C (the other being the Pacific sleeper shark, *S. pacificus*, Bigelow and Schroeder 1944). They reach a large size (of at least 6 m, Bigelow and Schroeder, 1948), feed on a both teleost and marine mammal prey in Cumberland Sound (Fisk et al., 2002), and move throughout the water column from near the surface to several hundred meters depth in arctic waters (Skomal and Benz, 2004). Greenland sharks were sampled via bottom long line (set times = 2–24 h) baited with either seal blubber or squid. Greenland sharks were sampled for the present study in April 2008 ($n = 18$, 4 females, 14 males, total length = 265.3 ± 32.9 cm) as part of a larger field campaign, which included subsequent sampling dates in August 2008, April 2009 and August 2009. Because trends between Greenland shark and prey FA profiles were similar when considering data from only April 2008 or all sampling dates combined, data for the latter-sampled three seasons were retained for a separate investigation of seasonal and inter-annual diet variability in Cumberland Sound Greenland sharks. Greenland sharks were euthanized upon capture via an incision made through the dorsal surface (immediately behind the head, anterior to the gills) to sever the spinal cord and dorsal aortae, followed directly by a second incision through the brain. Blood was allowed to flow from the dorsal cut for several seconds before being collected into a centrifuge tube with no additives or lining. Blood was centrifuged immediately in the field, the plasma portion removed using a sterile pipette and transferred into a 5 mL cryovial. Plasma was only obtained from 12 of the 18 Greenland sharks sampled due to equipment failure in the field. Approximately 5 grams of white dorsal muscle were collected ~2 cm above the vertebrae, and placed into a 5 mL cryovial. Liver samples were taken by removing a cross section of one lobe, at approximately the mid-way point along the lobe's length, dissecting ~5 g from the center and placing into a 5 mL cryovial. All samples for FAs were immediately put on ice, and were frozen via dry ice or liquid nitrogen within 1 h from the time of collection. Stomach contents were identified to as low a taxonomic level as possible and counted for all 18 Greenland sharks (Table 1). Squid and mammal bait identified in the sharks' stomachs were not included in these counts. However, hooked Greenland halibut that were found in the sharks' stomachs were included in counts because Greenland sharks are known to eat hooked halibut off of fishing lines during the winter artisinal fishery in Cumberland Sound (McMeans, Fisk, unpublished data), and because we used shark-specific fishing gear (i.e. metal leads and gangions), these halibut were not associated with our fishing operations. The exploitation of halibut off of Inuit fishermen's lines would affect the FA profile of the sharks and is therefore relevant to the present study.

2.2. Prey sampling

Known prey of the Cumberland Sound Greenland sharks sampled here, based on stomach contents (Table 1), were sampled for FA analysis and included: arctic skate (*Amblyraja hyperborea*, Collett 1879), Greenland halibut and ringed seal. In addition, several potential prey of Greenland sharks were also sampled for FA analysis, which have been previously identified in the stomachs of Greenland sharks from Cumberland Sound (B.C. McMeans, A.T. Fisk, unpublished data), and included arctic char (*Salvelinus alpinus*, Linnaeus 1758),

Table 1
Stomach contents from 18 Greenland sharks (*Somniosus microcephalus*) sampled in Cumberland Sound, Nunavut, Canada during April 2008.

Sampling date	% Occurrence[a]	Tissue found
Invertebrates		
Buccinum cyaneum	22.2	Whole or operculum
Strongylocentrotus droebachiensis	11.1	Whole
Squid spp.	11.1	Beaks
Gorgonocephalus arcticus	5.6	Whole
Scavanging amphipods[b]	33.3	Whole
Elasmobranchii		
Amblyraja hyperborea	11.1	Sections of wing
Teleostei		
Reinhardtius hippoglossoides	72.2	Whole or pieces of skin and muscle[3]
Myoxocephalus scorpius	27.8	Whole
Lycodes reticulatus	11.1	Whole
Lumpfish	5.6	Pieces of skin and muscle
Mammalia		
Pusa hispida	16.7	Pieces (blubber/muscle) or intact body sections
Pusa hispida pup	16.7	Whole
Other		
Skate egg	5.6	Whole
Unidentified teleost	38.9	Pieces of muscle
Total fish[c]	77.8	
Total mammal	33.3	

[a] % occurrence = # of stomachs containing that prey · total # of stomachs^{-1} * 100.
[b] Including *Orchomenella* spp., *Onisimus* spp., *Menigrates* spp.
[c] Total fish and mammal = # of stomachs containing any fish or mammal species, respectively, divided by total # stomachs.

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harp seal (*Phoca groenlandica*, Erxleben 1777) and narwhal (*Monodon monoceros*, Linnaeus 1758). The data for potential prey were included in the present study to assess how Greenland shark tissue FA profiles compared to that of both known prey (Table 1) and potential prey that may not have been captured by the sharks' stomachs contents. All prey species were collected from the shark sampling sites (near the mouth of Pangnirtung fjord, see McMeans et al., 2012 for a map of sampling locations). Char were collected via gill nets, skate and halibut via bottom long line (set times 2–4 h) and marine mammals were harvested by local Inuit hunters. Narwhal were sampled during August 2007, skate and halibut during April 2008 and Arctic char, ringed seal and harp seal during August 2008. Because fish were found in shark stomachs either whole or as fragments of muscle and bone (Table 1), muscle was sampled from the dorsal surface of fish for FA analysis. The FA profile of Greenland halibut muscle reported here (Table 2), which was the dominant teleost prey of the Greenland sharks (Table 1), is similar to that reported for conspecifics homogenized and analyzed whole (Andersen et al., 2004). For example, Greenland halibut analyzed whole (Andersen et al., 2004) and as muscle only (Table 2) had 18:1n9 in high and similar proportions (mean ± SD = 15.8 ± 0.2 and 15.5 ± 2.3%, respectively). Thus, muscle likely accounted for most of the elasmobranch and teleost biomass consumed by Greenland sharks, and is a useful proxy for the FA profile of these prey. Because marine mammal tissue was found in Greenland shark stomachs predominantly as pieces of blubber (Table 1), and because blubber would contribute the most lipids to Greenland shark consumers (vs. other mammal tissues), the inner half of the dorsal surface blubber layer was sampled from mammals for FA analysis. All muscle and blubber samples for FA were placed in cryovials, immediately immersed in ice and frozen via dry ice or liquid nitrogen within 1 h after sampling.

