Comparison of fin and muscle tissues for analysis of signature fatty acids in tropical euryhaline sharks

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Abstract

Fatty acid (FA) analysis can provide an effective, non-lethal method of elucidating the trophic ecology of fish. One method utilized in the field is to collect biopsied muscle tissue, but this can be problematic in live sharks due to a thick dermal layer with extensive connective tissue. The aim of this research was to determine whether fin and muscle tissue yield similar FA profiles in three species of tropical euryhaline sharks: *Carcharhinus leucas*, *Glyphis garricki* and *Glyphis glyphis*. Fatty acid profiles were detectable in fin clips as small as 20 mg (~5 mm x 6 mm) and muscle biopsies >10 mg mass. Overall profiles in relative (%) FA composition varied significantly between fin and muscle tissues for *C. leucas* and *G. garricki* (global *R*-values = 0.204 and 0.195, $P < 0.01$), but not *G. glyphis* (global *R*-value = 0.063, $P = 0.257$). The main FAs that contributed to these differences were largely 18:0 for *C. leucas*, 20:4ω6 for *G. garricki* and 20:5ω3 for *G. glyphis*, which reflect the different physiological functions and turnover rates of the two tissues. Notably, no significant differences were detected between tissue types for the major classes of FAs and abundant dietary essential FAs. It was concluded that FA profiles from either fin clips or muscle tissue may be used to examine the trophic ecology of these tropical euryhaline sharks when focusing on dietary essential FAs. Given that some non-essential FAs were different, caution should be applied when comparing FA profiles across different tissue types.

Keywords: elasmobranchs, trophic ecology, *Glyphis*, *Carcharhinus,* biochemical tracers

1. Introduction

Many shark, ray and chimaera species (Class Chondrichthyes) are susceptible to severe population reductions as a result of negative anthropogenic influences such as over-exploitation and habitat destruction, with an estimated 24% of chondrichthyan species considered to be threatened (Dulvy et al., 2014). Reductions in the abundance of apex or meso-predators such as sharks can cause changes in ecosystems through competitive release, resulting in the alteration of fish population dynamics (Stevens et al., 2000). It is important, therefore, to understand the trophic ecology of sharks to evaluate the consequences of reductions in their abundance. Given the rarity and/or threatened status of many shark species, non-lethal and minimally intrusive methods for determining diet are often required.

Prey consumption analyses in sharks have traditionally involved stomach content analyses, which require major intervention (e.g., gastric lavage) or lethal dissection (Barnett et al., 2010; Cortés, 1999). In recent times, less invasive, but still highly informative techniques have been used, such as stable isotopes (e.g., Hussey et al., 2011a; Speed et al., 2011) and lipid and fatty acid (FA) profiling (e.g., Couturier et al., 2013a; Rohner et al., 2013). Fatty acids have been validated in determining the dietary sources of sharks through comparisons with stomach content analysis (Pethybridge et al., 2011a) and *in vivo* (Beckmann et al., 2013). This concept works due to the inability of most high-order predators to synthesize specific FAs, such as

22:5ω3 and 22:6ω3 (Iverson, 2009) that are only found in primary producers or lower order consumers. The detection of such FAs within the tissues of a consumer suggests direct or secondary consumption of specific taxa such as autotrophic algae, diatoms and bacteria (Dalsgaard et al., 2003; Parrish et al., 2013). In addition to dietary information, FA analysis has been used to acquire information on elasmobranch (shark and ray) bioenergetics, life-history and physiology (Beckmann et al., 2014a; Pethybridge et al., 2014, 2011b).

Fatty acids are vital for cell and organelle function in living organisms, especially essential FAs (EFA) that are involved in critical physiological functions (Tocher, 2003). While many FAs can only be assimilated by consumers through their diet, some FAs necessary for physiological and structural functions are produced *de novo* (Tocher, 2003). Given the variety of tissue structure and functionality within multicellular animals, FA profiles can vary among tissue types. For instance, different shark tissues have been found to preferentially store higher saturated fats (SAT) and polyunsaturated fats (PUFA) in structural tissues (e.g., muscle), while higher monounsaturated fats (MUFA) are often found in tissues used for energy storage (e.g., liver, (Pethybridge et al., 2010)). While liver tissue can provide the most temporally sensitive indicator of dietary change in sharks (Beckmann et al., 2014b), it requires lethal sampling. Muscle tissue provides dietary information integrated over longer time periods, but can be problematic to collect in live sharks due to a thick dermal layer with extensive connective tissue (Tilley et al., 2013). Although fin clips are used extensively in shark genetic studies (e.g., Lewallen et al., 2007), and are recognised as a viable tissue for stable isotope analysis

(e.g., Hussey et al., 2011b; Olin et al., 2014), their utility for FA analysis has not yet been determined.

Shark fins consist of cartilage and some connective tissue, muscle and vascularisation, with an outer dermal layer covered with denticles. This composition of various tissue types has the potential to influence the FA profiles of fins versus muscle tissue, given the tissue-based differences reported for stable isotope analysis of $\delta^{13}C$ (Hussey et al., 2010). Here, FA profiles obtained from fin tissue and non-lethal muscle biopsies are examined to determine whether they differ from the same three species of tropical euryhaline elasmobranchs: Bull Shark *Carcharhinus leucas*, Northern River Shark *Glyphis garricki*, and Speartooth Shark *Glyphis glyphis*. River sharks (*Glyphis* species) are globally threatened and rare species (Pillans et al., 2009) with little information available on their biology, including trophic ecology. In doing so, the utility of fin tissue was explored as a non-lethal method for examining FA profiles in future dietary analyses of potentially important apex predators in tropical river ecosystems.

2. Methods

2.1. Ethics statement

This study was conducted with the approval of the Charles Darwin University animal ethics committee (Approval A12016 and A11041) in conjunction with permits from NT Fisheries and Kakadu National Park (Permit RK805).

2.2. Tissue sampling and preparation

Sharks from each of the three target species (Table 1) were captured from the South Alligator River, Kakadu National Park, Australia, between March 2013 and July 2014 using 4 or 6 inch gill nets, or hook and line. Tissues were collected from each temporarily restrained (<5 minutes) individual before they were released back into the water. All sharks were juveniles or sub-adults (Table 1). Muscle tissue biopsies (mean wet weight 0.025 g) were collected from the caudal peduncle using a 3–5 mm biopsy punch (Stiefel, USA), along with a fin clip sample (~15 mm^2 and 0.03 g) from the rear tip of a pectoral fin (Lewallen et al., 2007). Tissue samples were immediately placed in liquid nitrogen (–196°C) for up to 1 week during fieldwork, then transferred to a –20°C freezer. To avoid degradation of the sample from defrosting and refreezing, all frozen muscle samples were dissected in the freezer to remove dermal layers and as much connective tissue as possible to ensure only muscle tissue was sampled. While initial samples were extracted from wet tissue, these samples were freeze-dried for analysis.

