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TECHNICAL COMMENT

Experimental Evaluation of Fatty Acid Profiles as a Technique to Determine Dietary Composition in Benthic Elasmobranchs

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ABSTRACT

Fatty acid (FA) analysis is a tool for dietary investigation that complements traditional stomach content analyses. Controlled feeding experiments were used to determine the extent to which the FA composition of diet is reflected in the liver and muscle tissue of the Port Jackson shark *Heterodontus portusjacksoni*. Over 10 wk, two groups of sharks were fed prawns or squid, which have distinct FA profiles. The percentage of total FA was significantly different for shark liver and muscle tissue when comparing controls with prawn- and squid-fed sharks. Compared with experimentally fed sharks, control shark muscle and liver had higher levels of 18:1n-9 and 20:2n-9. When comparing prawn- and squid-fed sharks, only liver tissue showed a significant difference in FA profiles. The livers of prawn-fed sharks were comparatively higher in 18:1n-7, 22:5n-3, 20:0, and 18:1n-9, while the squid-fed sharks had higher levels of 16:0 and 22:6n-3. These FAs in shark liver tissue were all reflective of higher amounts in their respective dietary items, demonstrating the conservative transfer of FA from diet to liver tissue. This study shows that liver and muscle FA profiles can be used as indicators of dietary change through the comparison of controls and fed sharks. The timescale of this study may not have been sufficient for capturing the integration of FA into muscle tissue because only liver FA profiles were useful to distinguish between sharks fed different diets. These findings have important implications for sampling design where FA profiles are used to infer dietary preferences.

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Introduction

Accurate examination of the diet of a species can be difficult because diet information is often obtained by examining stomach contents only (Cortés 1997). Methods such as molecular identification of prey (Sigler et al. 2006; Dunn et al. 2010), stable isotopic analysis (Fisk et al. 2002; Estrada et al. 2003; Domi et al. 2005; Hussey et al. 2011), and fatty acid (FA) analysis (Schaufler et al. 2005; Pethybridge et al. 2010; Pethybridge et al. 2011) have recently become more popular in chondrichthyan research. There has been limited investigation into how prey selection influences the FA profiles of different predator tissues in a controlled environment. Combining biochemical methods such as FA and stable isotope analysis is likely to give the most accurate indication of time-integrated diet and may reduce the biases associated with analyzing stomach contents alone. Furthermore, lethal sampling is often not possible in highly mobile, threatened, and endangered species, with muscle biopsy being the only way to obtain dietary information in such species.

Lipid stores and their constituent FAs are an indicator of diet (Cowey et al. 1976; Kanazawa et al. 1979), and previous studies have shown that diet influences FA profiles in consumer tissues (Fraser et al. 1989; Kirsch et al. 1998). As most FAs are not synthesized by marine vertebrates, they are usually integrated through the diet and can act as biochemical indicators of food webs (Wilson et al. 2001; Dalsgaard et al. 2003; Thiemann et al. 2008). Long-chain polyunsaturated fatty acids (PUFAs) have more than one double bond and are most often used as dietary indicators because they cannot be biosynthesized in sufficient quantities to ensure optimal physiological performance (Tocher and Ghioni 1999; Turner and Rooker 2005). Monounsaturated fatty acids (MUFAs), which have one double bond, and saturated fatty acids (SATFAs), which do not contain double bonds, can be biosynthesized de novo. Dietary levels and enzyme availability can, however, affect the level to which this occurs (Tocher 2003). In most marine vertebrates, lipids

are stored in the adipose tissue found in muscle or blubber (Budge et al. 2011). Sharks are different from all other vertebrates because their ability to oxidize FA (obtained from lipids) is largely confined to the liver (Moyes et al. 1990; Ballantyne 1997). As a result, shark liver is the major site of both lipid storage and metabolism through mitochondrial and peroxisomal FA oxidation (Hallgren and Larsson 1962; Malins 1968; Bone and Roberts 1969; Medzihradsky et al. 1992). Several studies have indicated that shark tissues can accumulate dietary FA (Schaufler et al. 2005; Semeniuk et al. 2007; Pethybridge et al. 2010, 2011; Wai et al. 2011); however, the timescales of this integration are unclear and require further investigation.

Understanding the tissue-specific differences with respect to the processing of dietary lipids is fundamental to applying FA dietary tracer techniques to food-web studies. While taking a biopsy of muscle tissue would be the preferred method for dietary analysis because it is less invasive, liver tissue is known to act as a fat storage medium (Moyes et al. 1990; Ballantyne 1997). As a result, the effect of diet on both liver and muscle tissue needs to be investigated in a controlled setting to determine to what extent these tissues are reflective of diet. In this study, Port Jackson sharks *Heterodontus portusjacksoni* (Myer 1973), a common benthic elasmobranch (Last and Stevens 2009), were used as the study species. The purpose of this study was (1) to investigate which FAs drove the difference between experimentally fed and wild specimens (controls), (2) to determine which FAs drove the difference between experimentally fed sharks (prawn- and squid-fed sharks), and (3) to evaluate the differences between liver and muscle FA profiles as an indication of the diet consumed.

Material and Methods

Animal Maintenance

Port Jackson sharks were collected during fishery-independent surveys of the South Australian Prawn Trawling Fishery in Gulf St. Vincent (GSV), South Australia. Trawling was undertaken at night using standard 27–30-m double-rig demersal otter-trawl gear with two 14.6-m-wide nets and 4.5-cm diamond mesh cod ends, with trawl shots lasting for 30 min (Dixon et al. 2011). Sharks were transported to the marine facilities at the South Australia Research and Development Institute, Aquatic Sciences Centre, West Beach, and maintained in 200-L plastic containers. Between 8 and 48 h after capture, sharks were transferred to 5,000-L tanks provided with flow-through seawater, where they were housed for the duration of the project. The gender of all sharks was recorded based on the presence or absence of claspers. Sharks were also measured (total length), weighed (g), and tagged with Hallprint dart head tags to allow identification of individual sharks throughout the study. To assess the natural diet of sharks, four individuals (two males and two females) were euthanized before any experimental feeding to act as controls. The remaining 14 sharks were kept in four tanks (three or four sharks per tank) to prevent overcrowding. The sharks in each tank were fed the same diet, and each tank was held under identical conditions through a flow-through system and had an even distribution of sharks in terms of weight and length. To ensure there were no tank effects, temperature, pH, and dissolved oxygen concentration were regularly recorded and showed no significant differences between the tanks (ANOVA, temperature: 21.0°–23.9°C, $F = 0.822$, $P = 0.490$; pH: 7.26–7.79, $F = 0.020$, $P = 0.996$; dissolved oxygen (ppm): 4.82–10.45, $F = 0.188$, $P = 0.904$).

