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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 utilised 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 × 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*, 20:4 ω6 for *G. garricki* and 20:5 ω3 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.

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 synthesise specific FAs, such as 22:5 ω3 and 22:6 ω3 (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., 2015). 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., 2011b, 2014).

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

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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 in. gill nets, or hook and line. Tissues were collected from each temporarily restrained (<5 min) 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.

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

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 h, 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, 10 mg 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 h 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 min. 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™-1 fused silica capillary column (15 m × 0.1 mm internal diameter and 0.1 μm film thickness), a flame ionisation 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).