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Trophic niche partitioning of five sympatric shark species in the tropical eastern Pacific Ocean revealed by multi-tissue fatty acid analysis

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ABSTRACT

Fatty acid (FA) analysis of consumer tissues has recently shown utility in drawing further inferences about trophic niche dynamics of marine predators such as sharks. In this study, we examined liver, plasma, and muscle FAs in five coexisting pelagic sharks (blue (*Prionace glauca)*, silky (*Carcharhinus falciformis)*, bigeye thresher (*Alopias superciliosus)*, pelagic thresher (*Alopias pelagicus)*, and smooth hammerhead (*Sphyrna zygaena*)) inhabiting the tropical eastern Pacific Ocean. Results showed complex inter- and intra-individual and tissue variation among the five shark species. Based on multivariate analysis of the muscle FAs, *P. glauca* and *C. falciformis* have the largest FA niche widths, indicating diverse feeding habits or habitat isolation, whereas *A. pelagicus* and *S. zygaena* occupied a narrower niche width, reflecting increased trophic specialization. High percentages of muscle FA niche overlap indicated strong resource competition between *S.zygaena* and *C. falciformis* and a degree of dietary isolation by *P. glauca*. Interpretations of feeding ecology differed based on the analysis of plasma FAs, which could be attributed to higher dietary FA turnover rates. The liver was deemed unsuitable to examine FA niche metrics based on high and unexplained intra-specific variance in liver FAs as well as the unique lipid metabolism in chondrichthyans. Overall, our multi-tissue approach revealed the magnitude of potential competitive interactions among coexisting tropical shark species. It also expanded our understanding of inter-tissue variability and best practices when using FA analysis to estimate trophic niche metrics of sharks.

1. Introduction

Determining the mechanisms underlying the maintenance of biodiversity and species coexistence in resource-limited settings has long been a core focus of community ecology (Carrete et al., 2005). Trade-offs among coexisting species shape differential life-history strategies and thus trophic interactions, including the strength of resource competition (Vandermeer, 1972; Silvertown, 2004). In particular, trophic niche differentiation, based on how organisms utilize their dietary resources, is a necessary prerequisite for species coexistence (Every et al., 2017; Sardenne et al., 2019). In this context, oceanic pelagic sharks generally occupy a position at or near the top of food webs, and as such, contribute to regulating food web structure and function via top-down control (Heithaus et al., 2008). Currently, however, our understanding of the mechanisms underlying the ecological coexistence of sympatric pelagic sharks is still comparatively limited, particularly with respect to those species that survive in the relatively resource-poor (oligotrophic) open ocean. Five large-sized pelagic shark species are known to inhabit the tropical eastern Pacific Ocean, namely, the blue (*Prionace glauca*), silky (*Carcharhinus falciformis*), bigeye thresher (*Alopias superciliosus*), pelagic thresher (*A. pelagicus*), and smooth hammerhead (*Sphyrna zygaena*) sharks, which are considered to be apex predators (Essington et al., 2006; Galván-Magaña et al., 2013). Accordingly, we assume that a comparative study of the trophic niches of these sharks would go some way to revealing the mechanisms that facilitate the coexistence of sympatric oceanic apex predators.

In recent years, the analysis of consumer fatty acids (FAs) has emerged as a potentially valuable technology in the field of ecology, including trophic niche ecology (Brewster et al., 2016; Gong et al., 2018; Sardenne et al., 2016). As apex predators, sharks synthesize extremely

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few FAs *de novo*, which are mainly short-chain moieties with low levels of desaturation (Cárdenas-Palomo et al., 2018; Iverson, 2009). In addition, exogenously derived FAs obtained from dietary sources (dietary-derived FAs) are typically conservatively deposited in tissues, the accumulation of which thereby provides information on feeding patterns over certain periods of time (Cárdenas-Palomo et al., 2018; Kelly and Scheibling, 2012; Stowasser et al., 2009). In this regard, dietary-derived FAs can serve as indices in studies on the feeding habits and nutritional relationships of organisms. For example, C22:0 + C24:0 can retrace the feeding habits of organisms comprising more coastal based food webs (McGovern et al., 2018). The docosahexaenoic acid/-eicosapentaenoic acid (C22:6n3/C22:5n3, DHA/EPA) ratio potentially may be used to determine the degree of carnivory, because of the high conservativeness of DHA through the food web (Alderete-Macal et al., 2020; Dalsgaard et al., 2003; Kelly and Scheibling, 2012). Sharks exhibit complex and varied trophic ecologies owing to their array of morphologies, habitats and foraging strategies. The use of FA profiles in shark ecology extends to diet, habitat use, ontogenetic shifts and quantifying trophic niche amongst other applications (Every et al., 2017; Meyer et al., 2019; Pethybridge et al., 2011). In a study examining the FA profiles of two sympatric reef shark species, Bierwagen et al. (2019) succeeded in identifying interspecific dietary resources and established that the FA niche width of whitetip reef sharks (*Triaenodon obesus*) was smaller than that of grey reef sharks (*Carcharhinus amblyrhynchos*).

Multi-tissue FA analysis is a n-dimensional analytical method that can facilitate a detailed examination of shark trophic ecology (McMeans et al., 2012; Pethybridge et al., 2014). This approach is based on the concept that tissues have different turnover rates of FAs, thereby making them suitable for determining dietary composition over ecologically relevant timescales. However, for certain sharks, it remains unclear as to which tissues provide the most accurate information regarding diet, owing to their specific lipid metabolism and storage pathways (Meyer et al., 2019). The findings of previous studies have shown that the liver is an energy storage organ with an extremely high metabolic rate and FA composition reflects moderate-term (weekly) dietary intake of sharks (Beckmann et al., 2013; Iverson, 2009). Similarly, plasma functions as a medium to transport exogenous FAs via lipoproteins (e.g., chylomicrons) (Ballantyne, 1997; Metcalf and Gemmell, 2005; Mills et al., 1977), and thus plasma FAs represent short-term (daily) dietary intake (McMeans et al., 2012). The muscle tissues of sharks lack the enzymes necessary to catabolize FAs, and typically have low lipid levels (Ballantyne, 1997). Nevertheless, neutral storage lipids are still present in shark muscle, and muscle FAs are considered to provide integrated long-term (monthly) dietary information (Beckmann et al., 2013; Regost et al., 2003). Furthermore, McMeans et al. (2012) reported high levels of dietary FA modification in the liver of Greenland sharks (*Somniosus microcephalus*), whereas muscle FA profiles were generally more similar to those of prey.

In this study, we sought to investigate differences in the FA profiles of the liver, plasma, and muscles of the aforementioned five sympatric pelagic shark species inhabiting the tropical eastern Pacific Ocean. Specifically, our goals were to (1) explore inter and intraspecific variability which includes examining differences among species, tissues, sex and development/maturation levels (mature vs immature), and (2) estimate fatty acid niche metrics to assess the dietary differences and trophic relationships of these species and characterize the associated trophic niche partitioning and coexistence mechanisms.