Feeding and Sampling Regime

Shark feeding was initiated 3 d after capture (December 14, 2009). Sharks were fed one of two diets: either western king prawns *Penaeus latisulcatus* (Kishinouye 1900) or squid (southern calamari) *Sepioteuthis australis* (Quoy and Gaimard 1832) collected from the GSV prawn trawl fishery. Sharks were fed at the same time of day to satiation three times a week for 10 wk, and any uneaten food was removed from the tank approximately 2 h after feeding. Before feeding, after 5 wk of feeding, and at the conclusion of the experiment, sharks were measured (total length) and weighed (g) to track their progress. At the conclusion of the 10-wk experiment on February 22, 2010, 24 h after feeding, sharks were euthanized by spinal section and pithing and dissected. We collected 5–10 g of muscle and liver tissue and froze at −20°C until analyzed. To compare the FA profiles of prawn- and squid-fed *Heterodontus portusjacksoni* with their diet source, six whole prawns and six whole squid were individually homogenized in a blender for FA analysis.

Lipid Extraction and FA Analysis

Samples were analyzed by the FOODplus Fatty Acid Lab (Urrbrae, South Australia). Lipids were extracted from diet or shark tissue samples using a chloroform : methanol (2 : 1) method as described by Bligh and Dyer (1959). The percentage of lipid was calculated on a wet weight basis, and the lipid was extracted with chloroform. FA methyl esters (FAMEs) were produced by heating the extracted lipids in 1% H_2SO_4 in methanol for 3 h in a 70°C water bath. After cooling, distilled water was added along with 2.0 mL of *n*-heptane and was shaken and centrifuged, allowing the phases to separate. The extracted FAMEs were separated and quantified using a gas chromatograph (Palo Alto, CA) to determine FA composition. Samples were run on a gas chromatograph with a flame ionization detector, and an external standard was used with approximately 50 different FAME types.

Statistical Analysis

Bray-Curtis similarity matrices were calculated for square-root-transformed data to test the differences between dietary items, fed sharks, and control sharks using Primer, version 6.1.13 (http://www.primer-e.com). Subsequently, analysis of similarities (ANOSIM; Clarke 1993) was performed, and significant differences in FA profiles were identified using *R* values. The percent contribution of each FA to the separation between diets

Table 1: Length, weight, and reproductive parameters for control and fed *Heterodontus portusjacksoni*

Shark ID	Tank	Diet	Total length (mm)	Weight (g)			% change	Class
				Week 0	Week 5	Week 10		
1	1	Prawn	420	450	400	500	10.00	Immature
2	1	Prawn	610	1,450	1,600	1,550	6.45	Maturing subadult
3	1	Prawn	590	1,350	1,400	1,400	3.57	Maturing subadult
4	1	Prawn	550	950	1,050	1,150	17.39	Maturing subadult
5	4	Prawn	650	1,750	1,800	1,900	7.89	Maturing subadult
6	4	Prawn	490	850	850	900	5.56	Immature
7	4	Prawn	600	1,550	1,450	1,450	−6.90	Maturing subadult
8	2	Squid	580	1,350	1,450	1,600	15.63	Maturing subadult
9	2	Squid	660	1,850	1,900	2,100	11.90	Maturing subadult
10	2	Squid	460	550	550	700	21.43	Immature
11	3	Squid	580	1,300	1,300	1,400	7.14	Maturing subadult
12	3	Squid	550	900	1,100	1,200	25.00	Maturing subadult
13	3	Squid	520	750	900	1,100	31.82	Maturing subadult
14	3	Squid	580	1,150	1,250	1,300	11.54	Maturing subadult
C-1	C	Natural	590	1,688	...	...	...	Maturing subadult
C-2	C	Natural	450	929	...	...	...	Immature
C-3	C	Natural	410	598	...	...	...	Immature
C-4	C	Natural	540	1,299	...	...	...	Maturing subadult

and fed sharks was assessed using similarity percentage (SIMPER) analysis, which measures the top 90% of contributing variables (Clarke 1993). To complement ANOSIM, differences in FA composition between diets, tissues, and maturity status were also analyzed using perMANOVA+, version 1.0.3 (Anderson 2001), using 9,999 permutations under a reduced model and additional pairwise tests using the square root of the pseudo-*F* statistic (*t*-test). Unlike ANOSIM, perMANOVA is able to determine whether the interactions between diets, tissues, and maturity status were significant. The perMANOVA relies on comparing the observed value of a test statistic (pseudo-*F* ratio) against a recalculated test statistic generated from random reordering (permutation) of the data (Anderson 2001). This permutation approach is a "semiparametric" multivariate version of a univariate one-way ANOVA. The advantage of this is that the resulting test is "distribution free" and not constrained by many of the typical assumptions of parametric statistics. The *F* profiles were then depicted using nonmetric multidimensional scaling (MDS) represented by two-dimensional plots. Stress values of the MDS ordination are considered good when stress is lower than 0.1 (Kruskal and Wish 1978). ANOVA was also used to test the differences in weight and total fat percentage between fed and control sharks and diets.

Results and Discussion

The results of this study may affect future sampling designs because they indicate that dietary patterns may be detected in liver FA profiles when comparing animals feeding on different exclusive diets. Muscle FA profiles were not indicative of sharks fed exclusive diets, and more research is required into the time-scales of FA integration into this tissue to develop appropriate sampling designs that can be applied to studies of wild animals. Controlled experiments simulating dietary switches should be conducted in captivity, and subsamples should be taken at shorter intervals to detect dietary FAs, which are likely to be mediated to tissues for storage or utilization in the form of energy metabolism. Furthermore, extended feeding trials are required to compare changes in FA profiles over time and determine whether the FA composition of different tissues reaches a steady state or whether it is fluctuating in response to diet.

Size, Weight, and Maturity

Fourteen fed sharks and four controls were analyzed (table 1). The weight of control sharks was not significantly different from that of either prawn- or squid-fed sharks (ANOVA, $F < 0.001$, $P = 0.986$). The weight of prawn- and squid-fed sharks and controls was also not significantly different by week sampled (ANOVA, $F = 0.374$, $P = 0.690$), and there was no significant interaction between diet and week sampled (ANOVA, $F = 0.098$, $P = 0.906$). Squid-fed sharks gained significantly more weight by percentage than did prawn-fed sharks (ANOVA, $F = 17.016$, $P > 0.001$) and sharks were significantly different between week sampled ($F = 4.640$, $P = 0.016$). There was, however, no significant interaction between week sampled and diet (ANOVA, $F = 1.244$, $P = 0.300$). The differences in weight gain were consistent with significantly higher levels of total fat in the squid diet (ANOVA, $F = 16.928$, $P = 0.002$), with $1.9\% \pm 0.08\%$ compared to $1.1\% \pm 0.18\%$ seen in prawns. Furthermore, no squid-fed sharks lost weight throughout the duration of the experiment, indicating that differences in health

condition and growth rate were a result of diet and not differences between individuals.