2.3. Fatty acid analysis

Samples were freeze dried for 48 h, weighed to the nearest microgram (Sartorious ME5 microbalance), and homogenized in 2 mL of 2:1 (v/v) chloroform:methanol (Folch et al., 1957) to extract lipids. Non-lipid containing material was removed following centrifugation, and the resulting supernatants were combined in a 15 mL centrifuge tube. The lipid extract was calibrated to exactly 8 mL with 2:1 (v:v) chloroform:methanol. Then, 1.6 mL of a 0.9% NaCl in water solution was added, the phases were mixed and the upper aqueous layer was removed and discarded along with other non lipid containing material, leaving the solvent layer to be evaporated under nitrogen gas. The lipids were re-dissolved in 2 mL of 2:1 chloroform:methanol and, from this volume, duplicate 100 μL aliquots of sample extracts were added to pre-weighed, seamless, tin cups (Elemental Microanalyses Ltd. catalogue no. D4057) and evaporated at room temperature for gravimetric determination of percent total lipid (on a dry weight tissue basis). The remaining 1.8 mL of the lipid extract were evaporated to dryness under nitrogen and FA methyl esters (FAME) were produced by adding sulfuric acid in methanol (1:100) and toluene to the vials. The headspace was flushed with nitrogen, vortexed and incubated (16 h) at 50 °C in a water bath. After cooling, potassium hydrogen carbonate, isohexane:diethyl ether (1:1) and butylated hydroxytoluene (0.01%) were added to the vials, vortexed and centrifuged and the organic layer was transferred to another centrifuge tube. Isohexane:diethyl ether (1:1 vv^{-1}) was added to the original tube, which was shaken, vortexed, and centrifuged. All FAME containing layers were pooled and evaporated to dryness under nitrogen, FAME were dissolved in hexane, transferred to amber glass GC vials and separated using a Hewlett Packard 6890 GC with the following configuration: splitless injection; column = Supelco (SP-2560 column) 100 m × 0.25 mm ID × 0.20 μm thick film; oven = 140 °C (hold for 5 min) to 240 °C at 4 °C min^{-1}, hold for 12 min; carrier gas = helium, 1.2 mL min^{-1}; detector = FID at 260 °C; injector = 260 °C; total run time = 42 min per sample. Identification of FAs was accomplished using a 37-component FA standard (Supelco 47885-U) with methyl stearidonate (Fluka, 43959), 13-eicosenoic acid methyl ester (Sigma E3512), 9-eicosenoic acid methyl ester (Indofine Chemical, 20-2001-1), 16-docosatetraenoic acid methyl ester (Sigma D3534) and 19-docosapentaenoic acid methyl ester (Supelco, 47563-U) added. 11-docosenoic acid methyl ester was identified using a Triple Quadrupole GC/MS (Agilent 7890A with Agilent 7000 mass detector) and confirmed by comparing our mass spectrum with that from the American Oil Chemists' Society Lipid Library (http://lipidlibrary.aocs.org/index.html). Extraction efficiencies (estimated by adding a known amount of an internal standard, α-cholestane; Sigma catalog #: C-8003, to each of the tissue samples prior to grinding and extracting) were close to 100%. In the present study, "∑SAFA" is used to indicate

Table 2
Fatty acid proportions (% of total, mean ± SD) from the total lipid extract of *Somniosus microcephalus* and representative prey from Cumberland Sound.

Species	Tissue[a]	n	16:0	16:1n−7	18:0	18:1n−9	18:1n−7	18:2n−6	20:1n−9	18:3n−3	22:1n−11
S. microcephalus	BP	12	8.8 ± 1.4	4.3 ± 1	2.4 ± 2.2	15.3 ± 2.1	4.5 ± 1.4	1.1 ± 0.3	16.4 ± 3.4	0.5 ± 0.7	9.9 ± 3.1
	L	18	4.2 ± 0.8	4.0 ± 1.6	1.3 ± 0.3	18.3 ± 2.2	6.1 ± 1.2	0.7 ± 0.2	24.7 ± 4.3	1.6 ± 1.2	17.9 ± 4.6
	M	18	10.1 ± 0.8	6.7 ± 1.3	1.3 ± 0.3	19.8 ± 2.1	7.1 ± 1	1.1 ± 0.1	17.9 ± 2.9	1.5 ± 0.6	9.5 ± 2.1
A. hyperborea	M	5	18.1 ± 0.7	3.0 ± 0.3	4.4 ± 0.5	8.1 ± 0.4	6.3 ± 0.4	1.5 ± 0.1	4.4 ± 0.9	0.3 ± 0	0.8 ± 0.5
S. alpinus	M	7	12.3 ± 1.9	16.4 ± 6.1	2.1 ± 0.3	11 ± 1.7	3.4 ± 1	1.5 ± 0.5	10.6 ± 6.7	0.5 ± 0.6	10.5 ± 5.1
R. hippoglossoides	M	9	10.0 ± 0.4	10.5 ± 0.6	2.0 ± 0.2	15.5 ± 2.3	6.6 ± 1.1	0.9 ± 0.1	17.7 ± 1.1	0.9 ± 0.8	16.2 ± 2.3
P. hispida	B	8	5.5 ± 0.9	21.3 ± 4.5	0.7 ± 0.3	18.5 ± 4	7.2 ± 1.2	1.7 ± 0.2	7.8 ± 3	0.4 ± 0.1	2.3 ± 2.8
P. groenlandica	B	15	5.4 ± 2.1	16.8 ± 2.8	0.9 ± 0.3	16.5 ± 4.1	6 ± 1.1	1.9 ± 0.3	12 ± 2.6	0.6 ± 0.2	5.5 ± 4.6
M. monoceros	B	7	6.4 ± 0.8	24.9 ± 2.4	1.1 ± 0.3	22 ± 1.8	6 ± 0.4	1.1 ± 0.1	9.9 ± 1.1	0.4 ± 0	4.2 ± 1.1

Species	Tissue	22:1n−9	20:4n−6	20:5n−3	24:1n−9	22:5n−3	22:6n−3	∑SAFA	∑MUFA	∑PUFA	%Lipid[b]
S. microcephalus	BP	2.9 ± 0.6	2.4 ± 0.7	9.1 ± 1.8	1.5 ± 0.3	2.7 ± 1.7	10.6 ± 2.1	12.9 ± 4.3	58.9 ± 5.8	28.2 ± 3.7	14.4 ± 4.8
	L	3.9 ± 0.6	0.8 ± 0.3	3.1 ± 1	1.9 ± 0.5	1.5 ± 0.7	5.1 ± 1.9	6.5 ± 1.3	78.8 ± 4.2	14.6 ± 3.5	78.8 ± 11.2
	M	2.0 ± 0.2	1.6 ± 0.3	5.6 ± 0.9	0.8 ± 0.1	1.7 ± 0.4	8.8 ± 1.3	12.7 ± 0.8	65.4 ± 2.2	21.8 ± 2.2	56.8 ± 5.9
A. hyperborea	M	0.5 ± 0.1	3.3 ± 0.3	10.3 ± 1.3	0.3 ± 0.0	2.2 ± 0.2	30.5 ± 1.2	24.6 ± 0.9	25.3 ± 2.1	50.1 ± 1.9	6.6 ± 1.1
S. alpinus	M	1.8 ± 0.7	0.4 ± 0.1	7.3 ± 2.5	0.6 ± 0.2	1.3 ± 0.3	10.9 ± 2.3	19 ± 1.5	56.7 ± 6.2	24.3 ± 5.2	22.7 ± 10.6
R. hippoglossoides	M	2.3 ± 0.2	0.4 ± 0.1	3.7 ± 0.7	0.7 ± 0.1	0.6 ± 0.1	4.8 ± 1.5	15.9 ± 0.6	70.9 ± 3	13.3 ± 2.5	39.9 ± 10.2
P. hispida	B	0.5 ± 0.4	0.5 ± 0.2	8.5 ± 2	0.1 ± 0.1	5.4 ± 1.3	9.5 ± 1.5	10.6 ± 1.9	61 ± 3.6	28.3 ± 3.2	85.5 ± 11.7
P. groenlandica	B	0.9 ± 0.5	0.3 ± 0.1	7.8 ± 1.9	0.2 ± 0.1	4.4 ± 1.2	9.6 ± 2.3	10.6 ± 3.3	62 ± 4.8	27.4 ± 4.6	73.9 ± 19.6
M. monoceros	B	1 ± 0.2	0.3 ± 0	2.7 ± 0.7	0.1 ± 0.1	1.2 ± 0.3	2.5 ± 0.6	16.4 ± 1.4	73.9 ± 2.3	9.7 ± 1.7	70.1 ± 21