2. Materials and methods

2.1. Sample collection

A total of 168 shark specimens were collected from amongst the bycatch of tuna captured using longline fishing in the eastern Pacific Ocean from September 2019 to January 2020 (Table 1, Fig. 1). Individuals were graded according to the modified maturity index proposed by Walker (2005) (Table A1), and male maturity depended on clasper index ($C = 1–3$), while female maturity depended on ovarian state ($U = 1–5$). Muscle and liver tissue samples were taken from the vicinity of the dorsal fin and the front of any lobe of the liver tissue, respectively, and plasma samples were obtained from the tail artery and collected within lithium heparin blood collection tubes. The samples were vacuum packaged and transported to the laboratory at −20 °C, and then stored at −80 °C.

2.2. Fatty acid analysis

Muscle samples were dried in a freeze dryer (Christ Alpha 1–4) at −55 °C for 24 h after using ultra-pure water, and ground to a fine powder using a refrigerated mixing ball mill (Mixer mill MM440). Plasma samples were also freeze-dried for 24 h and then ground to a powder, whereas liver samples were just oven-dried for 24 h. To extract tissue lipids, samples of the three tissue types were placed into stoppered centrifuge tubes and homogenized in 12 mL of 2:1 (v/v) dichloromethane–methanol solution for approximately 24 h at near-room temperature. Approximately 100 mg of the liver and plasma homogenates were weighed (±20 mg), and following centrifugation, the non-lipid-containing material was removed, and the resulting supernatants were combined in a 15-mL centrifuge tube. The lipid extracts were made up to exactly 8 mL with 2:1 (v/v) dichloromethane–methanol, to which 4 mL of a 0.9% solution of NaCl in water was added. Having mixed the phases, the upper aqueous layer was removed and discarded along with other non-lipid-containing material. The lower dichloromethane layer was thereafter transferred to a round-bottomed flask, and after purging with nitrogen (using a nitrogen blowing device) to remove the organic reagents, 4 mL of sodium hydroxide–methanol solution (0.5 moL/L) was added to reconstitute the total lipid extract.

Fatty acid methyl esters (FAMEs) were analysed for each tissue sample using a modification of the GAQSIQ method (Parrish, 1999). The extracted lipids of each tissue sample were immediately subject to FAME analysis to minimize the likelihood of contamination and oxidation. The round-bottomed flask containing the total lipid extract was subsequently connected to water bath reflux device for 20 min, followed by the addition of 4 mL of boron trifluoride-methanol solution (14% w/v), and methyl esterification was performed in a 60 °C water bath. The flask was then cooled to near-room temperature, after which 4 mL of n-hexane was added, followed by shaking for 20 s, and subsequent addition of saturated sodium chloride solution to make up to volume. The resulting

Table 1
Collection and biological information of sharks sampled from the tropical eastern Pacific Ocean.

Shark species	Common name	Code	Sample size		Precaudal length (cm)			Total lipid in muscle (%)
			mature	immature	Mean ± SD	Min	Max	
Prionace glauca	Blue shark	BSH	11	19	176 ± 22	129	221	5.8 ± 2.7
Alopias superciliosus	Bigeye thresher shark	BTH	11	18	146 ± 30	81	190	NA
Carcharhinus falciformis	Silky shark	FAL	12	18	136 ± 31	65	182	7.0 ± 2.8
Alopias pelagicus	Pelagic thresher shark	PTH	7	14	139 ± 15	99	158	4.9 ± 2.3
Sphyrna zygaena	Smooth hammerhead shark	SPZ	7	20	179 ± 25	141	222	4.1 ± 1.4

NA: Not Available.

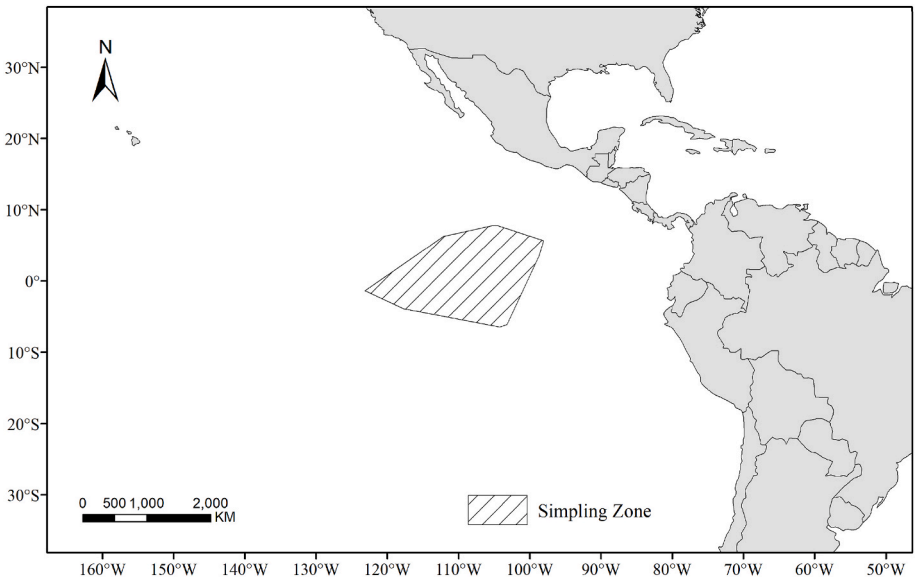

Fig. 1. Study area and sampling stations for pelagic sharks inhabiting the tropical eastern Pacific Ocean.

n-hexane layer containing FAMEs was then transferred to a back wall glass tube fitted with a screw-top Teflon cap. The FA profile for each sample was determined using an Agilent 7890B Gas Chromatograph incorporating an AgilentHP-88 capillary column (60 m × 0.25 nm × 0.20 μm) and coupled to a 5977A series Mass Spectrometer Detector (Agilent Technologies, Inc., USA). Chromatography was performed using a high-purity helium carrier gas with a split ratio of 10:1 and the inlet temperature was 250 °C. The heating program comprised an initial temperature of 125 °C, which was then increased at 8 °C/min to 145 °C, at which it was held for 26 min, then increased at 2 °C/min to 220 °C, held for 1 min, and finally increased to 227 °C at 1 °C/min and held for 1 min.

As standards, we used 37 FA mixed standards to which stearidonic acid methyl ester (Fluka, 43959), 13-eicosenoic acid methyl ester (Sigma E3512), 9-eicosenoic acid methyl ester (Indofine Chemical, 20-2001-1), 16-docosatetraenoic acid methyl ester (Sigma D3534), and 19-docosapentaenoic acid methyl ester (Supelco, 47563-U) were added, and methyl nonadenoate was used as an internal standard. On the basis of the qualitative analysis of FAs via a comparison of retention times, we used an internal standard method (GLC 37, Nu-Chek Prep Inc, Elysian) to quantitatively analyse FAs, with amounts being determined using the following equation:

$$X_i = F_i \times \frac{A_i}{A_{19}} \times \frac{m_{19}}{m} \times 100\%$$

where *X*i is the amount of component i in the sample (mg/g); *A*i is the peak area of component i; m is the mass of the sample; m19 is the mass of the internal standard methyl nonadenoate; *A*19 is the peak area of the internal standard in the sample; and *F*i is the ratio of the correction factor of component i to the internal standard, referred to as the relative correction factor.