2.3. Lipid and fatty acid extraction

Total lipid content was extracted using the modified Bligh and Dyer (1959) method using a one-phase dichloromethane (DCM):Methanol (MeOH):milliQ H_2O solvent mixture (10:20:7.5 mL) which was left overnight. After approximately 12 hours, the solution was broken into two phases by adding 10 mL of DCM and 10 mL of saline milliQ H_2O (9 g sodium chloride (NaCl) L^{-1}) to give a final solvent ratio of 1:1:0.9. The lower layer was drained into a 50 mL round bottom flask and concentrated using a rotary evaporator. The extract was transferred in DCM to a pre-weighed 2 mL glass vial. The solvent

was blown down under a constant stream of nitrogen gas, and the round bottom flask rinsed three times with DCM into the vial. The total lipid extract (TLE) was dried in the vial to constant weight and 200 µl of DCM was added. To release fatty acids from the lipid backbone, 10mg of TLE was added per 1.5 mL of DCM and transmethylated in MeOH:DCM:hydrochloric acid (HCl) (10:1:1 v/v) for 2 hours at 800°C. After cooling, 1.5 mL Milli-Q water was added and FA were extracted three times with 1.8 mL of hexane:DMC (4:1 v/v), after which individual tubes were vortexed and centrifuged at 2000 rpm for 5 mins. After each extraction, the upper organic layer was removed under a nitrogen gas stream. A known concentration of internal injection standard (19:0 FAME or 23:0 FAME) preserved in DCM was added before 0.2 µl of this solution was injected into an Agilent Technologies 7890B gas chromatograph (GC) (Palo Alto, California USA) equipped with an Equity^{TM}-1 fused silica capillary column (15 m x 0.1 mm internal diameter and 0.1 µm film thickness), a flame ionization detector, a splitless injector and an Agilent Technologies 7683B Series auto-sampler. At an oven temperature of 120°C, samples were injected in splitless mode and carried by helium gas. Oven temperature was raised to 270°C at 10°C min^{-1}, and then to 310°C at 5°C min^{-1}. Peaks were quantified using Agilent Technologies ChemStation software (Palo Alto, California USA). Confirmation of peak identifications was by GC-mass spectrometry (GC-MS), using an on-column of similar polarity to that described above and a Finnigan Thermoquest DSQ GC-MS system. Only fin and muscle tissue samples that were above 0.02 g and 0.01 g in mass, respectively, were used in these analyses, as lower sample masses compromised analytical detection.

Total FAs were determined in mg/g and calculated based on the total area of peaks of all FAs divided by the internal standard, times, the mass and volume of internal standard, the mass of the tissue and dilution factors.

2.4. Statistical analyses

Fatty acids were expressed as a percentage of total FAs in the sample, and FAs that accounted for less than 0.5% were excluded from statistical analyses. Paired *t*-tests were used to detect significant differences in the means of the major classes of total FAs (SAT, PUFA, MUFA) and four abundant EFAs within matched pairs of fin and muscle tissues from each individual for each shark species. *t*-tests were carried out on these EFAs to determine the extent of their influence in causing the differences between the tissues. Analysis of similarity (ANOSIM) was then applied to the multivariate FA profiles (31 FAs) obtained from each tissue type in a single factorial design to examine differences in overall FA profiles from the two tissue types. As fin and muscle tissues were extracted from the same individual, a dissimilarity matrix was used based on binomial deviance to accommodate the non-independence of samples (Clarke and Warwick, 2001). Where differences were detected by ANOSIM, similarities of variance (SIMPER) were used to determine the dietary FAs that contributed most to these differences, by indicating the percentage contribution of each FA based on the Euclidian dissimilarity of each pair. All multivariate analyses were performed using PRIMER (v6), while univariate analyses were performed using the base package of R (R Core Development Team, 2014).

3. Results

A total of 65 FAs were identified across the three shark species, with 31 FAs having relative mean values greater than 0.5% (Table 2). These 31 FAs made up 68–97% of total FAs, whereas the mean sum of the remaining 34 minor FAs ranged from 4–8%. Total FA was higher in muscle than fin in all sharks with large standard deviations in *C. leucas* and *G. garricki* whilst *G. glyphis* had less variation (Table 2).

3.1 Intraspecific tissue differences

No significant differences in the proportions of the main FA classes were detected between fin and muscle for these three species, with the exception of MUFA in *C. leucas* where higher amounts were found in muscle (Table 3; Fig.1). For all species, large intraspecific variability (standard deviations [SD]) in the major FA classes was observed in both fin and muscle tissues (Table 2; Fig 1). Standard deviations for most FAs were similar for both muscle and fin for a given species. There were, however, substantial differences in the degree of intraspecific variability in several FAs between muscle and fin. In *C. leucas*, for example, 16:0FALD, 17:0, 18:2ω6, 20:4ω6, 20:1ω5 and 22:4ω6 were more variable in muscle than fins, whilst the opposite was the case for 20:5ω3 and 20:2. In *G. garricki*, *i*17:0, 18:2b, 18:1ω9 were more variable in muscle, while16:0FALD, 17:1 and 18:0FALD were more variable in fins. In *G. glyphis*, 16:0FALD, 17:1, and 22:4ω6 were more variable in muscle, while 18:2b, 20:5ω3 and 24:1ω9 were more variable in fins.

In both the muscle and fin clips of *C. leucas*, the FAs with highest relative amounts were 18:0, 18:1ω9, 16:0, and 20:3ω9, in order of decreasing relative importance (Table 2). In *G. garricki* muscle, the 4 dominant FAs were 18.0, 18:1ω9, 16:0 and 20:4ω6, and although the same FAs were dominant in the fins, the order of importance was different (18:0, 20:4ω6, 18:1ω9 and 16.0; Table 2). For *G. glyphis* muscle and fin, the two dominant FAs were consistently 18:0 followed by 20:4ω6, however, the muscle had higher levels of 18:1ω9 than 16:0; the opposite was true for fins for this species. *t*-tests of the major EFAs (20:4ω6, 22:6ω3, 20:5ω3, 20:3ω9) found in the fins and muscles indicated no significant difference among tissue types, except for 20:4ω6 in *G. garricki* (Table 4, Fig 2).

Multivariate analysis revealed a large amount of overlap in the overall FA profiles obtained from the fins and muscles of each species (Fig. 3). The overall FA profile, however, had significant but weak differences that were detected between fins and muscles for *C. leucas* (global *R*-value = 0.204, $P < 0.01$) and *G. garricki* (global *R*-value = 0.195, $P < 0.01$), but not in *G. glyphis* (global *R*-value = 0.063, $P = 0.257$).

3.2 Interspecific differences

Similar relative amounts of SAT were observed in all three species (range 29.46 to 33.97%), while C*. leucas* had higher amounts of MUFA and lower amounts of PUFA than *G. garricki* and *G. glyphis*. Both *Glyphis* species had less variation in the SD of FAs between fin and muscle tissues than *C. leucas*. There were 11 EFAs that were detected in all species that were >0.5% and 10

EFAs that had minor contributions (<0.5%) for *C. leucas* and *G. glyphis*, and 8 in *G. garricki* (Table 2). Notably, the muscle of *C. leucas* consistently had higher relative amounts of all four EFAs, while in *G. garricki* and *G. glyphis* the relative amounts varied according to the specific EFA (Fig 2).

The FAs contributing to these significant but weak differences in the multivariate analysis varied among species (Table 2; Fig.4). In *C. leucas*, 18:0, 20:3ω9, 18:1ω9 and 16:0 contributed to 58% of the differences between fin and muscle, whereas in *G. garricki*, 56% of the differences were due to 20:4ω6, 18:1ω9, 20:3ω9 and 22:6ω9. The FAs contributing 60% of the difference between tissue types in *G. glyphis* were all EFAs, as well as 20:5ω3, 20:4ω6 and 22:4ω6. Fatty acids that appeared to be in similar amounts among tissue types were 16:0, 18:0, 20:0, 19:1, 20:1ω9, 20:1ω5, 20:3ω6 and 24:1ω9. There was considerable variation amongst individuals as shown by the large standard deviations for 20:5ω3 in *G. glyphis* (fin and muscle) and *C. leucas* (muscle), 20:4ω6 and 22:4ω6 in the fin of *C. leucas*, and 20:3ω9 in both tissue types in *G. garricki.* The mean ratio of ω3/ω6 FAs was higher in the muscle compared to the fins of all species.

4. Discussion

Overall FA profiles did appear to differ according to tissue type within the two shark species *C. leucas* and *G. garricki,* but not *G. glyphis*, which suggests caution must be applied when selecting which tissue type to use for future dietary studies in these and other chondrichthyan species. Sample size for *G. glyphis* was low which may partially account for the differences between the

species, however this species was included due to its rarity (Pillans et al., 2009). Differences in the overall FA profiles among tissue types were expected and are likely due to functional and dietary differences of certain FAs and their affiliation with different structural tissue types, which can be difficult to separate. Most of these differences in fin and muscle tissue were due to non-essential FAs and there were some important similarities that were apparent among the two tissue types in terms of key FAs. This included important EFAs, which suggests that the potentially less intrusive use of fin tissues may be effective for future studies wishing to explore dominant trophic patterns in these tropical euryhaline sharks.