[a] BP = blood plasma, M = muscle, L = liver, B = blubber.
[b] Percent (%) lipid = mass of lipid · dry weight of $sample^{-1}$.

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the sum of all FAs with zero double bonds, "∑MUFA" indicates the sum of all FAs with one double bond, and "∑PUFA" indicates the sum of all FAs with ≥2 double bonds.

2.4. Data analysis

Data were obtained for 50 individual FAs, but analyses were restricted to 15 FAs that contributed mean values >1% to at least one shark tissue (these FA are listed in Table 2). Together, these 15 FAs accounted for 92% of total shark plasma FAs and 95% of total muscle and liver FAs. Coefficients of variation were calculated to compare variability in each FA proportion among Greenland shark tissues. Principal components analysis (PCA) was used to explore relationships between Greenland shark and prey FAs. Separate PCAs were performed on proportional data (Table 2) and absolute μg mg^{-1} data (Table 3). FAs reported as μg mg^{-1} are highly sensitive to the % lipid of a sample, but are a useful way to ensure that, for example, high proportions of one FA are not driven by low proportions of another FA. PCA was a straightforward way to identify: 1) which FAs explained the largest amount of variance in the data, and 2) which shark tissue was most similar to prey tissues based on underlying similarities in FA profiles. FA data were standardized to a mean of 0 and variance of 1 prior to inclusion in the PCAs and were left untransformed because logit transformation (i.e. $\log[FA_i/(1-FA_i)]$), which is affective for increasing normality and linearity of proportional data (Warton and Hui, 2011), did not alter PCA outputs. FA variable weights were extracted 'unscaled' (i.e. scaling = 0) from the first two principal components (PC1 and PC2), and FA 'loadings' (i.e. correlations between each FA variable and each PC axis) were calculated by multiplying the unscaled FA weight by the square root of the eigenvalue for that principal component (McGarigal and Cushman, 2000). Variables with loadings >0.63 were considered highly influential to that component (McGarigal and Cushman, 2000). Non metric multidimensional scaling (performed in 2 dimensions using Euclidean distances) was also performed on the proportional (stress = 0.09) and μg mg^{-1} data (stress = 0.07) and produced a similar ordination to the PCAs, lending support to our application of PCA to identify patterns in the data. All analyses were performed in R (R_Development_Core_Team, 2010) and package 'vegan' (Oksanen et al., 2010) was used for the PCA and NMDS.

3. Results

Greenland halibut and ringed seal (adults and pups) were the most commonly identified prey in the Greenland sharks' stomachs (% occurrence = 72.2 and 33.3%, respectively, Table 1). Mass data were not available for the sharks' stomach contents, but the high % occurrence of Greenland halibut and ringed seal likely translated to a high contribution by mass because these species are often consumed whole (i.e. whole Greenland halibut and intact white-coat seal pups, Table 1) or as large pieces of tissue (e.g. an entire shoulder, forearm and flipper from an adult ringed seal, estimated weight = 4 kg, B.C. McMeans, personal observation). Previously reported data for Svalbard (Norway) Greenland sharks supports this contention because the most commonly consumed prey by these sharks, a teleost (Atlantic cod, *Gadus morhua*) and ringed seal (% occurrence = 39.4 and 30%, respectively), were also the dominant prey by mass (contributing 49.1 and 17.7% to the total prey biomass, respectively, Leclerc et al., 2012). Thus, although we cannot quantify their exact lipid mass contribution, both Greenland halibut and ringed seal (which both have high % lipid: 40 and 86% on a dw basis, respectively, Table 2) could reasonably have contributed to the lipid budget of the Greenland sharks sampled here.

Greenland halibut muscle and ringed seal blubber had different FA profiles, with the former being dominated by proportions of 18:1n−9, 20:1n−9, and 22:1n−11, and the latter having 16:1n−7, 18:1n−9 and DHA in the highest proportions (Table 2). FAs reported on a μg mg^{-1} basis supported this pattern (Table 3). Greenland shark plasma, liver and muscle had 18:1n−9 and 20:1n−9 in the highest proportions (Table 2). Plasma also had high proportions of DHA (10.6 ± 2.1%) and EPA (9.1 ± 1.8%) and muscle had high proportions of 16:0 (10.1 ± 0.8%) and DHA (8.8 ± 1.3%). Liver, on the other hand, had 22:1n−11 and 18:1n−7 in the next highest amounts, and combined with 18:1n−9 and 20:1n−9, these four MUFA contributed to 67.0% of total liver FAs (Table 2). Blood plasma and liver were more variable than shark muscle based on their higher CV for FA proportions (Table S1).

The PCA of shark and prey proportions (Fig. 1A) revealed that the greatest amount of variance in the data was explained by the difference between Greenland shark liver and marine mammal blubber, based on the observation that PC1 separated shark liver from mammal blubber due to higher proportions (positive loadings) of

Table 3
Absolute fatty acid values (μg mg^{-1} dry tissue, mean ± SD) of *Somniosus microcephalus* and representative prey.