The perMANOVA model showed that maturity, assessed according to Powter and Gladstone (2008), had no significant effect on FA composition, and there was no interaction between maturity and tissue or treatment (table 2). As only immature and subadult male sharks were examined, the effects of sex and size could not be investigated, and it is unknown whether the results are applicable to female or adult specimens. Previous work on teleosts has shown that specific FAs are required for gonad development, including utilization of specific FAs during the process of yolk deposition in eggs in female gonads (Ballantyne et al. 1996). Furthermore, female sharks have been shown to store more lipids in their livers because they require sufficient nutrients for reproduction and embryo development (Bone et al. 1995). The effect of sex-specific differences is, however, expected to be minimal in this study because animals were not sexually mature, and the lipid content within shark liver has been shown to be more strongly affected by seasonal differences than by sex (Jayasinghe et al. 2003). In addition, previous dietary studies on Port Jackson sharks have not detected diet differences between sexes (Powter et al. 2010). The difference between immature and subadult sharks was tested, and stage of maturity was also not found to have an effect on FA profiles. However, previous work by Wai et al. (2011) identified differences between adult and juvenile bamboo sharks *Chiloscyllium plagiosum* (Bennett 1830).

Table 2: Statistical test of fatty acid profile differences as a result of diet (control, prawn diet, squid diet), tissue (liver, muscle), and maturity (immature, maturing subadult) between control *Heterodontus portusjacksoni* and fed sharks by two-way factorial nonparametric MANOVA

	df	MS	*F*	*P*
Diet	2	805.150	23.058	<.001*
Tissue	1	3,093.600	88.594	<.001*
Maturity	1	31.708	.908	.384
Diet × tissue	2	280.550	8.034	<.001*
Diet × maturity	2	54.579	1.563	.179
Tissue × maturity	1	15.872	.454	.673
Diet × tissue × maturity	2	46.590	1.334	.246

*$P < 0.05$.

FA Composition and Effects

FA analysis of diet and shark tissues identified 52 individual FAs; 19 of these had a mean of more than 0.3% (fig. 1*a*; a comprehensive list can be found in table A1, available in the online edition of *Physiological and Biochemical Zoology*). Based on the Bray-Curtis similarity index, the FA compositions of Port Jackson sharks were significantly different by diet and tissue (table 2). There were no significant differences when analyzing sharks by FA fraction (SATFA, MUFA, PUFA) in liver (perMANOVA, pseudo-$F = 1.1418$, $P = 0.3419$; ANOSIM, $R = 0.183$, $P = 0.036$) or in muscle (perMANOVA, pseudo-$F = 0.582$, $P = 0.662$; ANOSIM, $R = -0.125$, $P = 0.982$). There was a significant interaction between diet and tissue, but no other significant interactions were identified (table 2). The MDS results based on FA profiles suggest that prawn- and squid-fed sharks cannot be distinguished using muscle tissue (fig. 2*a*). Three clusters, which can be separated at the 90% similarity level, are shown on the MDS plot: group A comprised the four control sharks, group B comprised five squid-fed sharks (8, 9, 12, 13, and 14) and five prawn-fed sharks (1, 3, 5, 6, and 7), while group C comprised two squid-fed sharks (10 and 11) and two prawn-fed sharks (2 and 4). There was clear separation of the control sharks from both groups of fed sharks. However, there was no separation between prawn- and squid-fed sharks. Significant differences were identified between muscle FA profile clusters (perMANOVA, pseudo-$F = 8.9937$, $P > 0.001$; ANOSIM, $R = 0.516$, $P = 0.050$), with pairwise tests indicating that groups A and B were significantly different from group C (table 3). The dietary shift in FA composition of liver tissue is more clearly visualized in the MDS scatterplot (fig. 2*b*). Five clusters, which can be separated at the 90% similarity level, have been shown on the MDS plot: group A comprises six prawn-fed sharks (1, 2, 3, 4, 6, and 7) and one squid-fed outlier (8), group B comprises three squid-fed sharks (10, 12, and 14) and one prawn-fed outlier (5), group C comprises three squid-fed sharks (9, 11, and 13), group D comprises three control sharks (C-1, C-3, C-4), and group E contains another control shark (C-2). Significant differences were identified between liver FA profile clusters (perMANOVA, pseudo-$F = 22.34$, $P > 0.001$; ANOSIM, $R = 0.94$, $P > 0.001$). Pairwise tests showed that groups A and B, B and C, A and D, A and C, and B and D were significantly different (table 3). There were no trends in terms of tank or shark size, weight, or maturity that explain the outliers (table 1).

Tissue-specific differences in lipid storage are indicated by differing FA profiles in relation to diet. Shark liver is metabolically different from other vertebrates because it behaves like adipose tissue (Medzihradsky et al. 1992) and is the main site of both lipid storage and metabolism (Hallgren and Larsson 1962; Malins 1968; Bone and Roberts 1969). This was demonstrated by the liver of controls containing 35.9% ± 2.55% total lipid compared to only 0.69% ± 0.06% in the muscle. While FA oxidation (one of the major processes of lipid metabolism) does not occur outside the liver (Moyes et al. 1990), the lipids stored there must be transported to other tissues to fuel a variety of metabolic functions (Ballantyne et al. 1993). The utilization of FAs outside of the liver, such as in muscle tissue, depends largely on the availability of enzymes required to facilitate oxidation (Tocher 2003; Turner and Rooker 2005). It has thus been suggested that ketone bodies may be the most important fat fuels and that there is a preference for ketone bodies rather than lipids as oxidative substrates (Zammit and Newsholme 1979; Ballantyne 1997). As a result, the majority of metabolic energy stores is likely to be derived from lipids stored in the liver while the muscle utilizes proteins (Pethy-