Total amounts of FAs were determined on a dry tissue weight (mg/g dry weight) basis, and the amounts of individual FAs are expressed in terms of a percentage of the total (Phillips et al., 2001). The FAs were classified into the following three main FA classes: saturated fatty acids (SFAs), representing all FAs with no double bonds; monounsaturated fatty acids (MUFAs), representing all FAs with a single double bond; and polyunsaturated fatty acids (PUFA), representing all FAs with two or more double bonds.

2.3. Statistical analysis

Diet interpretations based on FA analysis are considered best inferred from data standardized to the total mass of FAs quantified according to Happel et al. (2017). Correspondingly, our FAs data were expressed as a percentage of total FAs, with those FAs occupying trace amounts (<0.5%) being excluded from statistical analyses to avoid influence of possible analytical error (Every et al., 2016; Happel et al., 2017).

To examine inter and intraspecific variability in individual FAs between shark species, tissues, sex, and maturation we first checked the data for normality using the Shapiro-Wilk test and for equality of variances using the Bartlett's test. When both conditions were met, one-way ANOVAs were performed followed by a multi-comparison post-hoc Tukey's HSD test. Where conditions were not met or sample size was small (which was the case for sex and maturation comparisons), we used a non-parametric Kruskal-Wallis test, followed by Dunn's post-hoc test with a Bonferroni adjustment.

To examine differences in FA profiles between shark species and tissues, we considered nine FAs that were both abundant and considered to best infer the trophic ecology of sharks, including: C16:0, C18:0, C16:1n7, C18:1n9, C22:1n9, C24:1n9, C18:2n6, C20:4n6, EPA and DHA (reviewed in Meyer et al., 2019). After standardizing the selected FAs, by subtracting the respective means and dividing by the standard deviations, we used principal component analysis (PCA) to reduce the dimensionality of the data (Pedro et al., 2020). PCA was selected due to its ability to (1) identify patterns or groupings from large and complex datasets, and (2) establish which FAs explained the largest variance in the data obtained.

To calculate fatty acid niche breadth for each species and tissue, the scores derived from the dimensions 1 and 2 of the PCA were used to compute Bayesian standard ellipse areas (SEAc) that were corrected for small sample sizes following methods described in several FA trophic niche studies (Gong et al., 2020; Pedro et al., 2020; Sardenne et al., 2019). Overlap coefficients between groups were calculated as the ratio between the Bayesian estimate (4000 iterations) for the overlap area and the minimal ellipse area filled by a FA profile with 0% indicating no overlap and 100% indicating full overlap in trophic niches. The approach for FA niche calculations is analogous to the Layman metrics for stable isotopes (Layman et al., 2007).

The Origin 2021 pro software was used for all univariate statistical analyses and PCA. The R statistical software (v4.0.2) and SIAR package to calculate FA niche metrics (Parnell et al., 2013). All results are

reported as mean ± standard deviation (SD).

3. Results

Among the five assessed shark species, we identified a total of 35 FAs, 21 of which were found to have relative mean values greater than 0.5%, accounting for 87.3%–93.5% of all FAs (Table 2).

3.1. Interspecific and intraspecific variability of FA trophic tracers

In liver samples, we detected significant differences between species in total MUFAs (means ranging from 22.3% to 32.3%, ANOVA, F-value = 7.94; $P < 0.05$, Table A2), whereas no significant differences were detected for either total SFAs (20.3%–25.4%, $F = 2.17$; $P > 0.05$) or PUFAs (47.4%–53.7%, $F = 1.53$; $P > 0.05$) (Table 2). The most commonly identified FAs, in decreasing order of importance, were typically C16:0 (4.1%–10.7%), C22:6n3 (30.7%–39.6%), C20:5n3 (2.3%–7.1%), C20:4n6 (3.3%–6.9%), C18:1n9 (9.5%–17.8%), and C18:0 (4.6%–7.4%), all of which significant differences were detected among species (ANOVA, F = 3.64, 4.00, 16.85, 7.32, 4.11 and 3.06, respectively; $P < 0.05$, Table A2). *A. superciliosus* had the highest levels of C18:1n9 and C16:1n7, whereas *P. glauca* had the highest levels of C20:5n3 and C20:4n6, and *S. zygaena* had the highest levels of C22:6n3. Moreover, significant differences in C22:0 + C24:0 (1.0%–1.4%, ANOVA, F = 2.92; $P < 0.05$) were found among species, with *P. glauca* and *A. superciliosus* having slightly lower levels than the other four sharks. Species differences in the DHA/EPA ratio were also detected (ANOVA, F = 20.88; $P < 0.05$) with highest levels in *A. superciliosus* (16.2), followed by *A. pelagicus* (13.9), *S. zygaena* (13.1), *C. falciformis* (7.2), and *P. glauca* (5.1).

With respect to plasma, we detected significant differences in total SFAs (39.1%–45.7%), MUFAs (20.4%–24.8%), and PUFAs (28.9%–37.2%) among species (ANOVA, F = 14.13, 7.87 and 17.96, respectively; $P < 0.05$, Table A2) (Table 3). The most commonly identified FAs in plasma were typically C16:0 (6.2%–12.6%), C22:6n3 (8.3%–18.6%), C20:5n3 (4.1%–4.6%), C20:4n6 (0.7%–1.2%), C18:1n9 (6.7%–11.9%), and C18:0 (12.1%–15.5%), all of which differed significantly among species (ANOVA, F = 15.75, 28.00, 4.94, 9.76, 20.12 and 19.98, respectively; $P < 0.05$, Table A2). *A. superciliosus* was found to have the highest levels of C16:0, C16:1n7, and C18:1n9, whereas *S. zygaena* had the highest levels of C22:6n3, and *P. glauca and A. pelagicus* had the highest levels of C20:5n3. We also detected significant differences in C22:0 + C24:0 (4.7%–7.4%, ANOVA, F = 28.33; $P < 0.05$) among species, with *A. superciliosus* reporting much lowest levels than all other species. In contrast to the liver, highest plasma DHA/EPA ratios (ANOVA, F = 20.60; $P < 0.05$) were detected in *S. zygaena* (4.3), followed by *A. pelagicus* (3.2), *A. superciliosus* (3.0), *P. glauca* (2.2), and *C. falciformis* (1.9).