Species	Tissue[a]	16:0	16:1n−7	18:0	18:1n−9	18:1n−7	18:2n−6	20:1n−9	18:3n−3	22:1n−11
S. microcephalus	BP	4.3 ± 2.2	2.1 ± 1.4	1.1 ± 0.8	7.6 ± 4.4	2.4 ± 1.9	0.5 ± 0.3	8 ± 4.8	0.4 ± 0.7	4.9 ± 3.4
	L	26.8 ± 6.9	25.4 ± 12.6	8.3 ± 1.7	116.1 ± 23.5	38.4 ± 9.2	4.7 ± 1.5	148 ± 65.8	10.7 ± 8.2	114.8 ± 36.5
	M	50.9 ± 6.9	34.5 ± 10.8	6.7 ± 1.8	100.2 ± 17.8	35.9 ± 7.3	5.7 ± 1.1	89.6 ± 16.7	7.2 ± 3.1	47.9 ± 12
A. hyperborea	M	5.4 ± 1.1	0.9 ± 0.3	1.3 ± 0.4	2.4 ± 0.6	1.9 ± 0.5	0.5 ± 0.1	1.4 ± 0.6	0.1 ± 0	0.3 ± 0.2
S. alpinus	M	22 ± 9	29.1 ± 12.9	3.7 ± 1.5	21.3 ± 11.5	7.1 ± 5.6	2.9 ± 1.5	22 ± 22.4	1.1 ± 1.3	23.4 ± 22.3
R. hippoglossoides	M	36.7 ± 9.3	39.6 ± 12.6	7.2 ± 1.7	58.7 ± 22.3	24.9 ± 9.5	3.4 ± 1.1	65.9 ± 19.2	3 ± 2.8	59.9 ± 18.5
P. hispida	B	43.3 ± 9.8	173.4 ± 61.1	5 ± 1.8	150.1 ± 53.7	58.3 ± 20	13.1 ± 2.9	59.9 ± 22.4	3.1 ± 1.1	15.9 ± 18
P. groenlandica	B	32.7 ± 15.1	103 ± 35.5	4.9 ± 1.7	102.5 ± 45.6	37.2 ± 14.4	11.6 ± 3.1	73.6 ± 27.3	3.3 ± 1.8	33.5 ± 26.2
M. monoceros	B	34.2 ± 12.9	133.4 ± 44.6	5.8 ± 1.9	116.7 ± 36.4	32.5 ± 11.9	5.8 ± 2	52.3 ± 18.9	2.1 ± 0.8	22.4 ± 11.2

Species	22:1n−9	20:4n−6	20:5n−3	24:1n−9	22:5n−3	22:6n−3	∑SAFA	∑MUFA	∑PUFA
S. microcephalus	1.4 ± 0.7	1.2 ± 0.9	4.4 ± 2.6	0.7 ± 0.3	1.1 ± 0.5	5 ± 2.6	6.1 ± 3.2	28.7 ± 15.9	13.5 ± 7.5
	24.8 ± 6	4.9 ± 1.6	20.1 ± 8.7	12.2 ± 3.4	9.7 ± 5.1	33.1 ± 14.2	41.1 ± 9.9	504.1 ± 94.4	94.5 ± 31.9
	10.0 ± 1.8	8.0 ± 1.8	28.6 ± 7.2	3.9 ± 0.8	8.6 ± 3.2	44.3 ± 10.2	64.2 ± 9.5	331 ± 51.7	110.6 ± 22.1
A. hyperborea	0.2 ± 0.1	1.0 ± 0.1	3.1 ± 0.6	0.1 ± 0	0.7 ± 0.1	9.2 ± 1.9	7.4 ± 1.6	7.7 ± 2.2	15 ± 2.8
S. alpinus	3.6 ± 2.4	0.6 ± 0.1	12.4 ± 3.8	1.3 ± 0.8	2.3 ± 0.9	18.8 ± 5.6	35.4 ± 17.3	112.6 ± 73.3	42.3 ± 14.1
R. hippoglossoides	8.3 ± 2	1.3 ± 0.3	13.3 ± 2.6	2.6 ± 0.7	2 ± 0.6	16.5 ± 2.4	58.7 ± 15.5	265 ± 81.6	47.1 ± 7.6
P. hispida	3.7 ± 2.8	4.1 ± 2.7	69.2 ± 26	0.5 ± 0.5	43.9 ± 17	75 ± 17.9	83.2 ± 15.7	487.8 ± 119.2	227.8 ± 62.6
P. groenlandica	5.5 ± 3.6	1.9 ± 0.7	48.1 ± 18.1	1 ± 0.8	27 ± 11.6	59.6 ± 23.3	64.4 ± 25.5	381.5 ± 119.9	168.3 ± 57.5
M. monoceros	5.4 ± 2	1.5 ± 0.6	15 ± 8	0.4 ± 0.5	6.7 ± 3.4	14 ± 7.5	88.3 ± 30.3	393.6 ± 127.2	53.4 ± 25.2

[a] BP = blood plasma, M = muscle, L = liver, B = blubber.

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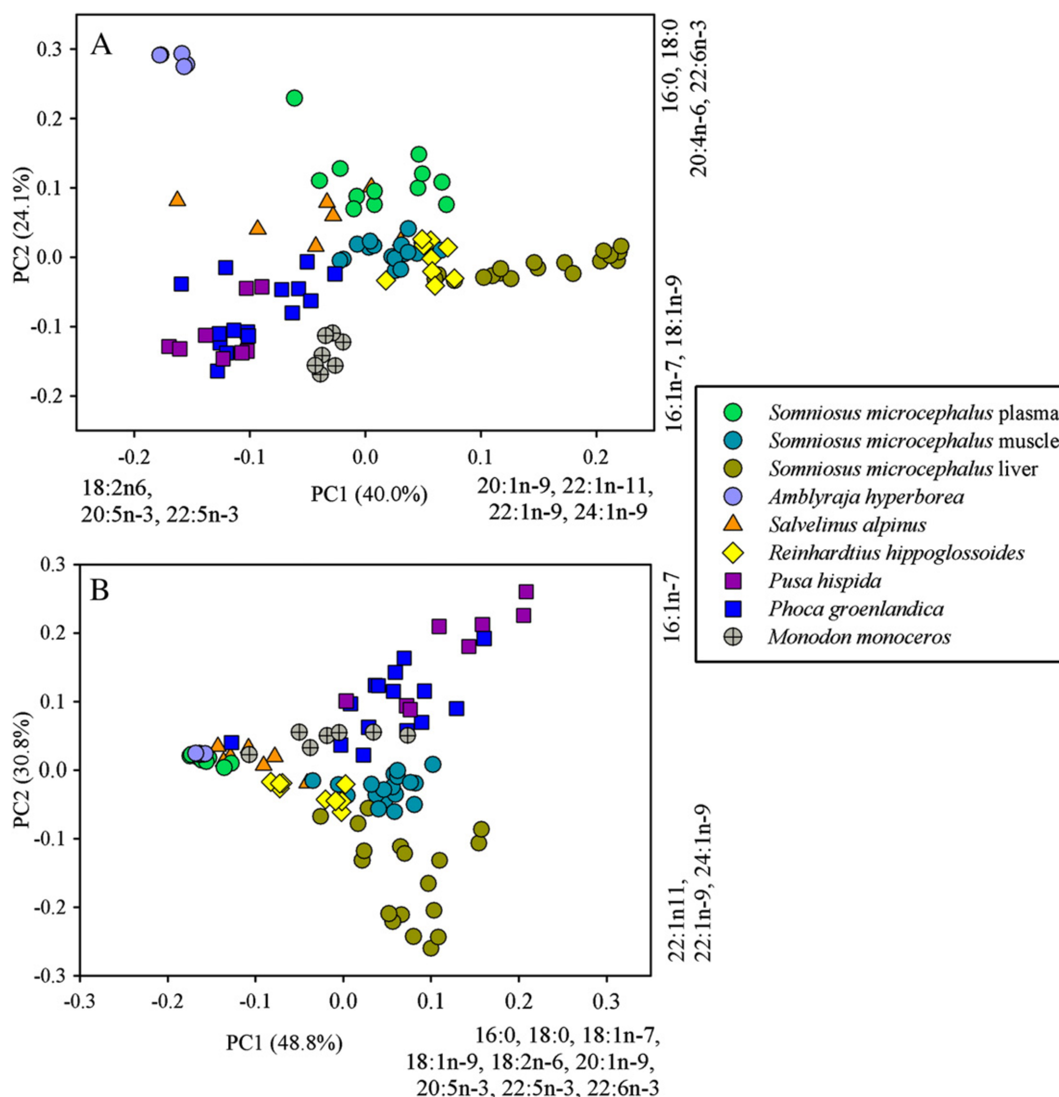

Fig. 1. Principal component analysis of fatty acid proportions (A: % of total) and μg fatty acid mg dry tissue^{-1} values (B) of Greenland sharks (*Somniosus microcephalus*) (muscle, liver and plasma) and several known teleost and marine mammal prey. Fatty acids that were highly correlated (>0.63) with each principal component axis are listed.