Similarity in the proportions of major classes of FAs among tissues types and species suggest they are most likely involved with structural or physiological functions common to tropical sharks. Conversely, FAs in higher quantities in either the muscle or fin (e.g., 17:0, 22:4ω6 and 20:3ω9) could be linked to specific structures, physiology or functions (e.g., locomotion) of those tissues (Pethybridge et al., 2010) or indicate temporal differences in diet (discussed below). Notably, our study species' muscle tissues were dominated by PUFA, as has been found in the Port Jackson Shark *Heterodontus portusjacksoni* (Beckmann et al., 2014b) and deep water shark species (Pethybridge et al., 2010). Polyunsaturated FAs also dominates in the sub-dermal tissue of the Reef Manta Ray *Manta alfredi* and the Whale Shark *Rhincodon typus* (Couturier et al., 2013b) and, typically in the muscle tissues of teleost fish (Belling et al., 1997; Økland et al., 2005). In contrast, shark liver tissue, which

has been shown to be more representative of diet (Beckmann et al., 2014b), is typically dominated by energy-rich MUFA.

Using signature FA analysis to better understand a species' trophic ecology should take into account known trophic markers and EFA, particularly if they show highly variable patterns among tissues types. Commonly used estuarine-based trophic markers, detected in this study that were variable between fin clips and muscle, included those produced by bacteria (17:0, *i*17:0), diatoms, algae, mangroves and terrestrial plants (18:2ω6, 20:4ω3, 20:4ω6, and 20:5ω3), and dinoflagellates (22:6ω3; (Alfaro et al., 2006; Kelly and Scheibling, 2012; Sargent et al., 1989)). Many other FAs are considered to be trophic markers for particular taxon or trophic groups and were also variable between the fin clips and muscles. For example, 18:1ω7 is characteristic of bacteria (Kelly and Scheibling, 2012), 20:1ω9, 20:1ω11 and 22:1ω11 of copepods (Falk-Petersen et al., 2002; Kelly and Scheibling, 2012), 16:1ω7 of diatoms and mangrove (Kelly and Scheibling, 2012; St. John and Lund, 1996), and 22:0 and 24:0 of mangrove and terrestrial plants (Joseph et al., 2012; Rossi et al., 2008). That these particular FAs were variable between the tissue types indicates tissue differences, however the fact that these known markers were found in the fins supports their utility for dietary studies.

Determining the importance of FA profile differences between fins and muscle for dietary analysis requires differentiation between FAs that are assimilated from an individual's diet (such as EFAs) from those produced *de novo* (Tocher, 2003). Essential FA profiles found in muscle and fin tissue of these

tropical shark species were dominated by the ω6 FAs, which are formed through the linoleic pathway. In this pathway, 20:4ω6 is elongated to 22:4ω6 (Tocher, 2010) and as there are only small amounts of precursors to 20:4ω6 it is likely that it has been accumulated by diet. Importantly, the differences between tissues in 20:4ω6 and 22:4ω6 were proportional across tissues within species, suggesting similar processes are occurring in the fin and muscle. These processes may be occurring at different rates since 20:4ω6 in *G. garricki* was the only significantly different EFA in univariate analysis. As only one EFA differed the combination of non-essential FAs may be more important in influencing differences than individual EFAs. Therefore the lack of significant differences between most fin and muscle EFAs, the low *r* values in the ANOSIM and that similar processes are likely occurring in fin and muscle suggests that both tissue types are appropriate for trophic studies.

Variation in a range of FAs among tissue types can indicate variable uptake of particular tissues over time. For example, the EFA 20:3ω9 was a major contributor to differences between fin and muscle in both *C. leucas* and *G. garricki*. This unusual FA has also been detected in some *C. leucas* in the Florida Everglades and, along with other ω6 and ω3 PUFA were linked to deficiency in EFA in these sharks (Belicka et al., 2012). It was also found that 18:1ω9 contributed to the dissimilarity of fin and muscle FA profiles in *C. leucas* and *G. garricki*. Present in high relative levels in a range of organisms, this FA can often be an indication of carnivory (Falk-Petersen et al., 2002; Kelly and Scheibling, 2012).

The fins in all species did accumulate FAs that are linked to diet and many of the FAs, particularly the EFAs, varied between the fin clips and muscle in similar ways. This suggests that the same processes are occurring in both tissues. Differences in the FA profiles of various elasmobranch tissues is now becoming well established (Beckmann et al., 2013; Pethybridge et al., 2010), with the first controlled experiments indicating the uptake of FA can vary considerably across shark muscle, liver and blood serum (Beckmann et al., 2014b).

Saturated FAs (SFA), such as 16:0 and 18:0, also contributed to differences between fins and muscle in *C. leucas* (and to some extent *G. garricki*), which is interesting because these SFA are ubiquitous in animals and variations are expected among tissue types according to rates of cellular metabolism (Tocher, 2003). Most fin tissue is cartilage, and so would be expected to have slower metabolism and tissue turnover rates than muscle (Malpica-cruz et al., 2012). Certainly, studies measuring stable isotopes have found that cartilage and fin have a slower turnover rate than muscle and blood (MacNeil et al., 2006; Malpica-cruz et al., 2012). It is therefore likely that the FA profiles of fins are representing another time period in the diet and habitat usage of these sharks. Such variances in FA profiles among fins and muscle could be particularly useful in providing scientists with key insights into the trophic ecology of species occupying dynamic tropical river environments that experience a monsoonal wet–dry cycle (Warfe et al., 2011).

This study found highly variable amounts of total FA in the muscle and fin both within and between species emphasising the importance of adequate sample sizes. Researchers could maximise the utility of such tissue samples in rare/threatened species, especially when sampling adults with larger shark fins, as some of the muscular tissue layers could be dissected and used to obtain stable isotope evidence (Hussey et al., 2011a). Moreover, comparisons could be made between muscle tissue profiles and connective tissue/cartilage profiles to explore temporal differences.

Apart from intraspecific differences across FA profiles there were also interspecific differences such as the variation in 20:4ω6 across species. These differences may be indicative of dietary and perhaps environmental change as ω6 have been identified as environmental indicators of temperature and increases in the relative amounts of the FA, 20:4ω6, and dominance of ω6 pathways have been linked to tropical waters (Couturier et al., 2013b; Sinclair et al., 1986). Furthermore, experimental work with seals and salmon found 18:1ω9 was assimilated into muscle and adipose fins directly from their diet (Budge et al., 2004; Skonberg et al., 1994). Therefore the differences in the amount of 18:1ω9 in these shark species may suggest separation between their trophic levels. Since more 18:1ω9 was found in the muscle than the fin, this could indicate an increase in consumption of higher order consumers with age. It could, however, also be due to *de novo* synthesised 14:0 and 16:0 (Dalsgaard et al., 2003).

Ontogeny, sex-based physiology and different movement patterns can all be reflected in FA profiles of different tissues (Belicka et al., 2012; Parrish et al., 2013). All the sharks studied here were juvenile to sub-adult individuals and as such were not sexually mature, with some individuals showing open umbilical scars indicating they were neonates, which implies a short period of active feeding. Consequently, it is highly likely that the fins of some small individuals (e.g. <100 cm total length) may be reflecting a stronger maternal signature than muscle tissue, due to differences in metabolism and structural turnover among the two tissues types (Belicka et al., 2012). Such effects may also explain some of the high degree of variation found within species, as these sharks were not only sampled from different stages of ontogeny, but also across a range of seasons (Sargent et al., 1999; Tocher, 2010). While it is difficult to obtain a fully replicated stratified sample of tissues among a range of developmental stages and body sizes in rare and/or difficult to sample animals, the potential for ontogenetic and sex-based influences upon FA profiles should be considered in future studies, where possible.