In muscle tissues, we detected significant differences in total MUFAs (20.9%–23.8%) and PUFAs (35.2%–40.2%) among species (ANOVA, F = 7.16 and 7.99, respectively; $P < 0.05$, Table A2), whereas differences in total SFAs were not significant (38.8%–41.2%, $F = 2.34$; $P > 0.05$) (Table 4). The most commonly identified FAs in muscle tissue were similar to those detected in liver and plasma, namely, C16:0 (11.1%–17.4%), C22:6n3 (10.8%–20.2%), C20:5n3 (3.2%–4.2%), C20:4n6 (2.9%–5.0%), C18:1n9 (7.1%–10.1%), and C18:0 (7.1%–11.2%), the amounts of which differed significantly among species (ANOVA, F = 20.33, 23.34, 6.02, 18.76, 5.46 and 33.02, respectively; $P < 0.05$, Table A2). *A. superciliosus* was found to have the highest levels of C16:0 and C22:6n3, *S. zygaena* the highest levels of C20:4n6 and C20:5n3, and *P. glauca* the highest levels of C18:1n9. We also detected significant differences among species with respect to C22:0 + C24:0 (3.0%–4.3%, ANOVA, F = 20.86; $P < 0.05$), with mean relatively levels lowest in *A. superciliosus* and highest in *S. zygaena*. Furthermore, species differences in muscle DHA/EPA ratios (ANOVA, F = 26.28; $P < 0.05$) were detected and were highest in *A. superciliosus* (7.2), followed by *A. pelagicus* (4.7), *P. glauca* (3.5), *C. falciformis* (2.8), and *S. zygaena* (2.6). There were only a limited number of consistent species-specific trends in known FA tracers between the tissues analysed. Across all tissues, *A. supercilosus* had statistically lowest levels of C20:5n3 and C22:0 + C24:0 whilst *C. falciformis* had statistically lowest DHA/EPA ratios and *A. pelagicus* had statistically lowest ∑MUFA. There were other examples of similar trends across two tissue types, including for C16:1n7, C18:1n9, C22:6n3 (between the liver and plasma), for 16:0 (between muscle and plasma) and for C20:5n3 (between the liver and plasma and between muscle and liver). On a number of occasions there were also opposite trends noted, such as for *A. supercilisus* for which levels of C16:1n7, ∑MUFA, and C22:6n3 were highest in the liver and lowest in the muscle.

Table 2
Fatty acid profiles (% of total FA ± standard deviation) of liver tissue of five oceanic pelagic shark species.

Fatty Acids	*Prionace glauca*	*Alopias superciliosus*	*Carcharhinus falciformis*	*Alopias pelagicus*	*Sphyrna zygaena*
C14:0	5.7 ± 2.3^{a}	3.2 ± 2.4^{b}	3.9 ± 1.8^{b}	2.0 ± 0.7^{c}	1.4 ± 0.7^{c}
C16:0	4.1 ± 6.7^{b}	5.8 ± 6.7^{b}	5.5 ± 7.0^{b}	10.7 ± 7.3^{a}	8.5 ± 6.5^{ab}
C17:0	2.0 ± 0.7^{a}	1.4 ± 0.3^{b}	2.2 ± 0.7^{a}	1.4 ± 0.4^{b}	1.8 ± 0.4^{a}
C18:0	4.6 ± 3.5^{b}	6.4 ± 2.5^{ab}	7.4 ± 4.5^{a}	6.9 ± 1.1^{ab}	7.1 ± 3.7^{a}
C20:0	0.7 ± 0.3^{ab}	0.5 ± 0.1^{c}	0.8 ± 0.2^{a}	0.6 ± 0.3^{abc}	0.6 ± 0.2^{bc}
C22:0	0.5 ± 0.3	0.5 ± 0.1	0.6 ± 0.2	0.6 ± 0.3	0.5 ± 0.2
C23:0	0.4 ± 0.3^{b}	0.5 ± 0.2^{b}	0.5 ± 0.2^{ab}	0.7 ± 0.4^{a}	0.5 ± 0.2^{ab}
C24:0	0.6 ± 0.4^{b}	0.6 ± 0.2^{b}	0.7 ± 0.2^{a}	0.8 ± 0.4^{ab}	0.7 ± 0.3^{ab}
∑SFA	20.5 ± 6.9^{ab}	20.3 ± 6.2^{b}	23.4 ± 7.2^{ab}	25.4 ± 7.5^{a}	22.3 ± 7.5^{ab}
C16:1n7	4.3 ± 2.9^{ab}	5.7 ± 2.7^{a}	4.8 ± 3.9^{a}	2.7 ± 1.2^{bc}	1.8 ± 1.2^{c}
C18:1n9	9.5 ± 7.7^{b}	17.8 ± 13.1^{a}	12.5 ± 5.6^{ab}	13.0 ± 4.5^{ab}	11.5 ± 5.3^{b}
C20:1	7.9 ± 3.7^{a}	3.8 ± 1.6^{b}	3.5 ± 2.0^{b}	2.5 ± 0.9^{b}	7.1 ± 3.0^{a}
C22:1n9	2.5 ± 0.8^{a}	1.5 ± 0.6^{b}	0.8 ± 0.5^{c}	0.9 ± 0.2^{c}	0.9 ± 0.3^{c}
C24:1n9	2.1 ± 0.8^{a}	1.4 ± 0.5^{bc}	1.8 ± 0.6^{ab}	1.2 ± 0.5^{cd}	1.0 ± 0.2^{d}
∑MUFA	27.8 ± 7.7^{ab}	32.3 ± 9.2^{a}	25.0 ± 5.8^{bc}	22.3 ± 4.3^{c}	24.0 ± 6.7^{bc}
C18:2n6	1.8 ± 0.9^{a}	2.0 ± 0.5^{a}	2.1 ± 0.6^{a}	1.9 ± 0.4^{a}	1.3 ± 0.4^{b}
C20:2	1.3 ± 0.5^{a}	0.8 ± 0.2^{c}	1.0 ± 0.3^{bc}	1.1 ± 0.3^{ab}	1.3 ± 0.3^{a}
C22:2n6	0.8 ± 0.7^{b}	1.0 ± 0.3^{ab}	1.2 ± 0.4^{a}	1.2 ± 0.5^{a}	1.2 ± 0.3^{a}
C20:3n3	1.4 ± 1.4^{b}	2.1 ± 0.8^{ab}	2.3 ± 0.8^{a}	2.4 ± 1.8^{a}	1.9 ± 0.8^{ab}
C20:3n6	0.6 ± 0.3^{b}	0.7 ± 0.2^{b}	0.7 ± 0.2^{b}	0.9 ± 0.4^{a}	0.7 ± 0.2^{b}
C20:4n6	6.9 ± 3.8^{a}	4.3 ± 1.6^{bc}	4.4 ± 1.4^{bc}	6.2 ± 3.9^{ab}	3.3 ± 2.2^{c}
C20:5n3	7.1 ± 4.4^{a}	2.3 ± 1.1^{c}	4.6 ± 1.8^{b}	3.0 ± 1.2^{bc}	3.4 ± 1.7^{bc}
C22:6n3	30.7 ± 7.2^{b}	33.2 ± 9.4^{b}	34.0 ± 8.1^{ab}	34.2 ± 8.0^{ab}	39.6 ± 8.3^{a}
∑PUFA	51.7 ± 11.2	47.4 ± 11.9	51.6 ± 9.6	52.4 ± 5.9	53.7 ± 9.5
C22:0 + C24:0	1.0 ± 0.6^{b}	1.1 ± 0.3^{b}	1.4 ± 0.5^{ab}	1.4 ± 0.8^{a}	1.2 ± 0.5^{ab}
DHA/EPA	5.1 ± 2.3^{b}	16.2 ± 5.5^{a}	8.0 ± 2.3^{b}	13.8 ± 8.3^{a}	14.5 ± 7.8^{a}

Alphabetic characters infer statistically differences in individual FAs between species (as determined by Tukey HSD). The absence of alphabetic characters following values indicates no statistical difference.