20:1n−9, 22:1n−11, 22:1n−9, and 24:1n−9 in the former and higher proportions (negative loadings) of 18:2n−6, EPA and 22:5n−3 in the latter (Fig. 1A). PC2 revealed a separation between skate (positive scores) and narwhal (negative scores) (Fig. 1A). All three Greenland shark tissues overlapped to some extent with at least one prey species, but shark muscle, liver and plasma all overlapped with Greenland halibut on PC1 (Fig. 1A). Shark muscle and plasma separated closer to marine mammal on the PCA than liver, and based on their overlap on PC1, shark muscle was more similar to plasma than liver (Fig. 1A).

Absolute μg mg^{-1} data supported results from the FA proportions because shark muscle was more similar to the dominant prey, halibut and ringed seal, whereas shark liver was the most distant from the prey samples on the μg mg^{-1} PCA (Fig. 1B). Thus, Greenland shark muscle was more similar to prey FA in both relative proportions (Fig. 1A) and absolute μg mg^{-1} values (Fig. 1B) than shark liver. Plasma had much lower FAs on a μg mg^{-1} basis relative to shark muscle and liver, and therefore separated from the other shark tissues on the μg mg^{-1} PCA (Fig. 1B).

Qualitative comparisons of FA proportions were made among: 1) individual Greenland sharks and 2) individual dominant prey samples (halibut and ringed seal) (Fig. 2). Based on this comparison, FAs can be grouped into one of three categories as follows: 1) FAs that were generally similar (i.e. within a few %) in all three shark tissues to Greenland halibut and ringed seal (i.e. 18:0, 18:1n−7, 18:2n−6, 22:5n−3, Fig. 2A), 2) FAs that were higher in shark liver than prey values (i.e. 20:1n−9, 22:1n−11, 22:1n−9, 24:1n−9, Fig. 2B), and 3) FAs that were higher in plasma and/or muscle than prey tissues (i.e. 18:1n−9, ARA, EPA, DHA, Fig. 2C). Specifically, for the latter group, shark muscle (and liver) were higher in proportions of 18:1n−9, shark muscle and plasma were higher in ARA, and several shark plasma samples were higher in EPA and DHA relative to halibut and ringed seal (Fig. 2C, Table 2). Absolute μg mg^{-1} data for shark muscle and liver (but not plasma due to low FA μg mg^{-1}) generally supported the above categorizations. For example, from group 1, mean 18:1n−7 of shark muscle and liver was between 36 and 39 μg mg^{-1}, and fell in between the values exhibited by Greenland halibut and ringed seal (24.9 and 58.3 μg mg^{-1}, respectively, Table 3). However, ringed seal had higher μg mg^{-1} values of 22:5n−3 (43.9 ± 17.0 μg mg^{-1}) than shark tissues, but proportionally, all three shark tissues fell within the extreme values of 22:5n−3 exhibited by Greenland halibut and ringed seal (Table 2, Fig. 2A). From group 2, all prey had mean 22:1n−11 less than 60 μg mg^{-1}, but shark liver had values of 115 μg mg^{-1} (Table 3). From group 3, ARA was higher in shark muscle (8 μg mg^{-1}) than in any of the prey (all means ≤4.1 μg mg^{-1}) (Table 3), which supports the proportional data (Table 2).

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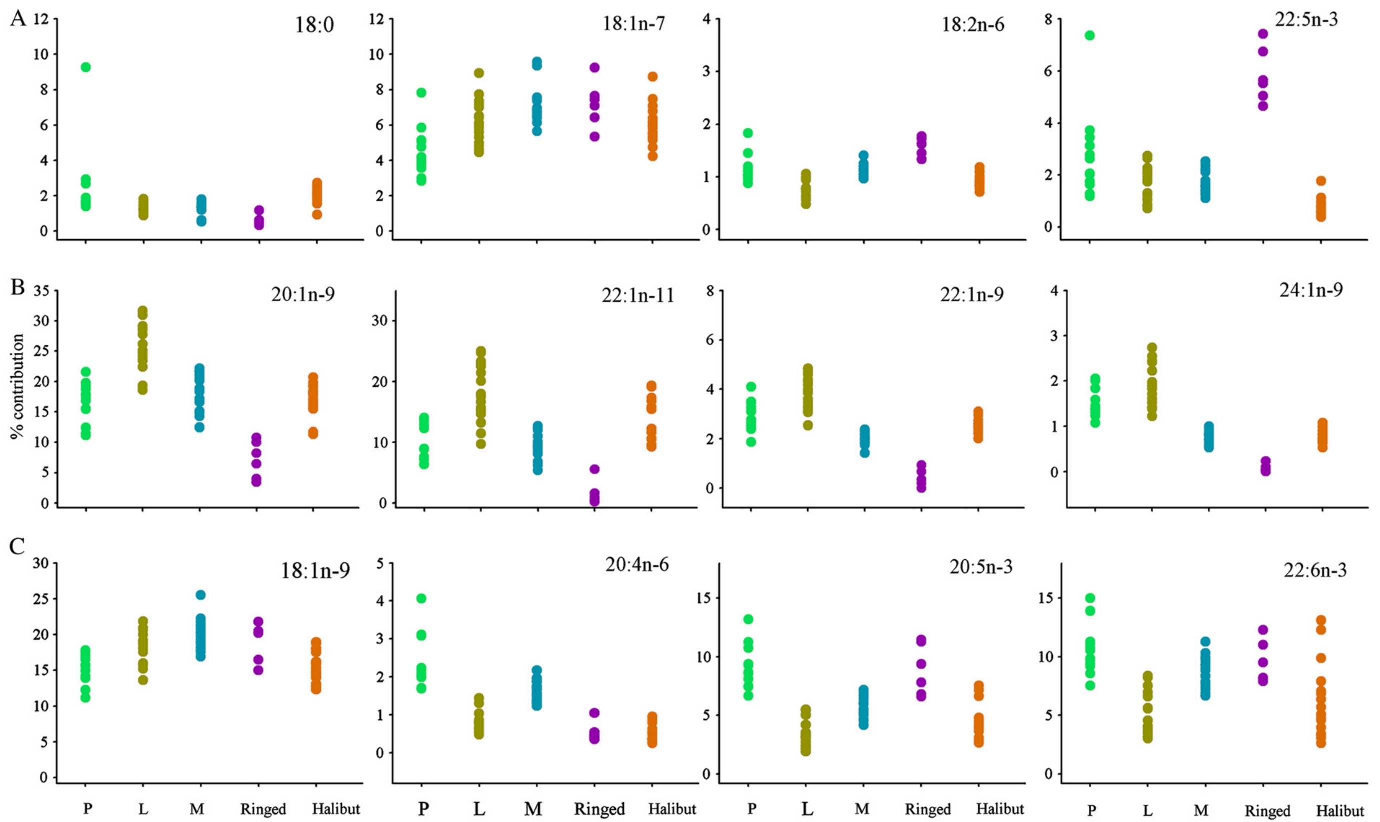