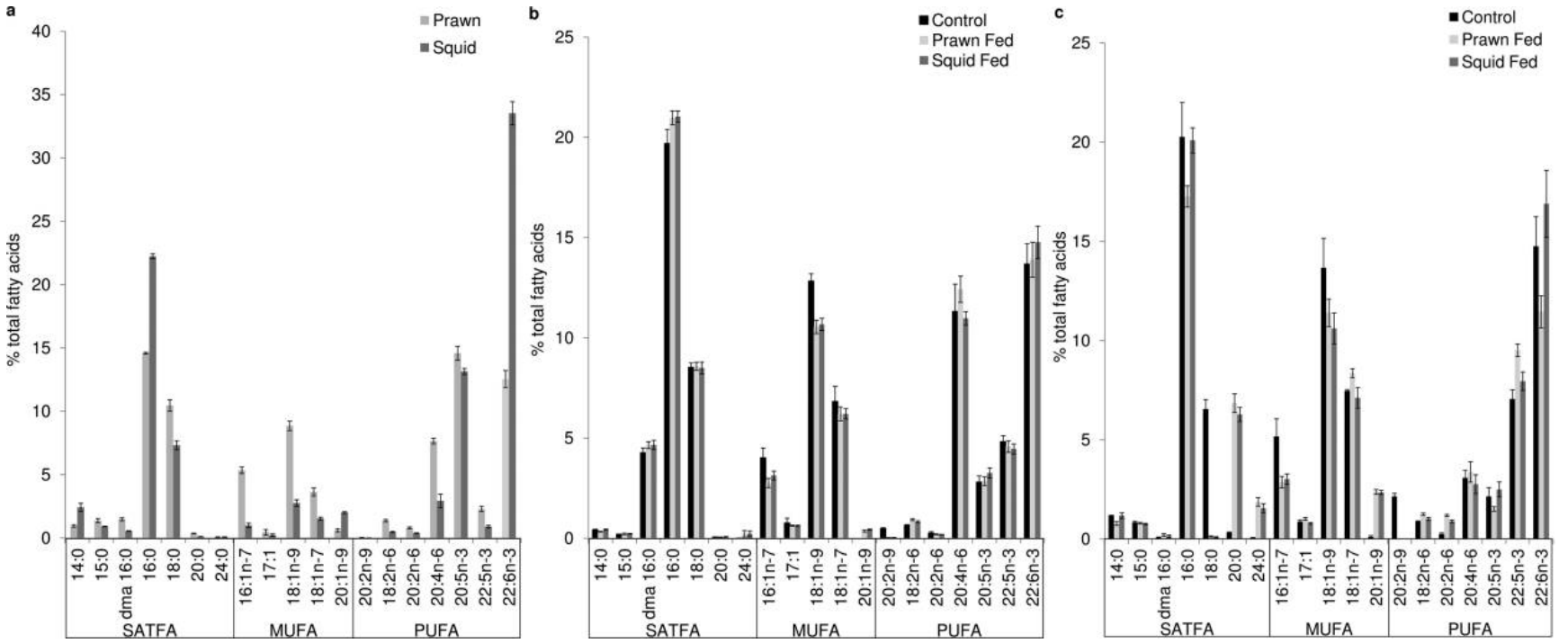

Figure 1. Fatty acids (FAs; 19 of 52 identified) with an overall mean of ≥0.3% of total FAs that represent the largest differences between the prey items fed to Port Jackson sharks (*a*), the muscle profiles of sharks fed different diets (*b*), and the liver profiles of sharks fed different diets (*c*). Bars are means and vertical lines are standard errors.

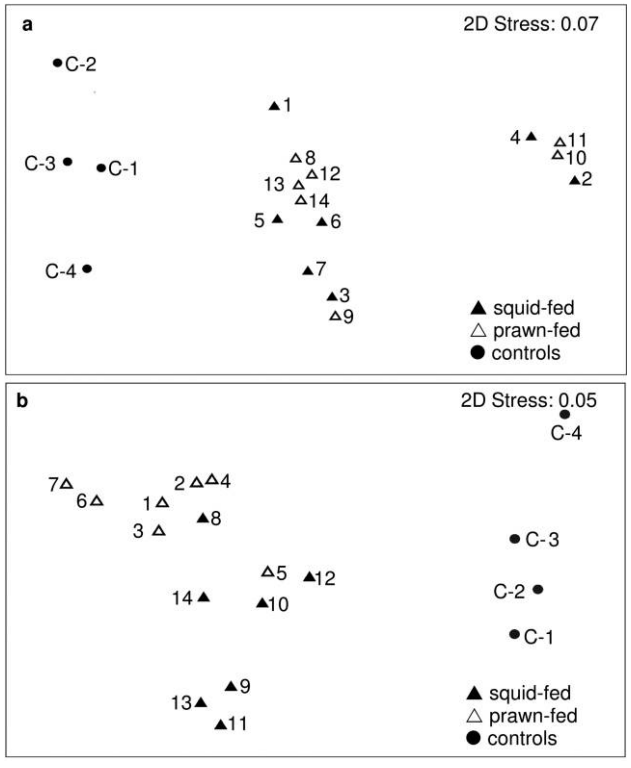

Figure 2. Multidimensional scaling plot of muscle (*a*) and liver (*b*) fatty acid composition of squid-fed, prawn-fed, and control sharks (after 10 wk of feeding). Numbers represent shark identification.

bridge et al. 2010). Because of the similar FA composition in the muscle tissues of sharks fed different diets over a 10-wk period, this study demonstrates that muscle may not be a suitable tissue to target when using FA profiles to investigate differences in diet.

FA Profiles of Diet Fed to Sharks

Prawns and squid had significantly different FA profiles (ANOSIM, $R = 0.989$, $P = 0.002$; perMANOVA, pseudo-$F = 3.976$, $P < 0.001$), with SIMPER analysis showing that diets were 25% dissimilar and that PUFA was the dominant contributor to the dissimilarity. Docosahexaenoic acid (DHA; 22:6n-3), oleic acid (18:1n-9), and palmitoleic acid (16:1n-7) all contributed more than 5% to the dissimilarity observed between prawns and squid. Palmitoleic acid was 4.4% higher in prawns than in squid, and oleic acid was 6.1% higher in prawns than in squid, while DHA was 20.1% higher in squid than in prawns (fig. 1*a*). Diets were also significantly different when analyzed by FA class (perMANOVA, pseudo-$F = 19.5$; $P = 0.002$; ANOSIM, $R = 0.99$, $P = 0.002$). Diets showed a 7% dissimilarity, which was driven by MUFA (47%) and PUFA (34%). Prawns contained 12.5% more MUFA than squid, while squid contained 4.1% more SATFA than prawns and 11.5% more PUFA than prawns.

FA Profiles of Experimentally Fed Sharks

PerMANOVA and ANOSIM showed that the FA profiles of muscle tissue were not significantly different between prawn- and squid-fed sharks (table 4; ANOSIM, $R = 0.03$, $P = 0.553$; perMANOVA, pseudo-$F = 0.712$, $P = 0.627$). This was in contrast to liver FA profiles, which were significantly different between prawn- and squid-fed sharks (table 4; ANOSIM, $R = 0.109$, $P = 0.039$; perMANOVA, pseudo-$F = 2.6$, $P = 0.002$). SIMPER indicated that prawn- and squid-fed sharks were 14% dissimilar (fig. 3), which was largely driven by PUFA, contributing 50% to the total dissimilarity. This suggests that FA may not be deposited in the muscle tissue and instead is stored in the liver. This could take place during periods of low physiological demand, when FA is stored in the liver and not transported to other tissues such as muscles. Alternatively, dietary FAs could have also been transported to muscle tissues to fulfil a shark's immediate metabolic requirements. Dietary lipids are absorbed through the intestine, packaged into lipoprotein particles (such as chlyomicrons), and transported to the cells to be stored as triacylglycerols. Therefore, it is possible that ingested FAs are directly transported to the muscle from the intestine to satisfy sharks' immediate energy requirements. In such cases, changes in FA profiles as a result of differing dietary FA signatures would have been detected in muscle tissue earlier than in liver tissue and might have already equilibrated by week 10. The ability to detect changes in FA signature in various tissues may, therefore, be dependent on the sampling time following feeding. Previous work has indicated that substantial variation occurs in lipid composition among tissues, and similarities in lipid classes based on physiological function