Table 3
Fatty acid profiles (% of total FA ± standard deviation) of plasma tissue of five oceanic pelagic shark species.

Fatty Acids	*Prionace glauca*	*Alopias superciliosus*	*Carcharhinus falciformis*	*Alopias pelagicus*	*Sphyrna zygaena*
C14:0	1.1 ± 0.3^{a}	1.2 ± 0.2^{a}	1.0 ± 0.2^{b}	1.1 ± 0.3^{a}	0.8 ± 0.1^{c}
C16:0	7.8 ± 3.9^{b}	12.6 ± 4.4^{a}	6.2 ± 3.3^{b}	10.9 ± 3.9^{a}	7.1 ± 3.4^{b}
C17:0	4.5 ± 0.9^{ab}	3.5 ± 0.7^{d}	4.8 ± 0.6^{a}	3.7 ± 1.2^{cd}	4.0 ± 0.7^{bc}
C18:0	14.0 ± 3.3^{a}	12.2 ± 2.0^{b}	15.5 ± 1.6^{a}	12.1 ± 3.3^{b}	12.9 ± 1.6^{b}
C20:0	2.6 ± 0.8^{a}	2.0 ± 0.4^{b}	2.9 ± 0.4^{a}	2.1 ± 0.7^{b}	2.3 ± 0.7^{b}
C22:0	1.8 ± 0.5^{ab}	1.5 ± 0.3^{d}	2.1 ± 0.3^{a}	1.5 ± 0.5^{cd}	1.7 ± 0.3^{bc}
C23:0	2.8 ± 1.3^{b}	2.6 ± 0.6^{b}	3.6 ± 0.8^{a}	2.6 ± 1.1^{b}	2.7 ± 1.1^{b}
C24:0	4.9 ± 1.4^{ab}	3.4 ± 1.3^{d}	5.3 ± 0.7^{a}	3.6 ± 1.5^{cd}	4.2 ± 1.2^{bc}
∑SFA	44.6 ± 3.0^{ab}	42.1 ± 3.7^{c}	45.7 ± 3.5^{a}	42.6 ± 3.9^{bc}	39.1 ± 3.9^{d}
C16:1n7	2.2 ± 0.2^{a}	2.3 ± 0.3^{a}	2.3 ± 0.2^{a}	2.0 ± 0.6^{a}	1.8 ± 0.5^{b}
C18:1n9	7.2 ± 2.1^{b}	11.9 ± 3.8^{a}	6.7 ± 2.2^{b}	7.8 ± 2.1^{b}	6.9 ± 2.1^{b}
C20:1	4.7 ± 11.1^{a}	1.8 ± 0.3^{b}	1.6 ± 0.3^{c}	1.3 ± 0.4^{c}	1.8 ± 0.4^{ab}
C22:1n9	1.4 ± 0.3^{a}	1.0 ± 0.2^{c}	1.3 ± 0.4^{ab}	1.1 ± 0.3^{c}	1.1 ± 0.3^{bc}
C24:1n9	6.4 ± 1.4^{ab}	4.8 ± 1.0^{c}	6.3 ± 1.9^{a}	4.1 ± 2.2^{c}	5.2 ± 1.5^{bc}
∑MUFA	22.6 ± 2.8^{b}	24.8 ± 3.4^{a}	22.6 ± 3.4^{b}	20.4 ± 2.6^{c}	20.9 ± 3.4^{bc}
C18:2n6	3.6 ± 2.3^{b}	3.7 ± 2.1^{b}	5.1 ± 2.9^{a}	2.8 ± 1.7^{b}	2.8 ± 1.6^{b}
C20:2	2.9 ± 0.9	2.4 ± 0.5	2.8 ± 1.3	2.6 ± 0.9	2.8 ± 0.5
C22:2n6	2.2 ± 0.8^{a}	1.8 ± 0.3^{b}	2.1 ± 0.4^{a}	2.0 ± 0.5^{a}	2.0 ± 0.3^{ab}
C20:3n3	3.7 ± 0.8	3.4 ± 0.5	3.5 ± 0.3	3.3 ± 1.3	3.7 ± 0.4
C20:3n6	1.3 ± 1.0^{ab}	1.0 ± 0.6^{ab}	1.0 ± 1.0^{ab}	1.2 ± 0.6^{a}	0.7 ± 0.8^{b}
C20:4n6	1.3 ± 0.8^{a}	1.6 ± 0.7^{a}	0.5 ± 0.4^{b}	1.7 ± 1.5^{a}	1.6 ± 0.8^{a}
C20:5n3	4.6 ± 0.5^{a}	4.1 ± 0.7^{b}	4.4 ± 0.4^{ab}	4.6 ± 0.5^{a}	4.4 ± 0.5^{ab}
C22:6n3	10.1 ± 3.2^{cd}	11.7 ± 2.7^{c}	8.3 ± 2.7^{cd}	14.9 ± 4.5^{b}	18.6 ± 6.5^{a}
∑PUFA	29.8 ± 3.0^{b}	30.7 ± 3.3^{b}	28.9 ± 3.7^{b}	34.4 ± 4.8^{a}	37.2 ± 6.0^{a}
C22:0 + C24:0	6.4 ± 1.6^{ab}	4.7 ± 1.7^{d}	7.4 ± 1.0^{a}	5.1 ± 1.9^{cd}	5.9 ± 1.5^{bc}
DHA/EPA	2.2 ± 0.8^{cd}	3.0 ± 1.2^{bc}	1.9 ± 0.6^{cd}	3.2 ± 1.0^{b}	4.3 ± 1.7^{a}

Alphabetic characters infer statistically differences in individual FAs between species (as determined by Tukey HSD). The absence of alphabetic characters following values indicates no statistical difference.

Table 4
Fatty acid profiles (% of total FA ± standard deviation) of muscle tissue of five oceanic pelagic shark species.