Fig. 2. Fatty acid proportions (% of total, individual values) of Greenland shark plasma (P), liver (L), and muscle (M), and values for the sharks' dominant prey, Greenland halibut (muscle) and ringed seal (blubber). Fatty acids are separated by shark–prey: A) similarity, B) higher values in shark liver, and C) higher values in shark plasma and/or muscle. Note the different scales of the y axes.

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4. Discussion

The Greenland sharks sampled here consumed predominantly Greenland halibut and ringed seal based on stomach contents, which agrees with previously reported contaminant and stomach content data from Cumberland Sound Greenland sharks (Fisk et al., 2002). Greenland shark muscle, liver and plasma FAs supported a greater consumption of halibut, based on shark tissues-halibut overlap on PC1 of the proportions PCA. All three tissues will therefore provide some information about diet for future explorations focused on the feeding ecology of these sharks. Because elasmobranch liver turns over more quickly than muscle (MacNeil et al., 2006), and plasma is known to be a short term dietary indicator (Käkelä et al., 2009), differences in the relative magnitude of FAs between slow and fast turnover tissues could be used to identify differences in feeding behavior over time. However, in agreement with previous findings from 16 species of Chondrichthyan (Pethybridge et al., 2010), inter-tissue differences were apparent, and Greenland sharks had higher PUFA in their muscle ($\sum$PUFA = 21.8% versus liver = 14.6%) and higher MUFA in their liver ($\sum$MUFA = 78.8% versus muscle = 65.4%). This pattern hints at underlying, baseline differences between the tissues that are likely related to different tissue requirements and roles in FA metabolism (Ballantyne, 1997; Tocher, 2003), and stresses the need to consider tissue differences when interpreting FAs as indicators of shark diet.

Greenland sharks appeared to retain 18:0, 18:1n−7, 18:2n−6, and 22:5n−3 in their tissues in generally similar proportions that existed in their diet. Proportions of 18:0 and 18:1n−7 were similar between Greenland halibut and ringed seal and all three shark tissues (Fig. 2A), whereas proportions of 18:2n−6 and 22:5n−3 in shark tissues generally fell in between the values exhibited by dominant prey. Thus, the tissues of shark consumers should reflect a mixture of prey sources in these FA. Muscle and liver of European sea bass (*Dicentrarchus labrax*) were also similar to dietary proportions of 18:0, 18:1n−7, 18:2n−6 and 22:5n−3 regardless of whether fish were fed a diet of 100% fish oil, or a mixed diet (40% fish and 10% rapeseed oil, Mourente and Bell, 2006). It is noteworthy that several individual Greenland sharks had high proportions of plasma 22:5n−3 that matched those observed in ringed seal (Fig. 2A), which could reflect recent seal consumption.

Lower 16:1n−7 proportions (Table 2) and $\mu g\ mg^{-1}$ values (Table 3) in shark tissues vs. prey indicates that this FA was selectively catabolized or was continually elongated to 18:1n−7 (Tocher, 2003). Higher C_{20}–C_{22} MUFA in shark liver vs. prey, on the other hand, indicates either selective retention of these FA from the diet, or accumulation as the products from chain shortening (partial beta oxidation, e.g. 22:1n−9 to 20:1n−9). Differences between shark liver and prey FA profiles, and high variability in liver FA among individual Greenland sharks, supports our prediction, and the perception of liver as a dynamic tissue that exhibits a high degree of FA modification, likely to meet requirements associated with metabolism and buoyancy (Ballantyne, 1997).

Greenland shark muscle was the most similar of the tissues sampled to both halibut and ringed seal FA profiles, suggesting that dietary FAs are incorporated into the shark's muscle with little modification, and that Greenland shark muscle provides an accurate view of diet. Lower CV of muscle FAs indicates that proportions of muscle FAs were likely regulated to meet tissue-specific requirements to a greater degree than liver or plasma (Parrish, 2009). Atlantic salmon muscle proportions of EPA and DHA did not reflect differences obtained in their experimental diet, further suggesting that these FA are maintained at species-specific levels (Budge et al., 2011). However, calculated CVs were often similar among muscle, liver and plasma (e.g. 20:1n−9 CV: plasma = 0.21, liver = 0.18, muscle = 0.16, Table S1), indicating that variability was present in muscle FAs among individual Greenland sharks that could have arisen from dietary differences among individuals.

Because polar lipids respond less to changes in diet than neutral lipids (Regost et al., 2003), one might expect shark muscle to be less responsive to diet than tissues like liver that are dominated by neutral storage molecules. The similarity between Greenland shark muscle and prey FA profiles is therefore somewhat surprising considering that shark muscle lacks the enzymes necessary to catabolize FAs (Zammit and Newsholme, 1979), and typically has low % lipid and high contribution of phospholipids (Pethybridge et al., 2010). However, shark species differ in their muscle total lipid (Davidson et al., 2011) and % contribution of neutral lipids (Sargent et al., 1973), suggesting that the ability to store FAs in muscle, and subsequently the responsiveness of shark muscle to diet, may also differ among shark species. Interestingly, Greenland shark muscle has higher % lipid (19 ± 4%, ww, converted from dw values provided on Table 2 using % water content of individual samples) than other shark species analyzed to date (e.g. range among species reported in Pethybridge et al., 2010: 0.37–1.87% ww). Squalids, including *S. acanthias* and the Pacific sleeper shark, also appear to have a higher contribution of storage molecules in their muscle than other sharks (e.g. *S. acanthias* muscle TAG + DAG = 84.2%, Malins et al., 1965; Pacific sleeper shark muscle = 73% TAG, Schaufler et al., 2005). Therefore, the muscle of sharks with lower % lipid may not align so closely with prey FA profiles as observed here in Greenland sharks. Clearly, further work is required to determine the role of muscle lipids in some sharks, which could function as additional energy stores, or in buoyancy regulation (Malins and Barone, 1970).