Table 3: Pairwise statistical tests of dietary differences in muscle and liver tissue fatty acid profiles between control *Heterodontus portusjacksoni* and sharks fed prawn or squid by two-way factorial nonparametric MANOVA (perMANOVA) and analysis of similarities (ANOSIM)

	perMANOVA		ANOSIM	
Tissue and group	*t*	*P*	*R*	*P*
Liver:				
A, B	3.150	.003*	.802	.003*
B, C	2.904	.030*	.981	.029*
D, A	6.373	.011*	1	.008*
A, C	4.746	.009*	1	.008*
D, B	4.742	.028*	1	.029*
D, C	5.902	.106	1	.1
D, E	2.349	.246	1	.25
E, A	4.734	.125	1	.125
E, B	4.375	.198	1	.2
E, C	6.698	.250	1	.25
Muscle:				
A, C	3.60	.002*	.864	.001*
A, B	1.48	.136	−.01	.436
C, B	5.11	.008*	1	.008*

*$P < 0.05$.

Table 4: Pairwise statistical tests of dietary differences in muscle and liver tissue fatty acid profiles between control *Heterodontus portusjacksoni* and sharks fed prawn or squid diets by two-way factorial nonparametric MANOVA (perMANOVA) and analysis of similarities (ANOSIM)

	perMANOVA		ANOSIM	
Tissue and diet	*t*	*P*	*R*	*P*
Muscle:				
Prawn, squid	.712	.627	.030	.553
Prawn, control	2.879	.003*	.599	.002*
Squid, control	2.960	.003*	.643	.003*
Liver:				
Prawn, squid	2.600	.002*	.109	.039*
Prawn, control	7.612	.003*	.391	.091
Squid, control	7.145	.003*	.461	.118

*$P < 0.05$.

have previously been shown (Pethybridge et al. 2010). The lack of reflection of diet in muscle FA profiles may be further explained by the physiological function of muscle tissue and the fact that dietary FAs have already turned over (equilibrated) and that the muscle FA profile has reverted back to those FAs that are needed by that tissue. While there are no previous data available on the turnover rates of FA in sharks, Turner and Rooker (2005) reported a turnover rate of 1 wk in the homogenized tissue of whole pelagic juvenile cobia *Rachycentron canadum* (Linnaeus 1766).

Several individual FAs contributed more than 5% to the total dissimilarity of the liver FA profiles of squid- and prawn-fed sharks, including palmitic acid (16:0) and arachidic acid (20:0), DHA (22:6n-3) and docosapentaenoic acid (DPA; 22:5n-3), and oleic acid (18:1n-9) and vaccenic acid (18:1n-7). Palmitic acid was 2.8% higher in the liver of squid-fed sharks compared with that of prawn-fed sharks and 0.06% higher in the muscle of squid-fed sharks compared with that of prawn-fed sharks; DHA was 5.4% higher in the liver of squid-fed sharks compared with that of prawn-fed sharks and 0.9% higher in the muscle of squid-fed sharks compared with that of prawn-fed sharks (fig. 1*c*). This was reflective of a 7.6% increase of palmitic acid and a 21% increase of DHA in squid diet compared with prawn diet (fig. 1*a*). Compared with squid-fed sharks, prawn-fed sharks had 0.8% more oleic acid, 1.6% more DPA, 0.6% more arachidic acid, and 1.2% more vaccenic acid (fig. 1*c*). This was reflected in dietary items, with prawns containing 6.1% more oleic acid, 1.4% more DPA, and 0.3% more arachidic acid than squid (fig. 1*a*).

Palmitic acid and arachidic acid are SATFAs and can both be created through de novo FA synthesis. Despite this, dietary levels were still shown to influence the FA composition of fed sharks, indicating that de novo synthesis was limited. Vaccenic acid can also be derived from palmitic acid via the metabolic pathway. Levels of vaccenic acid in the diet were, however, consistent with the levels of this FA in the liver tissue, suggesting that vaccenic acid is, regardless, a good marker of diet. DHA and DPA are omega-3 PUFAs that are largely incorporated from the diet, and the reduced ability of marine fish to form DHA and its metabolic precursor DPA through desaturation and elongation makes them useful as dietary tracers (Tocher 2003).

FA Profiles of Control Sharks and Experimentally Fed Sharks

The FA profiles of the muscle and liver tissues of both groups of experimentally fed sharks were significantly different from those of control sharks (table 4). SIMPER analysis indicated that the muscle FA profiles of experimentally fed sharks and controls were 14% dissimilar and that the liver FA profiles of fed sharks and controls were 24%–26% dissimilar (fig. 3). The dissimilarity between groups was driven by PUFA for muscle and by SATFA for liver. Although control sharks had higher percentages of dietary-derived PUFA in their muscle tissue and liver (33%–35%) than fed sharks, muscle PUFA levels were still low compared with those previously reported for this species (43%; Dunstan et al. 1988) and other chondrichthyans (fig. 4*a*). Values of PUFA in liver tissues also appear to vary widely across chondrichthyan species (fig. 4*b*).

The overall dissimilarity of the muscle of fed sharks and controls was driven by octadecanal-dimethylacetal (DMA; 18:0), 8,11-cis-eicosadienoic acid (20:2n-9), and eicosenoic acid (20:1n-9) in prawn-fed sharks and controls, while erucic acid (22:1n-9) and mead acid (20:3n-9) contributed more than 5% to the overall dissimilarity observed between squid-fed and control sharks. The overall dissimilarity observed between the liver of fed sharks and controls was driven by arachidic acid (20:0), stearic acid (18:0), palmitic acid (16:0), DHA (22:6n-3), DPA (22:5n-3), oleic acid (18:1n-9), and eicosenoic acid (20:1n-9), which all contributed more than 5% to the dissimilarity observed between groups. The liver of control sharks had 6.4% more stearic acid, 3% more palmitic acid, 3.3% more DHA, and 2.3% more oleic acid than seen in the liver of prawn-fed sharks (fig. 1*c*). The percentage of oleic acid was 2.3% higher in the muscle of control sharks compared with that of prawn-

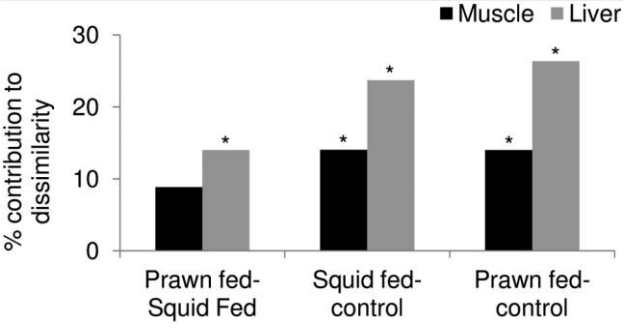

Figure 3. Similarity percentage analysis expressed as a percentage of overall dissimilarity based on a Bray-Curtis similarity matrix. Sharks fed prawns, sharks fed squid, and controls are compared by tissue type (muscle and liver). An asterisk represents significant differences between the total fatty acid profiles of feeding groups; nonparametric MANOVA and analysis of similarities values are presented in table 4.