Fatty Acids	*Prionace glauca*	*Alopias superciliosus*	*Carcharhinus falciformis*	*Alopias pelagicus*	*Sphyrna zygaena*
C14:0	2.1 ± 0.5^{ab}	2.0 ± 0.6^{b}	2.7 ± 0.5^{a}	2.2 ± 0.7^{ab}	2.2 ± 0.6^{ab}
C16:0	14.9 ± 4.5^{b}	17.5 ± 3.7^{a}	11.7 ± 1.8^{c}	15.6 ± 2.9^{ab}	11.1 ± 1.4^{c}
C17:0	2.2 ± 0.4^{b}	2.2 ± 0.7^{b}	2.7 ± 0.6^{a}	2.6 ± 0.8^{ab}	2.8 ± 0.8^{a}
C18:0	9.2 ± 1.3^{b}	7.1 ± 1.5^{c}	11.2 ± 2.0^{a}	7.4 ± 1.2^{c}	9.9 ± 1.7^{b}
C20:0	2.0 ± 0.4^{bc}	1.9 ± 0.8^{c}	2.5 ± 0.6^{a}	2.4 ± 0.8^{ab}	2.6 ± 0.8^{a}
C22:0	1.6 ± 0.6^{b}	1.6 ± 0.8^{b}	2.5 ± 0.8^{ab}	2.4 ± 0.9^{ab}	2.6 ± 0.9^{a}
C23:0	1.9 ± 0.6	1.4 ± 1.1	1.6 ± 1.2	1.5 ± 1.2	1.8 ± 1.4
C24:0	1.7 ± 0.9^{ab}	1.3 ± 1.0^{b}	2.2 ± 1.1^{a}	1.8 ± 1.2^{ab}	2.1 ± 1.4^{a}
∑SFA	39.6 ± 3.6	38.9 ± 3.0	41.3 ± 1.6	40.0 ± 2.7	40.1 ± 3.9
C16:1n7	2.7 ± 0.7^{a}	2.0 ± 0.6^{b}	2.3 ± 0.4^{ab}	1.9 ± 0.7^{b}	2.3 ± 0.6^{ab}
C18:1n9	10.2 ± 1.8^{a}	9.4 ± 4.0^{a}	8.8 ± 1.5^{a}	7.2 ± 1.4^{b}	9.0 ± 1.8^{a}
C20:1	2.6 ± 2.3	2.1 ± 0.4	2.2 ± 0.3	2.5 ± 0.4	2.2 ± 0.6
C22:1n9	1.6 ± 0.3^{ab}	1.5 ± 0.6^{b}	1.9 ± 0.4^{a}	1.6 ± 0.6^{ab}	1.9 ± 0.7^{a}
C24:1n9	2.2 ± 0.4^{c}	2.0 ± 0.7^{c}	3.1 ± 0.5^{a}	2.7 ± 0.5^{b}	2.6 ± 0.6^{b}
∑MUFA	23.8 ± 2.6^{a}	20.9 ± 3.7^{b}	23.5 ± 1.7^{a}	21.6 ± 1.2^{b}	23.0 ± 2.1^{a}
C18:2n6	2.2 ± 1.1	2.3 ± 0.8	2.7 ± 1.0	2.9 ± 1.7	2.8 ± 1.7
C20:2	1.7 ± 0.4^{bc}	1.6 ± 0.6^{c}	2.1 ± 0.4^{a}	1.9 ± 0.7^{abc}	2.0 ± 0.6^{ab}
C22:2n6	2.2 ± 0.4^{ab}	2.0 ± 0.9^{b}	2.6 ± 0.5^{a}	2.4 ± 0.8^{ab}	2.5 ± 0.7^{a}
C20:3n3	3.8 ± 0.9^{b}	3.3 ± 0.8^{bc}	3.8 ± 0.4^{b}	3.1 ± 0.8^{c}	4.5 ± 1.4^{a}
C20:3n6	1.8 ± 0.4	1.7 ± 0.7	2.1 ± 0.5	2.0 ± 0.7	2.0 ± 0.6
C20:4n6	4.1 ± 1.0^{b}	3.2 ± 1.0^{cd}	3.7 ± 0.5^{bc}	3.0 ± 0.7^{d}	5.1 ± 1.4^{a}
C20:5n3	4.0 ± 0.6^{a}	3.2 ± 1.1^{b}	4.1 ± 0.8^{a}	3.9 ± 1.2^{a}	4.5 ± 1.1^{a}
C22:6n3	13.8 ± 3.5^{bc}	20.2 ± 6.4^{a}	10.9 ± 3.0^{c}	16.6 ± 4.9^{b}	10.8 ± 3.3^{c}
∑PUFA	36.6 ± 5.3^{bc}	40.2 ± 4.2^{a}	35.2 ± 2.0^{c}	38.7 ± 2.7^{ab}	37.0 ± 4.1^{bc}
C22:0 + C24:0	3.3 ± 1.0^{bc}	3.0 ± 1.3^{c}	4.2 ± 1.4^{ab}	3.8 ± 1.4^{abc}	4.3 ± 1.9^{a}
DHA/EPA	3.5 ± 1.0^{bc}	7.2 ± 3.5^{a}	2.8 ± 1.2^{c}	4.7 ± 1.8^{b}	2.6 ± 0.9^{c}

Alphabetic characters infer statistically differences in individual FAs between species (as determined by Tukey HSD). The absence of alphabetic characters following values indicates no statistical difference.

3.2. Tissue, maturation and sex effects

For all species, the plasma and muscle had higher relative levels of SFAs and lower levels of PUFAs compared to the livers (Fig. 2). Moreover, based on calculated SD values, the proportions of all three classes of FAs appeared to show greater variability in liver tissues than in either plasma or muscle (e.g., SFA SD: liver = 6.2–7.5, plasma = 3.0–3.9, muscle = 1.6–3.9; PUFA SD: liver = 5.9–11.9, plasma = 3.0–6.0, muscle = 2.0–5.3, Fig. A1). It was also noteworthy that we detected relatively higher variability in the plasma of mature *P. glauca* (e.g., MUFA SD = 12.9), whereas the variability in muscle of all shark individuals was typically low.

Maturity stage (immature vs mature) was found to significantly effect most individual FA tracers for at least one or more different tissues and shark species, with the exception being for C20:5n3 (determined by Kruskal-Wallis tests; Table A3). Notably, maturity significantly influenced five of the six FA tracers examined (excluding C20:5n3) for the plasma tissue of *S. zygaena* (Fig. A2). Two FA tracers (C20:4n6 and DHA/EPA) in the liver of *S. zygaena* were also significantly influenced by maturation; higher relative levels of PUFA's were found in mature compared to immature individuals (Fig. 2). The only other species to show any effect of maturation was *C. falciformis* which showed differences in liver C22:6n3 and C22:0 + C24:0 and muscle C20:4n6 (Fig. 2). For the plasma and muscle tissues of most shark species, relative levels of MUFA were typically lower in mature compared to immature individuals (Fig. 2).

Sex was also factor that influenced variability in individual FA tracers of two shark species, *A. pelagicus* and *P. glauca* (determined by Kruskal-Wallis tests; Table A3). In the case of *A. pelagicus*, C22:6n3, C18:1n9 and C22:0 + C24:0 showed significant differences between sexes in liver (Fig. A2). Similarly, there were significant differences in C22:6n3 and C20:5n3 (Table A3) for liver and muscle based on the sex of