Because most extra-hepatic shark tissues utilize ketone bodies, not FA, for energy (Speers-Roesch and Treberg, 2010), Greenland shark plasma FAs were expected to closely reflect dietary FAs. In support of this prediction, plasma FA proportions were similar to the Greenland halibut and/or ringed seal for most FAs (Fig. 2). The similarity between shark plasma and muscle proportions supports the contention that muscle FA were of dietary origin. However, in addition to neutral FAs like TAG, the total lipid fraction of shark plasma also contains polar lipids (Craik, 1978). The presence of polar lipid in our plasma samples, which could feasibly be of both dietary and non dietary origin (e.g. inter-tissue routing of membrane lipids), could explain the higher ARA, EPA and DHA proportions observed in Greenland shark plasma vs. dominant prey, as well as overlap with arctic char on ordinations (which were not identified in the sharks' stomachs, but have high EPA and DHA, Table 2). Closer agreement between prey and plasma FA would be expected if we had analyzed the isolated the chylomicron fraction (e.g. Cooper et al., 2006) instead of whole plasma. Plasma FA were also variable among individual sharks (based on CV), indicating that plasma FA are likely dynamic in time, and sensitive to the duration since the last meal, the item being digested and perhaps other physiological factors associated with shark movement or reproductive status. Future work is needed to explore these potential factors that could alter the plasma FA profile of sharks from that obtained in the diet.

Our results for Greenland shark muscle, liver and plasma support findings from previous research in teleosts (Mourente and Bell, 2006) and seabirds (Käkelä et al., 2009) that the relationship between consumer and dietary FAs is not always 1:1. The FA profile of Greenland sharks and prey could vary with season and/or year due to changes in diet (e.g. for ringed seal, Thiemann et al., 2007), which is an important consideration because prey were sampled for the present study during both 2007 and 2008. However, because between—is often greater than within-species FA variability (Budge et al., 2002), temporal changes in FA profiles within-species are unlikely to alter the between-species trends reported here (e.g. Fig. 2). Fatty acids should be useful for exploring differences in diet within-species, or between closely related species (e.g. with space or time), though, because sharks that consume a greater quantity of certain FA should have higher proportions of that FA versus sharks that consume less. For example, C_{20} and C_{22} MUFA are biosynthesized by *Calanus* copepods and should differ among sharks that obtain different amounts of these FA in their diet. In fact,

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Greenland sharks from Cumberland Sound had 18 and 25% 20:1n−9 in their muscle and liver, respectively (Table 2), but Pacific sleeper shark muscle and liver from the Gulf of Alaska, as well as blubber from the Pacific sleeper sharks' stomachs, had <6% (Schaufler et al., 2005). Cumberland Sound halibut had high 20:1n−9 proportions (17.7 ± 1.1%) and are a likely source of observed 20:1n−9 proportions in Greenland shark tissues. It therefore appears that C_{20} and C_{22} MUFA are useful dietary indicators in sharks, as long as similar proportions are not expected between shark liver and prey.

Results from our field study provide new information about the degree that tissue FA profiles of a large shark differ from those of dominant prey. Further work is required to establish if the observed similarities and differences in shark–prey FA proportions (i.e. Fig. 2) are applicable to other elasmobranchs. Due to the multitude of habitats and temperatures that elasmobranchs occupy, and their wide range of locomotory modes and diets, differences among species are likely. Greenland sharks (and Pacific sleeper sharks) are clearly unique in their habitat (e.g. ice-covered seas), and their higher % lipid (Table 2) and contribution of MUFA to their muscle (Table S2). The deep-water *Centroscymnus coelolepis* and the coastal *Carcharhinus obscurus*, which are both highly mobile species (Compagno, 1984a, 1984b), come the closest to the muscle $\sum$MUFA of the Greenland shark at ~40% (Table S2), but further work is required to determine the ubiquity of shark muscle as a dietary indicator. The liver of Greenland sharks, on the other hand, has a more comparable FA composition to other shark species, but is most similar to that of mobile, deep water species like *Dalatias licha* (Table S3). Thus, our results for specific shark–prey FA differences may be most directly applicable to large, mobile squaliforms. Based on inspection of Tables S2 and S3, it is clear that future work should address what factors govern differences in FA profiles among shark species.

5. Conclusions

Our comparison provided several general conclusions that should be applicable to other sharks. First, researchers should not expect that shark FA profiles will exactly match that of dominant prey items, which is somewhat obvious and expected, but still an important consideration for inferring diet based solely on shark FA profiles. Second, liver FA should be interpreted with care, due to potentially high modification of dietary FA profiles by shark consumers. Third, muscle FA profiles of Greenland sharks were the most similar to that of known prey, but future studies are tasked with determining the ubiquity of this trend in other shark species. Finally, plasma FA are likely sensitive to the presence of some non-dietary lipid (if the total lipid fraction is analyzed), digestion and the timing since the last meal, but still appeared useful for inferring shark diet, which is important for future studies focused on large sharks that may be endangered or protected. Progression towards a more quantitative application of FAs to study shark diet will require a more precise understanding of the retention/metabolism of dietary FAs, which could be gained from other FAs like polymethylene-interrupted FAs and through controlled feeding studies performed on a variety of elasmobranch species.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jembe.2012.06.017.

References

Andersen, S.M., Lydersen, C., Grahl-Nielsen, O., Kovacs, K.M., 2004. Autumn diet of harbour seals (*Phoca vitulina*) at Prins Karls Forland, Svalbard, assessed via scat and fatty-acid analyses. Can. J. Zool. 82, 1230–1245.

Ballantyne, J.S., 1997. Jaws: the inside story. The metabolism of elasmobranch fishes. Comp. Biochem. Physiol. B 118, 703–742.

Bigelow, H.B., Schroeder, W.C., 1948. Sharks. In: Tee-Van, J., Breder, C.M., Hildebrand, S.F., Parr, A.E., Schroeder, W.C. (Eds.), Fishes of the Western North Atlantic. Part one. Lancelets, cyclostomes, sharks. Sears Foundation for Marine Research, Yale University, New Haven, USA, 576 p.

Budge, S.M., Iverson, S.J., Bowen, W.D., Ackman, R.G., 2002. Among- and within-species variability in fatty acid signatures of marine fish and invertebrates on the Scotian Shelf, Georges Bank, and southern Gulf of St. Lawrence. Can. J. Fish. Aquat. Sci. 59, 886–898.

Budge, S.M., Penney, S.N., Lall, S.P., 2011. Response of tissue lipids to diet variation in Atlantic salmon (*Salmo salar*): implications for estimating diets with fatty acid analysis. J. Exp. Mar. Biol. Ecol. 409, 267–274.

Compagno, L.J.V., 1984a. FAO Species Catalogue. Vol. 4. Sharks of the world. An annotated and illustrated catalogue of shark species known to date: Part 2—Carcharhiniformes. FAO Fish. Synop.

Compagno, L.J.V., 1984b. FAO Species Catalogue. Vol. 4. Sharks of the world. An annotated and illustrated catalogue of shark species known to date: Part 1—Hexanchiformes to Lamniformes. FAO Fish. Synop.

Cooper, M.H., Iverson, S.J., Rouvinen-Watt, K., 2006. Metabolism of dietary cetoleic acid (22: 1n−11) in mink (*Mustela vison*) and gray seals (*Halichoerus grypus*) studied using radiolabeled fatty acids. Physiol. Biochem. Zool. 79, 820–829.