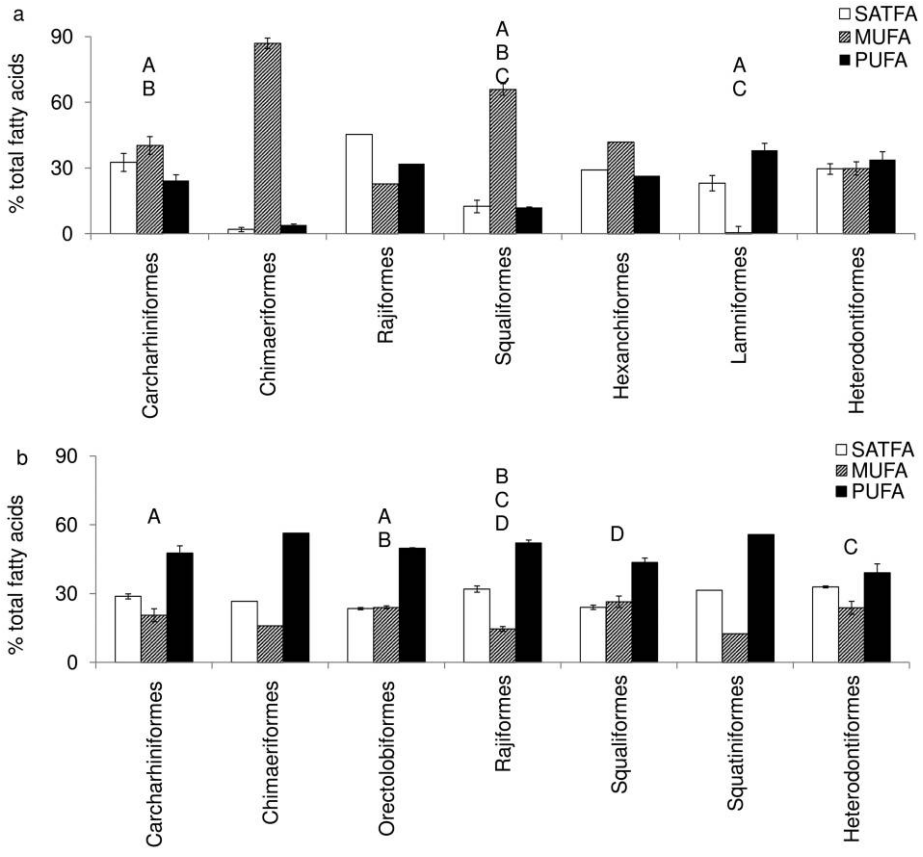

Figure 4. Average total fatty acid percentage of Port Jackson shark liver (*a*) and muscle (*b*) tissue by order. The average values for each order were calculated based on Dunstan et al. (1988), Nichols et al. (1998), Davidson and Cliff (2002), Jayasinghe et al. (2003), Schaufler et al. (2005), Néchet et al. (2007), Pethybridge et al. (2010), and Wai et al. (2011). Species-specific information can be found in tables A2 and A3, available in the online edition of *Physiological and Biochemical Zoology*. Significant differences between the fatty acid fractions of orders; nonparametric MANOVA and analysis of similarities values are presented in table 4 and are represented by the notation A, B, C, D.

fed sharks (fig. 1*b*). While the FA profile of wild diet is unknown, this suggests that the natural diet had increased availability of stearic acid, palmitic acid, DHA, and oleic acid compared with the experimental diet (fig. 1*a*). Prawn-fed sharks had 2.5% more DPA and 6.5% more arachidic acid in their liver than control sharks (fig. 1*c*). In comparison, the DPA and arachidic acid levels in the liver of squid-fed sharks were 1.6% and 0.6% less than in prawn-fed sharks but still 0.9% and 6% higher than in controls, respectively (fig. 1*c*). This was consistent with a 1.4% and a 0.3% increase in DPA and arachidic acid in prawns compared with squid, respectively (fig. 1*a*). Arachidic acid was detected only at trace levels in shark muscle, while levels of DPA were relatively similar, with muscle of control sharks containing 0.3% more DPA than that of prawn-fed sharks and 0.4% more than that of controls.

The liver of squid-fed sharks also contained 2.1% more DHA than the liver of controls (fig. 1*c*). In comparison, the liver of prawn-fed sharks contained 3.3% less DHA than that of controls, while squid-fed sharks contained 5.4% more DHA than prawn-fed sharks. The muscle of squid-fed sharks also contained 1.1% more DHA than that of controls, while the muscle of prawn-fed sharks only contained 0.2% more DHA than that of controls and 0.9% less DHA than that of squid-fed sharks (fig. 1*b*). Levels of DHA were 21% higher in squid compared with prawns (fig. 1*a*).

Compared with control sharks, experimentally fed sharks had 0.4% more eicosenoic acid (20:1n-9) in their muscle and 2.2%–2.3% more in their liver (fig. 1*b*, 1*c*). The muscle and liver of experimentally fed sharks contained 0.3%–0.4% and 0.2% more erucic acid (22:1n-9) than controls, respectively (fig. 1*b*, 1*c*). High levels of eicosenoic and erucic acid can be indicative of secondary consumption of zooplankton, which may indicate predation on animals such as crustaceans and squid (Phillips et al. 2003). However, these MUFAs are also potential products of the elongation and desaturation of oleic acid (18:1n-9) to mead acid (20:3n-9), which is known to occur in fish and other vertebrates (Tocher 2003). Experimentally fed sharks had 0.5% more mead acid in their muscle than the muscle of controls. However, in their liver, control sharks had 0.8% more mead acid than experimentally fed sharks, which had only trace levels. Because mead acid was not present in either diet, the level of