5. Conclusions

An understanding of differences in FA profiles obtained from different tissue types is important when utilizing FAs to elucidate the trophic ecology of higher order consumers such as sharks. Fatty acid profiles in the fins and muscles reflected FAs, which have previously been used as biomarkers in trophic studies of marine predators (Dalsgaard et al., 2003; Kelly and Scheibling, 2012). Similar proportions of dominant FAs, particularly EFAs, were found to occur among the muscle and fin tissues from these tropical euryhaline shark

species, along with some strong similarities between the two *Glyphis* species (which potentially could be explained by their genetic similarity (Wynen et al., 2009)). Collectively, this suggests comparable assimilation and usage processes may be occurring in both tissue types for these major FAs. Whilst muscle and fins are not directly interchangeable in dietary analyses, both tissue types have measurable quantities of dietary EFAs in the FA profiles of both tissues, suggesting that diet is being reflected and should have utility in future shark trophic studies.

Slight differences in the proportion of some EFAs within the different tissue types can provide key opportunities (e.g., temporal hindcasting of seasonal prey consumption), but also signal caution in applying these analyses to understanding patterns of diet. As fins consist of multiple tissues, each tissue type may have slightly different proportions of FAs dependent on the physiological needs of that tissue as compared to muscle where only one tissue type is present. Temporal variations in habitat usage and ontogeny will be reflected at different time scales of tissues due to turnover rates of FA that are not yet well understood. A priority for future research should be exploring links between FA profiles in these tissues and rates of assimilation in the various chondrichthyan tissues, to provide opportunities for temporal exploration of diet. Where possible, this should also include investigation of potential prey sources in controlled settings to validate the dietary links and examine FA synthesis pathways. What is clear is the need for further work on elucidating fine scale differences between tissues in order to determine the suitability of tissue FA analysis for dietary studies.

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References

Alfaro, A.C., Thomas, F., Sergent, L., Duxbury, M., 2006. Identification of trophic interactions within an estuarine food web (northern New Zealand) using fatty acid biomarkers and stable isotopes. Estuar. Coast. Shelf Sci. 70, 271–286. doi:10.1016/j.ecss.2006.06.017

Barnett, A., Redd, K.S., Frusher, S.D., Stevens, J.D., Semmens, J.M., 2010. Non-lethal method to obtain stomach samples from a large marine predator and the use of DNA analysis to improve dietary information. J. Exp. Mar. Bio. Ecol. 393, 188–192. doi:10.1016/j.jembe.2010.07.022

Beckmann, C.L., Mitchell, J.G., Seuront, L., Stone, D.A.J., Huveneers, C., 2014a. From egg to hatchling: preferential retention of fatty acid

biomarkers in young-of-the-year Port Jackson sharks *Heterodontus portusjacksoni*. J. Fish Biol. 85, 944–952. doi:10.1111/jfb.12451

Beckmann, C.L., Mitchell, J.G., Seuront, L., Stone, D.A.J., Huveneers, C., 2013. Experimental evaluation of fatty acid profiles as a technique to determine dietary composition in benthic elasmobranchs. Physiol. Biochem. Zool. 86, 266–278. doi:10.1086/669539

Beckmann, C.L., Mitchell, J.G., Stone, D.A.J., Huveneers, C., 2014b. Inter-tissue differences in fatty acid incorporation as a result of dietary oil manipulation in Port Jackson sharks (*Heterodontus portusjacksoni*). Lipids 49, 577–590. doi:10.1007/s11745-014-3887-6

Belicka, L., Matich, P., Jaffé, R., Heithaus, M., 2012. Fatty acids and stable isotopes as indicators of early-life feeding and potential maternal resource dependency in the Bull Shark *Carcharhinus leucas*. Mar. Ecol. Prog. Ser. 455, 245–256. doi:10.3354/meps09674

Belling, G.B., Abbey, M., Campbell, J.H., Campbell, G.R., 1997. Lipid content and fatty acid composition of 11 species of Queensiand (Australia) fish. Lipids 32, 621–625. doi:10.1007/s11745-997-0079-z

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol. 37, 911–917. doi:10.1139/o59-099

Budge, S.M., Cooper, M.H., Iverson, S.J., 2004. Demonstration of the deposition and modification of dietary fatty acids in pinniped blubber

using radiolabelled precursors. Physiol. Biochem. Zool. 77, 682–687. doi:10.1086/420945

Clarke, K., Warwick, R., 2001. Change in marine communities: An approach to statistical analysis and interpretation, 2nd ed. PRIMER-E, Plymouth.

Cortés, E., 1999. Standardized diet compositions and trophic levels of sharks. ICES J. Mar. Sci. 56, 707–717. doi:10.1006/jmsc.1999.0489

Couturier, L.I.E., Rohner, C.A., Richardson, A.J., Marshall, A.D., Jaine, F.R.A., Bennett, M.B., Townsend, K.A., Weeks, S.J., Nichols, P.D., 2013a. Stable isotope and signature fatty acid analyses suggest reef manta rays feed on demersal zooplankton. PLoS One 8, e77152. doi:10.1371/journal.pone.0077152

Couturier, L.I.E., Rohner, C.A., Richardson, A.J., Pierce, S.J., Marshall, A.D., Jaine, F.R.A., Townsend, K.A., Bennett, M.B., Weeks, S.J., Nichols, P.D., 2013b. Unusually high levels of n-6 polyunsaturated fatty acids in whale sharks and reef manta rays. Lipids 48, 1029–1034. doi:10.1007/s11745-013-3829-8

Dalsgaard, J., St. John, M.A., Kattner, G., Müller-Navarra, D., Hagen, W., 2003. Fatty acid trophic markers in the pelagic marine environment. Adv. Mar. Biol. 46, 225–340. doi:10.1016/S0065-2881(03)46005-7

Dulvy, N.K., Fowler, S.L., Musick, J.A., Cavanagh, R.D., Kyne, P.M., Harrison, L.R., Carlson, J.K., Davidson, L.N., Fordham, S. V, Francis, M.P., Pollock, C.M., Simpfendorfer, C.A., Burgess, G.H., Carpenter, K.E.,

Compagno, L.J.V, Ebert, D.A, Gibson, C., Heupel, M.R., Livingstone, S.R., Sanciangco, J.C., Stevens, J.D., Valenti, S., White, W.T., 2014. Extinction risk and conservation of the world's sharks and rays. eLife 3, e00590. doi:10.7554/eLife.00590

Falk-Petersen, S., Dahl, T.M., Scott, C.L., Sargent, J.R., Gulliksen, B., Kwasniewski, S., Hop, H., Millar, R.M., 2002. Lipid biomarkers and trophic linkages between ctenophores and copepods in Svalbard waters. Mar. Ecol. Prog. Ser. 227, 187–194. doi:10.3354/meps227187

Hussey, N.E., Brush, J., McCarthy, I.D., Fisk, A.T., 2010. δ15N and δ13C diet-tissue discrimination factors for large sharks under semi-controlled conditions. Comp. Biochem. Physiol. A. Mol. Integr. Physiol. 155, 445–453. doi:10.1016/j.cbpa.2009.09.023

Hussey, N.E., Chapman, D.D., Donnelly, E., Abercrombie, D.L., Fisk, A.T., 2011a. Fin-icky samples: an assessment of shark fin as a source material for stable isotope analysis. Limnol. Oceanogr. Methods 9, 524–532. doi:10.4319/lom.2011.9.524

Hussey, N.E., Dudley, S.F.J., Mccarthy, I.D., Cliff, G., Fisk, A.T., 2011b. Stable isotope profiles of large marine predators: viable indicators of trophic position, diet, and movement in sharks? Can. J. Fish. Aquat. Sci. 68, 2029–2045. doi:10.1139/F2011-115

Iverson, S.J., 2009. Tracing aquatic food webs using fatty acids: from qualitative indicators to quantitative determination lipids in aquatic ecosystems, in: Kainz, M., Brett, M.T., Arts, M.T. (Eds.), Lipids in Aquatic

Ecosystems. Springer New York, New York, NY, pp. 281–307. doi:10.1007/978-0-387-89366-2

Joseph, M.M., Renjith, K.R., Ratheesh Kumar, C.S., Chandramohanakumar, N., 2012. Assessment of organic matter sources in the tropical mangrove ecosystems of cochin, southwest India. Environ. Forensics 13, 262–271. doi:10.1080/15275922.2012.676600

Kelly, J., Scheibling, R., 2012. Fatty acids as dietary tracers in benthic food webs. Mar. Ecol. Prog. Ser. 446, 1–22. doi:10.3354/meps09559

Lewallen, E.A., Anderson, T.W., Bohonak, A.J., 2007. Genetic structure of leopard shark (*Triakis semifasciata*) populations in California waters. Mar. Biol. 152, 599–609. doi:10.1007/s00227-007-0714-0

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.