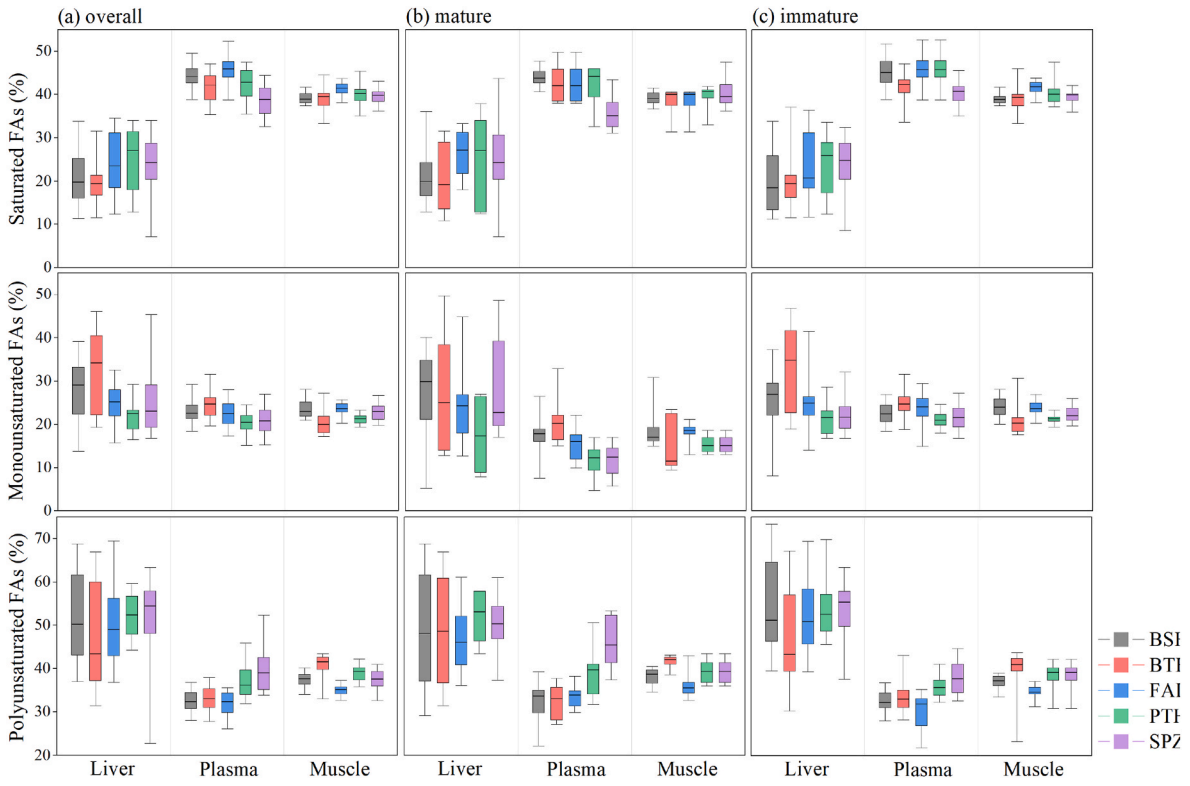

Fig. 2. Boxplots of the relative means of saturated, monounsaturated, and polyunsaturated fatty acid (FA) profiles based on the liver, plasma, and muscle tissues taken from five shark species *Prionace glauca* (BSH), *Alopias superciliosus* (BTH), *Carcharhinus falciformis* (FAL), *Alopias pelagicus* (PTH) and *Sphyrna zygaena* (SPZ) from the tropical eastern Pacific Ocean. Box upper and lower edges are the interquartile range (IQR), the line within each box is the mean, and the whiskers represent the minimum and maximum values.

P. glauca. There was no effect of sex for *S. zygaena, A. superciliosus* or *C. falciformis* or for any muscle tissue.

3.3. Fatty acid profile differences and trophic niche metrics

PCA plots illustrated that among all shark species, there are more dispersed FA profiles for liver and plasma compared to muscle, particularly for *A. superciliosus* and *C. falciformis* (Fig. 3). In the muscle tissue, there were several FAs that explained differences between species with higher levels of C16:0 and C22:6n3 in *A. superciliosus* and *A. pelagicus*, C18:1n9 in *P. glauca,* C20:4n6 and C20:5n3 in *S. zygaena* and C20:5n3 and C24:1n9 in the muscle of *C. falciformis* (Fig. 3).

Calculations of SEAc showed that there were higher estimates of FA niche width based on the FA profiles of liver or plasma than muscle tissue (Table 5). With respect to liver, *S. zygaena* and *A. pelagicus* occupied slightly smaller FA niche widths, compared with those of *P. glauca* and *C. falciformis*, with a similar pattern being observed for muscle. Contrastingly, based on our analysis of plasma, *C. falciformis* and *P. glauca* occupied slightly smaller FA niche widths, whereas that occupied by *A. pelagicus* was much larger. Estimates of SEAc for *A. superciliosus* for all tissue types were consistently in the middle range across all species.

The FA overlap coefficients looking at resource partitioning between species also showed large differences between tissues, with a higher percentage overlap typically detected for liver and muscle (Table 6). For the liver, the highest percent of overlap was reported between *A. superciliosus* and both *A. pelagicus* (69%) and *C. falciformis* (66%). The largest overlap observed for plasma FAs was between *A. pelagicus* and *S. zygaena* (78.8%) with *P. glauca* showing some degree of overlap with all other species (overlap coefficients ranging between 12 and 56%; Table 6). For the muscle, most species observed complete partitioning (close to 0% overlap) with only two exceptions. There was a large overlap between the muscle FAs of *C. falciformis* and *S. zygaena* (67%) and then a smaller overlap between the two thresher sharks *A. superciliosus* and *A. pelagicus* (13%; Table 6).

4. Discussion

Consistent with the findings of previous comparative studies of shark FA profiles (Alderete-Macal et al., 2020; Davidson and Cliff, 2002; Every et al., 2017; McMeans et al., 2012; Meyer et al., 2021; Pethybridge et al., 2011), we detected distinct, yet at times complex, inter-species and inter-tissue differences among all five shark species examined in this study.

4.1. Inter-tissue variability and their utility in trophic ecology

The five pelagic shark species in this study were characterized by significantly higher PUFA contents in the liver (47.4–54.0%) and higher SFA contents in plasma and muscle tissue (39.0–46.0%). This pattern was somewhat different to other shark studies (Pethybridge et al., 2010; Beckmann et al., 2014; McMeans et al., 2012) that have typically reported higher levels of PUFA in muscle than liver tissue and much higher levels of MUFAs in the liver than those reported here (Davidson et al., 2007). While not as high as those reported in this study, high concentrations of PUFA have been reported in the liver of other large, oceanic shark species off southern Australia (Pethybridge et al., 2014) and South Africa (Davidson et al., 2011). The higher levels of PUFA seen in the pelagic shark species in this study could reflect unique physiological capabilities of these species or unique prey profiles in the study area, as there have been limited reports of FAs in these shark species or other marine species in the tropical eastern Pacific Ocean. Indeed, high concentrations of PUFA (59.1%) have been reported in Humboldt squid (*Dosidicus gigas*) sampled from within the study area (Gong et al., 2020)