Table 5: Pairwise statistical tests of dietary differences in liver and muscle tissue fatty acid fractions by two-way factorial nonparametric MANOVA (perMANOVA) and analysis of similarities (ANOSIM)

	perMANOVA		ANOSIM	
Tissue and order	*T*	*P*	*R*	*P*
Liver:				
Carcharhiniformes, Chimaeriformes	2.942	.082	.853	.091
Carcharhiniformes, Hexanchiformes	Negative	...	−.311	.909
Carcharhiniformes, Lamniformes	1.457	.117	.252	.035*
Carcharhiniformes, Rajiformes	.955	.182	.151	.273
Carcharhiniformes, Squaliformes	4.012	.002*	.474	>.001*
Chimaeriformes, Hexanchiformes	No test	...	...	...
Chimaeriformes, Lamniformes	7.563	.163	1.000	.167
Chimaeriformes, Rajiformes	No test	...	...	...
Chimaeriformes, Squaliformes	1.232	.219	.202	.231
Hexanchiformes, Lamniformes	1.247	.329	.280	.333
Hexanchiformes, Rajiformes	No test	...	...	...
Hexanchiformes, Squaliformes	1.647	.156	.295	.154
Lamniformes, Rajiformes	2.376	.170	.840	.167
Lamniformes, Squaliformes	4.149	>.001*	.541	.001*
Rajiformes, Squaliformes	2.631	.073	.803	.077
Muscle:				
Carcharhiniformes, Chimaeriformes	.909	.599	.000	.600
Carcharhiniformes, Orectolobiformes	2.318	.006*	.715	.002*
Carcharhiniformes, Rajiformes	1.631	.143	.241	.171
Carcharhiniformes, Squaliformes	1.415	.161	−.047	.530
Carcharhiniformes, Squatiniformes	1.229	.401	.167	.200
Carcharhiniformes, Heterodontiformes	1.314	.337	−.036	.600
Chimaeriformes, Orectolobiformes	3.894	.109	1.000	.111
Chimaeriformes, Rajiformes	1.479	.245	.333	.750
Chimaeriformes, Squaliformes	1.213	.236	.235	.235
Chimaeriformes, Squatiniformes	No test	...	...	...
Chimaeriformes, Heterodontiformes	2.454	.336	1.000	.333
Orectolobiformes, Rajiformes	6.954	.005*	1.000	.006*
Orectolobiformes, Squaliformes	1.161	.272	−.045	.620
Orectolobiformes, Squatiniformes	5.906	.111	1.000	.111
Orectolobiformes, Heterodontiformes	4.850	.024*	.996	.022*
Rajiformes, Squaliformes	2.416	.031*	.376	.052*
Rajiformes, Squatiniformes	1.027	.490	−.111	.500
Rajiformes, Heterodontiformes	3.422	.104	.917	.100
Squaliformes, Squatiniformes	1.647	.119	.481	.118
Squaliformes, Heterodontiformes	1.119	.222	.138	.261
Squatiniformes, Heterodontiformes	2.420	.341	1.000	.333
Carcharhiniformes, Chimaeriformes	.909	.599	.000	.600
Carcharhiniformes, Orectolobiformes	2.318	.006*	.715	.002*
Carcharhiniformes, Rajiformes	1.631	.143	.241	.171
Carcharhiniformes, Squaliformes	1.415	.161	−.047	.530
Carcharhiniformes, Squatiniformes	1.229	.401	.167	.200
Carcharhiniformes, Heterodontiformes	1.314	.337	−.036	.600
Chimaeriformes, Orectolobiformes	3.894	.109	1.000	.111
Chimaeriformes, Rajiformes	1.479	.245	.333	.750
Chimaeriformes, Squaliformes	1.213	.236	.235	.235
Chimaeriformes, Squatiniformes	No test	...	...	...
Chimaeriformes, Heterodontiformes	2.454	.336	1.000	.333
Orectolobiformes, Rajiformes	6.954	.005*	1.000	.006*

Table 5 (*Continued*)

Tissue and order	perMANOVA		ANOSIM	
	T	*P*	*R*	*P*
Orectolobiformes, Squaliformes	1.161	.272	−.045	.620
Orectolobiformes, Squatiniformes	5.906	.111	1.000	.111
Orectolobiformes, Heterodontiformes	4.850	.024*	.996	.022*
Rajiformes, Squaliformes	2.416	.031*	.376	.052*
Rajiformes, Squatiniformes	1.027	.490	−.111	.500
Rajiformes, Heterodontiformes	3.422	.104	.917	.100
Squaliformes, Squatiniformes	1.647	.119	.481	.118
Squaliformes, Heterodontiformes	1.119	.222	.138	.261
Squatiniformes, Heterodontiformes	2.420	.341	1.000	.333

Note. Average values for each order were calculated based on Dunstan et al. (1988), Nichols et al. (1998), Davidson and Cliff (2002), Jayasinghe et al. (2003), Schaufler et al. (2005), Néchet et al. (2007), Pethybridge et al. (2010), and Wai et al. (2011). Species-specific information can be found in tables A2 and A3, available in the online edition of *Physiological and Biochemical Zoology*.

*$P < 0.05$.

mead acid in fed sharks was either a result of the mediation from stores in the liver or a result of biosynthesis.

Control sharks had 0.5% more 8,11-cis-eicosadienoic acid (20:2n-9) in muscle (fig. 1*b*) and 2.1% more in liver (fig. 1*c*) compared with fed sharks as a result of an experimental diet largely deficient in this FA (fig. 1*a*). Elevated levels of cis-eicosadienoic acid can be a result of omega-3 FA deficiencies, which can result in the preferential utilization of omega-3 and omega-6 FAs by enzymes (Caballero et al. 2002). Control sharks had no DMA 18:0 in muscle tissue and only trace amounts in liver; squid-fed sharks had 0.03% more DMA 18:0 in their muscle than prawn-fed sharks, and prawn-fed sharks contained 0.4% more than squid-fed sharks in their liver. Squid contained 0.4% more DMA than prawns. Plasmalogen-derived DMAs such as DMA 18:0 play an important role in membrane fluidity and have previously been linked to PUFA-rich diets devoid in DHA (Glick and Fischer 2010).