Malpica-cruz, L., Herzka, S.Z., Sosa-nishizaki, O., Lazo, J.P., 2012. Tissue-specific isotope trophic discrimination factors and turnover rates in a marine elasmobranch: empirical and modeling results. Can. J. Fish. Aquat. Sci. 69, 551–564. doi:10.1139/F2011-172

Økland, H.M.W., Stoknes, I.S., Remme, J.F., Kjerstad, M., Synnes, M., 2005. Proximate composition, fatty acid and lipid class composition of the muscle from deep-sea teleosts and elasmobranchs. Comp. Biochem.

555 Physiol. B. Biochem. Mol. Biol. 140, 437–443.
556 doi:10.1016/j.cbpc.2004.11.008

557 Olin, J.A., Poulakis, G.R., Stevens, P.W., DeAngelo, J.A., Fisk, A.T., 2014.
558 Preservation effects on stable isotope values of archived elasmobranch
559 fin tissue: comparisons between frozen and ethanol-stored samples.
560 Trans. Am. Fish. Soc. 143, 1569–1576.
561 doi:10.1080/00028487.2014.954055

562 Parrish, C.C., Nichols, P.D., Pethybridge, H.R., Young, J.W., 2015. Direct
563 determination of fatty acids in fish tissues: quantifying top predator trophic
564 connections. Oecologia 177, 85–95. doi:10.1007/s00442-014-3131-3

565 Parrish, C.C., Pethybridge, H.R., Young, J.W., Nichols, P.D., 2013. Spatial
566 variation in fatty acid trophic markers in albacore tuna from the
567 southwestern Pacific Ocean—A potential “tropicalization” signal. Deep
568 Sea Res. Part II Top. Stud. Oceanogr. 1–9.
569 doi:10.1016/j.dsr2.2013.12.003

570 Pethybridge, H.R., Daley, R., Virtue, P., Nichols, P.D., 2010. Lipid
571 composition and partitioning of deepwater chondrichthyans: inferences of
572 feeding ecology and distribution. Mar. Biol. 157, 1367–1384.
573 doi:10.1007/s00227-010-1416-6

574 Pethybridge, H.R., Daley, R.K., Nichols, P.D., 2011a. Diet of demersal sharks
575 and chimaeras inferred by fatty acid profiles and stomach content
576 analysis. J. Exp. Mar. Bio. Ecol. 409, 290–299.
577 doi:10.1016/j.jembe.2011.09.009

Pethybridge, H.R., Daley, R.K., Virtue, P., Nichols, P.D., 2011b. Lipid (energy) reserves, utilisation and provisioning during oocyte maturation and early embryonic development of deepwater chondrichthyans. Mar. Biol. 158, 2741–2754. doi:10.1007/s00227-011-1773-9

Pethybridge, H.R., Parrish, C.C., Bruce, B.D., Young, J.W., Nichols, P.D., 2014. Lipid, fatty acid and energy density profiles of White Sharks: insights into the feeding ecology and ecophysiology of a complex top predator. PLoS One 9, e97877. doi:10.1371/journal.pone.0097877

Pillans, R.D., Stevens, J.D., Kyne, P.M., Salini, J.P., 2009. Observations on the distribution, biology, short-term movements and habitat requirements of river sharks *Glyphis* spp. in northern Australia. Endanger. Species Res. 10, 321–332. doi:10.3354/esr00206

Rohner, C.A., Couturier, L.I.E., Richardson, A.J., Pierce, S.J., Prebble, C.E.M., Gibbons, M.J., Nichols, P.D., 2013. Diet of Whale Sharks *Rhincodon typus* inferred from stomach content and signature fatty acid analyses. Mar. Ecol. Prog. Ser. 493, 219–235. doi:10.3354/meps10500

Rossi, S., Youngbluth, M.J., Jacoby, C.A., Pages, F., Garrofe, X., 2008. Fatty acid trophic markers and trophic links among seston, crustacean zooplankton and the siphonophore *Nanomia cara* in Georges Basin and Oceanographer Canyon (NW Atlantic). Sci. Mar. 72, 403–416.

Sargent, J., McEvoy, L., Estevez, A., Bell, G., Bell, M., Henderson, J., Tocher, D., 1999. Lipid nutrition of marine fish during early development: current

status and future directions. Aquaculture 179, 217–229. doi:10.1016/S0044-8486(99)00191-X

Sargent, J.R., Henderson, J.R., Tocher, D.R., 1989. The Lipids, in: Halver, J.E. (Ed.), Fish Nutrition. Academic Press, San Diego, pp. 153–218.

Sinclair, A.J., O'Dea, K., Naughton, J.M., 1986. Polyunsaturated fatty acid types in Australian fish. Prog. Lipid Res. 25, 81–82. doi:10.1016/0163-7827(86)90016-0

Skonberg, D.I., Rasco, B.A., Dong, F.M., 1994. Fatty acid composition of salmonid muscle changes in response to a high oleic acid diet. J. Nutr. 124, 1628–1638.

Speed, C.W., Meekan, M.G., Field, I.C., McMahon, C.R., Abrantes, K., Bradshaw, C.J.A., 2011. Trophic ecology of reef sharks determined using stable isotopes and telemetry. Coral Reefs 31, 357–367. doi:10.1007/s00338-011-0850-3

St. John, M.A., Lund, T., 1996. Lipid biomarkers: linking the utilization of frontal plankton biomass to enhanced condition of juvenile North Sea cod. Mar. Ecol. Prog. Ser. 131, 75–85. doi:10.3354/meps131075

Stevens, J.D., Bonfil, R., Dulvy, N.K., Walker, P.A. 2000. The effects of fishing on sharks, rays and chimaeras (chondrichthyans), and the implications for marine ecosystems. ICES J. Mar. Sci. 57, 476–494.

R Core Development Team, 2014. R: A Language and Environment for Statistical Computing.

Tilley, A., López-Angarita, J., Turner, J.R., 2013. Diet reconstruction and resource partitioning of a Caribbean marine mesopredator using stable isotope bayesian modelling. PLoS One 8, e79560. doi:10.1371/journal.pone.0079560.

Tocher, D.R., 2010. Fatty acid requirements in ontogeny of marine and freshwater fish. Aquac. Res. 41, 717–732. doi:10.1111/j.1365-2109.2008.02150.x

Tocher, D.R., 2003. Metabolism and functions of lipids and fatty acids in teleost fish. Rev. Fish. Sci. 11, 107–184. doi:10.1080/713610925

Warfe, D.M., Pettit, N.E., Davies, P.M., Pusey, B.J., Hamilton, S.K., Kennard, M.J., Townsend, S.A., Bayliss, P., Ward, D.P., Douglas, M.M., Burford, M.A., Finn, M., Bunn, S.E., Halliday, I.A., 2011. The “wet-dry” in the wet-dry tropics drives river ecosystem structure and processes in northern Australia. Freshw. Biol. 56, 2169–2195. doi:10.1111/j.1365–2427.2011.02660.x

Wynen, L., Larson, H.K., Thorburn, D.C., Peverell, S.C., Morgan, D.L., Field, I.C., Gibb, K., 2009. Mitochondrial DNA supports the identification of two endangered river sharks (*Glyphis glyphis* and *Glyphis garricki*) across northern Australia. Mar. Freshw. Res. 60, 554–562. doi:10.1071/MF08201

Table 1. Number and total length (TL) of specimens from which samples of fin and muscle tissue were taken for fatty acid analysis in three shark species from the South Alligator River, Australia (Size range +/- SD) .