Craik, J., 1978. The lipids of six species of shark. J. Mar. Biol. Assoc. U. K. 58, 913–921.

Davidson, B., Sidell, J., Rhodes, J., Cliff, G., 2011. A comparison of the heart and muscle total lipid and fatty acid profiles of nine large shark species from the east coast of South Africa. Fish Physiol. Biochem. 37, 613–618.

Ferretti, F., Worm, B., Britten, G.L., Heithaus, M.R., Lotze, H.K., 2010. Patterns and ecosystem consequences of shark declines in the ocean. Ecol. Lett. 13, 1055–1071.

Fisk, A.T., Tittlemier, S.A., Pranschke, J.L., Norstrom, R.J., 2002. Using anthropogenic contaminants and stable isotopes to assess the feeding ecology of Greenland sharks. Ecology 83, 2162–2172.

Folch, J., Lees, M., Sloane-Stanley, G.H., 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226, 497–509.

Iverson, S.J., 2009. Tracing aquatic food webs using fatty acids: from qualitative indicators to quantitative determination. In: Arts, M.T., Brett, M.T., Kainz, M.J. (Eds.), Lipids in Aquatic Ecosystems. Springer, New York, pp. 281–308.

Käkelä, R., Furness, R., Kahle, S., Becker, P., Käkelä, A., 2009. Fatty acid signatures in seabird plasma are a complex function of diet composition: a captive feeding trial with herring gulls. Funct. Ecol. 23, 141–149.

Leclerc, L.M., Lydersen, C., Haug, T., Bachmann, L., Fisk, A.T., Kovacs, K.M., 2012. A missing piece in Arctic food web puzzle? Stomach contents of Greenland sharks sampled in Svalbard. Norway, Polar Biol. http://dx.doi.org/10.1007/s00300-012-1166-7.

MacNeil, M.A., Drouillard, K.G., Fisk, A.T., 2006. Variable uptake and elimination of stable nitrogen isotopes between tissues in fish. Can. J. Fish. Aquat. Sci. 63, 345–353.

Malins, D.C., Barone, A., 1970. Glyceryl ether metabolism: regulation of buoyancy in dogfish *Squalus acanthias*. Science 167, 79–80.

Malins, D.C., Wekell, J.C., Houle, C.R., 1965. Composition of the diacyl glyceryl ethers and triglycerides of the flesh and liver of the dogfish (*Squalus acanthias*). J. Lipid Res. 6, 100–105.

McGarigal, K., Cushman, S., 2000. Multivariate statistics for wildlife and ecology research. Springer-Verlag, New York. 283 pp.

McMeans, B.C., Arts, M.T., Rush, S., Fisk, A.T., 2012. Seasonal patterns in fatty acids of *Calanus hyperboreus* (Copepoda, Calanoida) from Cumberland Sound, Baffin Island, Nunavut. Mar. Biol.

Mourente, G., Bell, J., 2006. Partial replacement of dietary fish oil with blends of vegetable oils (rapeseed, linseed and palm oils) in diets for European sea bass (*Dicentrarchus labrax* L.) over a long term growth study: effects on muscle and liver fatty acid composition and effectiveness of a fish oil finishing diet. Comp. Biochem. Physiol. B, 145, pp. 389–399.

Myers, R.A., Baum, J.K., Shepherd, T.D., Powers, S.P., Peterson, C.H., 2007. Cascading effects of the loss of apex predatory sharks from a coastal ocean. Science 315, 1846–1850.

Oksanen, J., Guillaume Blanchet, F., Kindt, R., Legendre, P., O'Hara, R.B., Simpson, G.L., Solymos, P., Henry, M., Stevens, H., Wagner, H., 2010. Vegan: Community Ecology Package. R package version 1.17-4. URL http://CRAN.R-project.org/package=vegan 2010.

Parrish, C.C., 2009. Essential fatty acids in aquatic food webs. In: Arts, M.T., Brett, M.T., Kainz, M.J. (Eds.), Lipids in Aquatic Ecosystems. Springer, New York, pp. 309–326.

Author's personal copy

Pethybridge, H., Daley, R., Virtue, P., Nichols, P., 2010. Lipid composition and partitioning of deepwater chondrichthyans: inferences of feeding ecology and distribution. Mar. Biol. 157, 1367–1384.

Pethybridge, H., Daley, R.K., Nichols, P.D., 2011. Diet of demersal sharks and chimaeras inferred by fatty acid profiles and stomach content analysis. J. Exp. Mar. Biol. Ecol. 409, 290–299.

R Development Core Team, 2010. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org.

Regost, C., Arzel, J., Robin, J., Rosenlund, G., Kaushik, S., 2003. Total replacement of fish oil by soybean or linseed oil with a return to fish oil in turbot (*Psetta maxima*): 1. Growth performance, flesh fatty acid profile, and lipid metabolism. Aquaculture 217, 465–482.

Sargent, J., Gatten, R., McIntosh, R., 1973. The distribution of neutral lipids in shark tissues. J. Mar. Biol. Assoc. U. K. 53, 649–656.

Schaufler, L., Heintz, R., Sigler, M., Hulbert, L., 2005. Fatty acid composition of sleeper shark (*Somniosus pacificus*) liver and muscle reveals nutritional dependence on planktivores. ICES CM/ N:05.

Semeniuk, C.A.D., Speers-Roesch, B., Rothley, K.D., 2007. Using fatty-acid profile analysis as an ecologic indicator in the management of tourist impacts on marine wildlife: a case of stingray-feeding in the Caribbean. Environ. Manage. 40, 665–677.

Skomal, G.B., Benz, G.W., 2004. Ultrasonic tracking of Greenland sharks, *Somniosus microcephalus*, under Arctic ice. Mar. Biol. 145, 489–498.

Speers-Roesch, B., Treberg, J.R., 2010. The unusual energy metabolism of elasmobranch fishes. Comp. Biochem. Physiol. A 155, 417–434.

Thiemann, G.W., Iverson, S.J., Stirling, I., 2007. Variability in the blubber fatty acid composition of ringed seals (*Phoca hispida*) across the Canadian Arctic. Mar. Mamm. Sci. 23, 241–261.

Tocher, D.R., 2003. Metabolism and functions of lipids and fatty acids in teleost fish. Rev. Fish. Sci. 11, 107–184.

Wai, T.C., Leung, K.M.Y., Sin, S.Y.T., Cornish, A., Dudgeon, D., Williams, G.A., 2011. Spatial, seasonal, and ontogenetic variations in the significance of detrital pathways and terrestrial carbon for a benthic shark, *Chiloscyllium plagiosum* (Hemiscylliidae), in a tropical estuary. Limnol. Oceanogr. 56, 1035–1053.

Warton, D.I., Hui, F.K.C., 2011. The arcsine is asinine: the analysis of proportions in ecology. Ecology 92, 3–10.

Zammit, V.A., Newsholme, E.A., 1979. Activities of enzymes of fat and ketone-body metabolism and effects of starvation on blood concentrations of glucose and fat fuels in teleost and elasmobranch fish. Biochem. J. 184, 313–322.