These FAs were indicative of changes to the relative composition of FA profiles in response to change in diet. This demonstrates the potential for the use of FA liver profile analysis to indicate dietary change. DHA and oleic acid were also among the major drivers of the dissimilarity observed between prawn and squid diet items. Furthermore, DHA is particularly useful as a dietary indicator because sharks have a reduced ability to produce it through desaturation and elongation (Tocher 2003). In comparison, previous work on deepwater shark species (Pethybridge et al. 2011) indicated that vaccenic acid was a main predictor of prey groups in both liver and muscle tissue, while DPA, palmitic acid, and DHA were main predictors of prey in muscle tissue and not in liver. Levels of dietary-derived DHA in liver tissue were similar to those reported in other studies; however, levels of DHA in muscle tissue were considerably lower in this study (fig. 1*b*). Vaccenic acid is a potential indicator of crustaceans, benthopelagic squid, and fish, while DPA, DHA, and palmitic acid are potential indicators of crustaceans, octopuses, and mesopelagic squid (Pethybridge et al. 2011). Vaccenic acid and long-chain SATFA arachidic acid have previously been used as biomarkers to identify the source of detritus consumed in bamboo sharks (Wai et al. 2011). High levels of oleic acid were found in shark liver (Pethybridge 2010); however, this FA may be considered a product of de novo FA synthesis and not a result of diet (Ballantyne 1997). The differing predictors and levels of FA seen in this study are likely to be a result of the restricted diet of only one item in this study compared with the variety of prey items consumed in the wild. Furthermore, as a majority of the literature focuses on deepwater sharks, the trends in FA composition may be affected by the different physiological and biological parameters associated with their habitat (Pethybridge et al. 2010).

The FA profiles of control and fed sharks were significantly different when comparing muscle and liver tissue. This was in contrast to the comparison of prawn- and squid-fed sharks where only the liver FA profiles showed a significant difference. The dietary differences between controls and fed sharks were likely to be much larger than the differences between the prawn and squid diets because of the range of available food items in the wild. This suggests that the extent of the dietary change determines whether it can be detected in the muscle, while the liver is suitable for detecting even relatively minor dietary changes.

Implications: Timescales

Many of the FA profiles of chondrichthyans found in the literature describe the FA content of either liver (Emokpae and Anekwe 1983; Bakes and Nichols 1995; Nichols et al. 1998; Davidson et al. 2002, 2011) or muscle (Hornung et al. 1994; Wai et al. 2011) tissues. However, most studies do not make comparisons between these tissues. Comparisons between liver and muscle profiles in the dogfish *Squalus acanthias* (Linnaeus 1758) revealed differing compositions of FA, with muscle containing high percentages of C20 and C22 polyenoic acids while the liver contained high concentrations of C20 and C22 monoenoic acids (Malins 1968). Although Port Jackson shark muscle

tissues had higher levels of C20 polyenoic acids than liver, C22 polyenoic acids and C20 and C22 monoenoic acids were all higher in liver tissue compared with muscle tissue.

A review of previous literature analyzing FA profiles in elasmobranchs revealed that the FA fractions in liver tissue were significantly different between shark taxonomical orders (perMANOVA, pseudo-$F = 7.35$, $P > 0.001$; ANOSIM, $R = 0.39$, $P = 0.004$). Pairwise tests detected differences between Squaliformes and Carcharhiniformes and between Squaliformes and Lamniformes (table 5; fig. 4*a*). Although the mean FA fractions of shark muscle FA profiles also showed significant differences using ANOSIM ($R = 0.18$, $P = 0.040$), no significant differences were found using perMANOVA (pseudo-$F = 2.82$, $P = 0.072$). Pairwise tests detected differences between Carcharhiniformes and Orectolobiformes, Orectolobiformes and Rajiformes, Orectolobiformes and Heterodontiformes, and Rajiformes and Squaliformes (table 5; fig. 4*b*).

While there have been no previous controlled studies investigating FA analysis in sharks, there have been several studies using stable isotopes that have assessed tissue turnover rates and discrimination factors (Hussey et al. 2010; Logan and Lutcavage 2010; Matich et al. 2010; Kim et al. 2012). Stable isotope tissue turnover rates for elasmobranchs have been investigated in a controlled setting for liver, whole blood, and white muscle, with isotope turnover shown to be slow for shark tissues (Logan and Lutcavage 2010). Both carbon and nitrogen have been shown to incorporate faster into blood plasma than in muscle and red blood cells, and the rate of incorporation of carbon into muscle is similar to patterns seen in other aquatic ectotherms (Kim et al. 2012). As a result, muscle isotope data would be unlikely to demonstrate seasonal migrations or diet switches in sharks, while liver and blood would be more likely to show shorter-term movement or shifts in diet (Logan and Lutcavage 2010).

There have been very few attempts to relate descriptions of lipid profiles with dietary patterns in chondrichthyans. As seen in Port Jackson sharks, large quantities of dietary-derived DHA have previously been observed in sharks (Pethybridge et al. 2010). Comparisons of Pacific sleeper shark *Somniosus pacificus* (Bigelow and Schroeder 1944) tissues have also revealed distinct FA compositions, with livers containing relatively high concentrations of MUFA and muscle having higher concentrations of PUFA (Schaufler et al. 2005). This differs from the results observed in Port Jackson sharks, which contained high levels of PUFA in both tissues (fig. 4). High levels of MUFAs cetoleic acid (22:1n-11) and eicosenoic acid (20:1n-9) in sleeper sharks were linked to secondary predation on calanoid copepods through scavenging on whale blubber (Schaufler et al. 2005). FA profiles of the whale blubber were retrieved from sleeper shark stomachs, and the FA profiles of these and other prey items were compared with sleeper shark tissue profiles. This indicates that FA profiles are useful directly after feeding has occurred. However, if the sleeper sharks had not recently ingested the whale blubber, it is not known whether the same link to copepods could be established. FA profiles have the potential to do more than indicate recent meals, and experimental manipulations of diet over time can provide insights into the integration of FAs into different tissues over time.

The conservative transfer of dietary FAs to shark tissues may provide a record of short-term diet history. Tissue-specific differences are apparent, particularly in relation to muscle and liver, and understanding differences in the timescales of FA integration is an important aspect of interpreting FA values. FA profiles are likely to be a complementary method to use in conjunction with more conventional dietary analysis techniques such as stomach contents and stable isotopes. A combination of these methods can result in unravelling trophic pathways in complex ecosystems with multiple dietary sources.

Conclusions

This study shows that liver and muscle FA profiles can be used as indicators of dietary change through the comparison of control and fed sharks. In contrast, the similar muscle FA profiles of sharks fed different diets demonstrates that muscle may not be a suitable tissue to target when using FA profiles to investigate sharks feeding on different diets. Furthermore, the timescale of this study may not have been sufficient in capturing the integration of FA into muscle tissue because only liver FA profiles were useful to distinguish between sharks fed different diets.

We suggest that further captive experiments are required to gain further understanding into the timescales of FA integration into tissues. The diets fed to captive animals should be dramatically different in order to stimulate changes and investigate FA pathways. Furthermore, the difference between an immediate dietary change, as demonstrated by the comparison between control and fed animals, and sharks fed exclusively different diets over time should be further investigated. The effect of diet on FA profiles in the short term (<10 wk) may provide more insight into the role of muscle in energy storage and mediation. In addition, longer feeding trials (>10 wk) may demonstrate how the FA profile changes over time and particularly how long it takes for the liver FA profile to become stable in alignment with the diet, suggesting that FA stores have been exhausted and complete FA turnover has occurred.

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