Species	***n***	**Min TL (cm)**	**Max TL (cm)**	**Mean TL (cm)**	**Sex ratio M:F**
Carcharhinus leucas	17	74.5	82.5	78.49±3.48	8:9
Glyphis garricki	11	75.5	140.5	96.45±19.60	7:4
Glyphis glyphis	4	71.0	85.0	76.80±6.25	1:3

Table 2. Comparisons of the relative abundance of fatty acids (FA) (mean % ± standard deviation) between fin and muscle tissue in *Carcharhinus leucas*, *Glyphis garricki* and *G. glyphis*, from the South Alligator River, Australia.

	C. leucas		***G. garricki***		***G. glyphis***	
	Muscle	**Fin**	**Muscle**	**Fin**	**Muscle**	**Fin**
16:0	10.63 ±5.12	10.00 ±4.92	11.13 ±2.68	10.24 ±2.99	9.54 ±1.80	11.29 ±3.63
17:0	0.51 ±0.44	0.91 ±0.35	0.71 ±0.12	1.13 ±0.39	0.80 ±0.17	1.14 ±0.31
18:0	17.94 ±5.54	19.85 ±5.69	17.51 ±4.04	17.18 ±2.63	17.64 ±1.93	17.01 ±2.55
20:0	0.63 ±0.64	0.59 ±0.26	1.30 ±2.81	1.01 ±2.02	0.32 ±0.03	0.32 ±0.09
22:0	0.51 ±0.37	1.43 ±2.14	2.08 ±3.74	0.81 ±0.63	0.59 ±0.16	0.67 ±0.21
24:0	0.42 ±0.28	1.17 ±0.63	0.30 ±0.08	0.74 ±0.29	0.54 ±0.19	0.78 ±0.3
15:1	1.35 ±1.33	0.96 ±0.81	2.30 ±1.53	0.94 ±0.61	1.42 ±0.56	0.57 ±0.3

16:1ω7	1.89 ±1.44	1.53 ±1.18	0.94 ±0.40	1.03 ±0.51	0.90 ±0.28	0.93 ±0.36
17:1	1.12 ±0.77	2.59 ±1.48	1.10 ±0.32	3.04 ±1.86	2.66 ±1.95	2.64 ±1.26
18:1ω9	16.50 ±6.35	14.52 ±3.43	12.19 ±5.51	10.97 ±2.49	10.35 ±1.7	10.34 ±0.92
18:1ω7	5.36 ±2.49	3.71 ±1.37	5.53 ±1.72	3.64 ±1.54	5.47 ±0.84	3.79 ±0.53
17:1ω6	0.51 ±0.19	1.00 ±0.46	0.64 ±0.25	0.65 ±0.18	0.83 ±0.40	0.74 ±0.20
19:1	0.41 ±0.19	0.67 ±0.25	0.35 ±0.12	0.34 ±0.08	0.30 ±0.23	0.41 ± 07
20:1ω9	1.21 ±0.66	0.83 ±0.35	0.86 ±0.60	0.78 ±0.66	0.57 ±0.26	0.68 ±0.20
20:1ω5	0.54 ±1.08	0.48 ±0.37	0.18 ±0.14	0.17 ±0.07	0.16 ±0.03	0.24 ±0.19
22:1ω11	2.13 ±5.76	0.25 ±0.51	0.19 ±0.24	0.18 ±0.16	0.12 ±0.40	0.07 ±0.02
24:1ω9	0.83 ±0.43	0.64 ±0.32	0.57 ±0.23	0.65 ±0.25	0.96 ±0.66	1.02 ±0.25
18:2b□	0.62 ±0.33	2.60 ±0.98	0.28 ±0.21	1.38 ±0.53	0.49 ±0.13	1.32 ±0.69
18:2c□	0.97 ±0.74	0.51 ±0.58	0.13 ±0.32	0.31 ±0.41	0.12 ±0.09	0.14 ±0.02
18:2ω6	0.55 ±0.91	0.56 ±0.55	2.34 ±1.14	1.83 ±0.68	2.36 ±0.97	1.58 ±0.38
20:2	3.02 ±2.23	1.00 ±1.29	0.55 ±1.02	0.56 ±1.12	0.29 ±0.15	0.21 ±0.07
20:2ω6	0.59 ±0.87	0.27 ±0.5	0.76 ±0.28	0.42 ±0.16	0.83 ±0.23	0.51 ±0.22
20:3ω9[#]	8.36 ±6.41	7.91 ±4.43	1.80 ±4.70	1.52 ±3.66	0.35 ±0.18	0.29 ±0.10
20:3ω6	0.32 ±6.41	0.28 ±0.25	0.67 ±0.33	0.56 ±0.20	0.55 ±0.26	0.65 ±0.37
22:3	0.94 ±0.79	0.82 ±0.71	1.33 ±1.13	2.29 ±1.17	2.57 ±1.37	1.45 ±0.85
20:4ω6	3.18 ±4.38	5.66 ±3.37	10.47 ±4.68	15.46 ±5.46	14.76 ±3.96	12.43 ±4.74
22:4ω6	1.51 ±2.09	2.50 ±1.26	4.44 ±1.85	6.07 ±2.48	3.91 ±3.00	6.96 ±2.28
20:5ω3	0.52 ±0.20	1.30 ±3.27	0.89 ±0.71	0.97 ±0.75	0.94 ±0.32	4.74 ±7.22
22:5ω3	1.91 ±1.40	3.01 ±2.12	0.80 ±1.27	0.34 ±0.72	1.47 ±1.92	0.24 ±0.42
22:5ω6	0.89 ±0.61	1.01 ±0.46	1.87 ±0.67	1.52 ±0.55	1.76 ±0.15	1.97 ±1.11
22:6ω3	4.25 ±2.39	2.22 ±1.37	7.38 ±3.82	4.10 ±1.90	7.97 ±2.99	4.32 ±2.28
ΣFAs <0.5%*	8.38 ±8.74	5.04 ±1.67	4.13 ±1.84	5.31 ±1.22	5.03 ±1.25	4.99 ±1.37
ΣSAT	30.66 ±8.75	33.97 ±17.81	33.05 ±7.54	31.14 ±6.18	29.46 ±3.16	31.23 ±7.56
ΣMUFA	32.01 ±6.71	27.26 ±5.15	24.55 ±7.99	22.09 ±4.31	23.18 ±4.44	20.74 ±1.17

ΣPUFA	27.60 ±12.20	29.7 ±7.89	33.63 ±8.32	37.08 ±5.33	37.66 ±6.72	36.20 ±9.15
ω3/ω6	0.99 ±0.45	0.65 ±1.20	0.44 ±0.65	0.23 ±0.33	0.43 ±0.61	0.38 ±1.08
***i*17:0**	0.71 ±0.34	1.95 ±1.08	1.78 ±4.06	0.75 ±0.73	0.87 ±0.31	0.97 ±0.44
16:0FALD	0.61 ±0.64	0.57 ±0.34	1.46 ±1.01	1.25 ±0.62	1.44 ±1.43	1.64 ±1.12
18:0FALD	0.88 ±0.40	1.49 ±1.02	0.86 ±0.46	1.74 ±1.80	0.98 ±0.58	2.82 ±0.99
TFAs (mg/g)	2.56 ±4.16	1.76 ±1.35	4.21 ±5.95	3.66 ±03.71	1.06 ±0.23	0.56 ±0.37

FAs <0.5% include 14:0 15:0, a15:0, 15:0, 14:1, 16:1ω13, 16:1ω9, 16:1ω7, 16:1ω5, 17:1ω8+a17:0, 18:1ω7, 18:1ω5, 18:1, 19:1, 20:1ω7, 20:1ω11, 20:1ω5, 22:1ω9, 22:1ω7, 24:1ω11, 24:1ω7, 16:4+16:3, 18:2a^{v}, 18:4ω3, 18:3ω6, 18:3ω3, 20:4ω3/20:2, 21:5ω3, 21:3, 22:2a^{v}, 22:2b^{v}, *i*16:0, 18:1FALD

20:3ω9 identified based on comparison with other *C. leucas* fatty acid literature; a standard was not available at the time of analyses. ^{v} = unable to identify bonds as standard was not available at the time of analyses. FA - Fatty acids, TFA – total fatty acids, SAT- saturated fatty acids, MUFA - monounsaturated fatty acids, PUFA - polyunsaturated fatty acids. FALD – fatty aldehyde analyzed as dimethyl acetal.

Table 3. Paired *t*-tests comparing the concentrations of three major fatty acid (FA) classes detected within the fin and muscle tissues from each of three euryhaline shark species, *Carcharhinus leucas*, *Glyphis garricki*, and *G. glyphis*, from the South Alligator River, Australia. Significant (*P*<0.05) result shown in bold.

Major FA Class	Species	t score	df	p-Value
Saturated	*C. leucas*	-1.595	16	0.130
	G. garricki	0.649	10	0.531

	G. glyphis	-0.775	3	0.494
Monounsaturated	*C. leucas*	2.279	16	**0.037**
	G. garricki	1.237	10	0.244
	G. glyphis	1.429	3	0.249
Polyunsaturated	*C. leucas*	-0.785	16	0.444
	G. garricki	-1.541	10	0.154
	G. glyphis	-0.990	3	0.395

Table 4. Paired *t*-tests comparing the concentrations of four essential fatty acids detected within the fin and muscle tissues from each of three euryhaline shark species, *Carcharhinus leucas*, *Glyphis garricki*, and *G. glyphis*, from the South Alligator River, Australia. Significant ($P<0.05$) result shown in bold.

Abundant EFA	Species	t score	df	p-Value
20:5ω3	*C. leucas*	0.97	16	0.34
	G. garricki	0.34	10	0.74

	G. glyphis	0.89	3	0.44
20:4ω6	*C. leucas*	2.02	16	0.06
	G. garricki	2.29	10	**0.04**
	G. glyphis	-0.58	3	0.59
22:4ω6	*C. leucas*	1.58	16	0.13
	G. garricki	1.74	10	0.11
	G. glyphis	1.20	3	0.31
20:3ω9	*C. leucas*	-0.31	16	0.76
	G. garricki	-0.14	10	0.89
	G. glyphis	-0.37	3	0.74

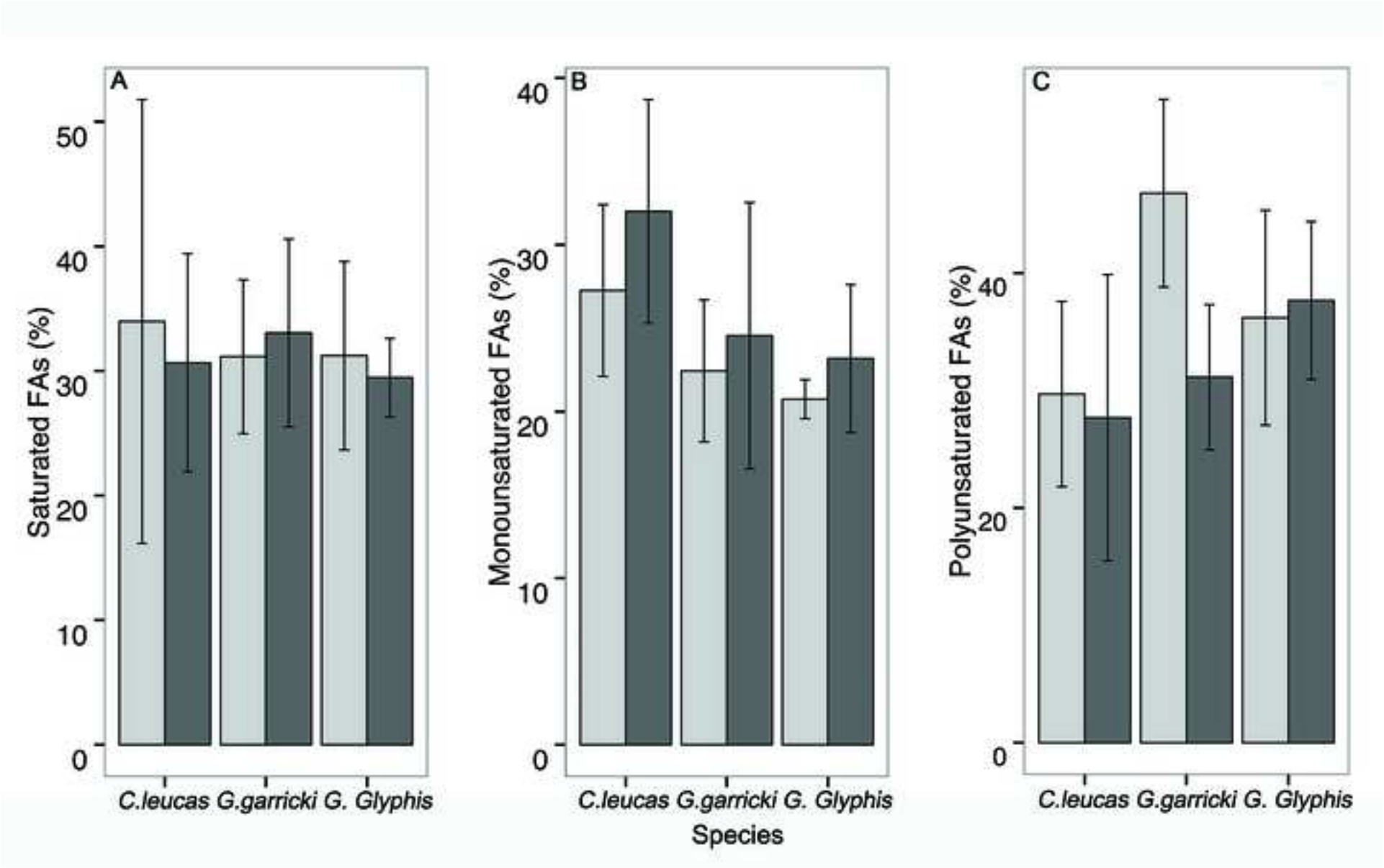
Figure legends

Fig. 1. Comparison of the relative means (± standard deviation) of (a) saturated, (b) monounsaturated, and (c) polyunsaturated fatty acid profiles based on fin and muscle tissues taken from three shark species (*Carcharhinus leucas, Glyphis garricki* and *G. glyphis*) from the South Alligator River, Kakadu National Park, Australia.

Fig. 2. Comparison of the fatty acid (a) 20:5ω3, (b) 20:4ω6, (c) 22:4ω6 and (d) 20:3ω6 (%) relative means (± standard deviation) within fin and muscle tissues taken from three shark species (*Carcharhinus leucas, Glyphis garricki* and *G. glyphis*) from the South Alligator River, Kakadu National Park, Australia.

Fig. 3. Ordination (nMDS) of fatty acid profiles from the fin and muscle tissues of the three shark species (a) *Carcharhinus leucas,* (b) *Glyphis garricki*, (c) *G. glyphis* from the South Alligator River, Kakadu National Park, Australia.

Fig. 4. % Contribution of fatty acids that caused the main differences between fin and muscle profiles from SIMPER analysis in (a) *Carcharhinus leucas* (b) *Glyphis garricki* and (c) *G. glyphis* from the South Alligator River, Kakadu National Park, Australia.



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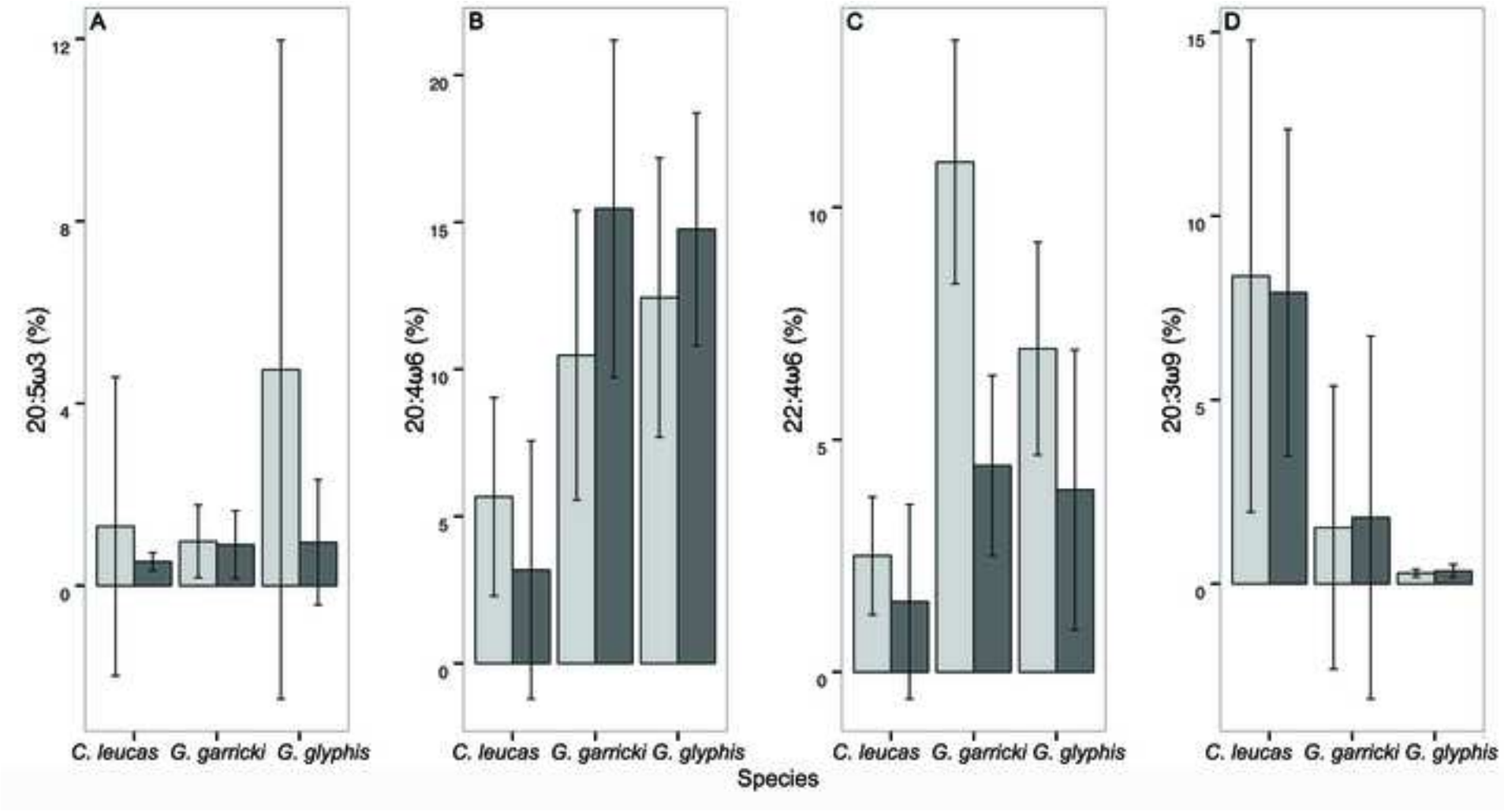

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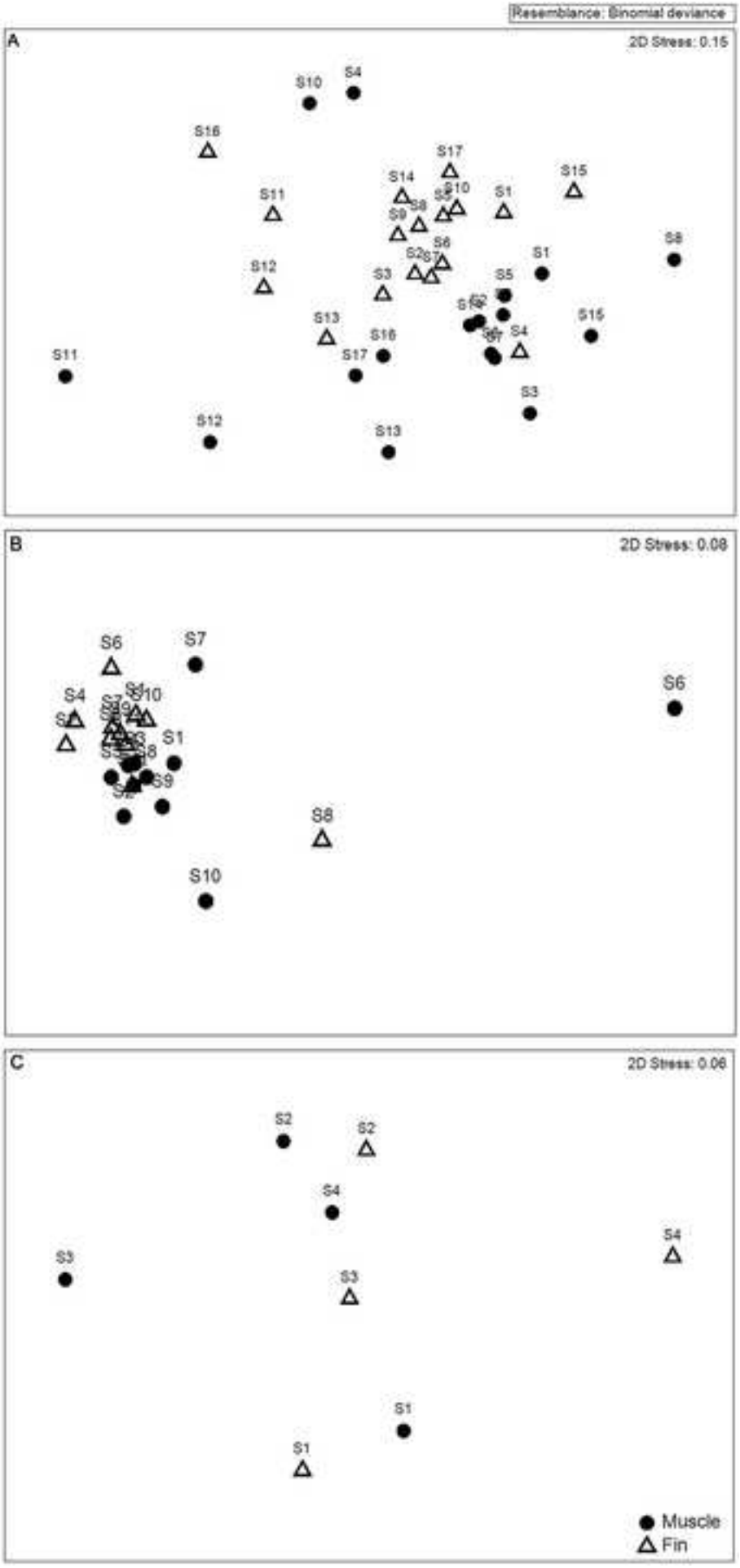

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