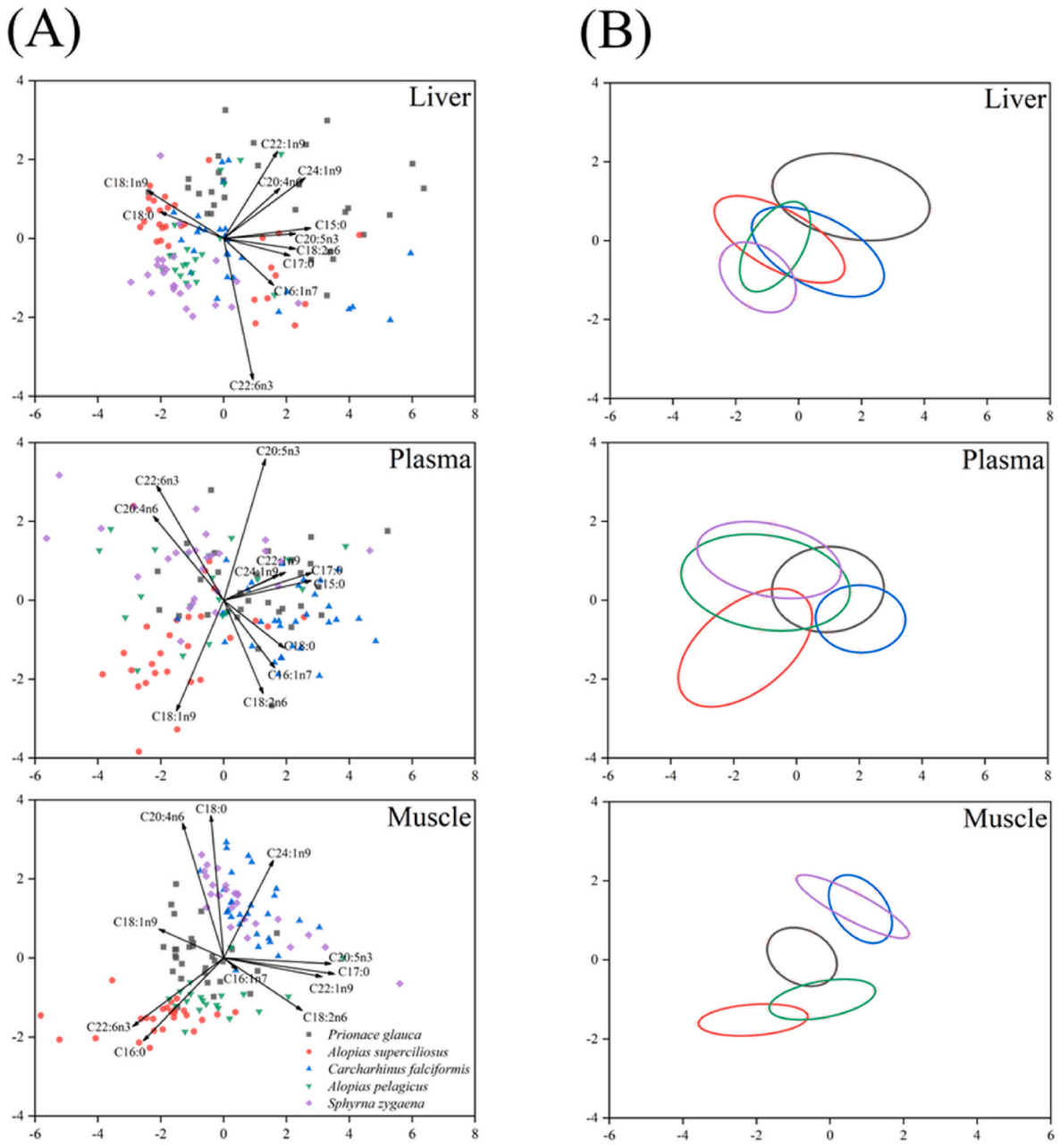

Fig. 3. Principal component analysis (PCA, A) and Standard Ellipse Areas (SEAc, B) of fatty acid profiles in the liver, plasma and muscle of the five shark species from the tropical eastern Pacific Ocean. PCA axes 1 and 2 explained 40.4% and 13.3% (liver), 47.6% and 15.3% (plasma), 42.9% and 19.0% (muscle) of the variation among shark species, respectively.

Table 5
Small sample size corrected standard ellipse area (SEAc) values for the liver, plasma, and muscle of five shark species from the tropical eastern Pacific Ocean. Higher values represent larger fatty acid niche widths.

Shark species	liver	plasma	muscle
Prionace glauca	7.8	5.7	2.0
Alopias superciliosus	5.1	8.0	1.9
Carcharhinus falciformis	5.9	3.6	2.0
Alopias pelagicus	3.1	9.2	1.7
Sphyrna zygaena	2.9	6.2	1.7

and known to be consumed by these shark species (Galván-Magaña et al., 2013). In contrast, comparable levels of PUFA in the muscle have been reported for a wide range of shark species including sharks inhabiting deep-sea (e.g., Pethybridge et al., 2010), coral reef (Bierwagen et al., 2019) and open ocean (Davidson et al., 2011) environments. Similar to that found by McMeans et al. (2012) the FA profiles of liver and plasma were much more variable than shark muscle.

Inter-tissue differences reported here and elsewhere indicate that elasmobranchs are selectively incorporating dietary FAs into different tissues based on their physiological roles and functions with respect to FA metabolism (Ballantyne, 1997; Pillans et al., 2009; Speers-Roesch et al., 2010). Despite a number of studies examining inter-tissue differences (Davidson et al., 2011b; Every et al., 2016), there remains some debate as to which tissue FA profiles are best aligned with those of prey and thus are best suited to infer trophodynamics of marine consumers (McMeans et al., 2012; Beckmann et al., 2013). An experimental study on a benthic coastal shark, *Heterodontus portusjacksoni*, observed that FA profiles of the liver and plasma changed more rapidly than muscle and

Table 6
The percentage overlap of the liver, plasma, and muscle fatty acid niche widths of five shark species from the tropical eastern Pacific Ocean (%). Higher percentages represent a larger degree of fatty acid niche overlap and resource partitioning.

Tissue	Shark species	*Alopias superciliosus*	*Carcharhinus falciformis*	*Alopias pelagicus*	*Sphyrna zygaena*
Liver	*Prionace glauca*	0.6	9.5	6.8	0.0
	Alopias superciliosus		65.8	69.0	16.6
	Carcharhinus falciformis			61.1	5.0
	Alopias pelagicus				41.2
Plasma	*Prionace glauca*	11.9	45.7	56.4	32.3
	Alopias superciliosus		0.0	24.8	2.0
	Carcharhinus falciformis			11.5	0.0
	Alopias pelagicus				78.8
Muscle	*Prionace glauca*	0.0	0.0	0.8	0.0
	Alopias superciliosus		0.0	12.7	0.0
	Carcharhinus falciformis			0.0	67.2
	Alopias pelagicus				0.0

that liver were more consistently aligned to known prey profiles (Beckmann et al., 2014). In contrast, field studies that have analysed both prey and multiple tissues FA profiles, have shown that the liver differs most from prey (McMeans et al., 2012) and that the liver is more likely to reflect MUFA-rich prey while muscle is likely to group with PUFA-rich prey (Pethybridge et al., 2010). Similar to these field studies, we share concerns for the utility of liver tissue, due to the very high inter and intra variability observed in this study. This result isn't surprising given that the liver of sharks functions as a major site of FA catabolism (i.e., beta oxidation), ketone body biosynthesis, and buoyancy regulation (mediated via the retention of lipids) (Ballantyne, 1997; Davidson and Cliff, 2011a; Käkelä et al., 2009). In contrast, we found that muscle tissue had lower SDs for nearly all individual FAs and tighter clustering as visualised by the PCA suggesting that it enables a more accurate assessment of long-term prey and differentiation between species (Pethybridge et al., 2014; Regost et al., 2003). The notable variation in the plasma FA profiles detected in this study was similar to other shark plasma studies (Bierwagen et al., 2019) and was considered to reflect a high temporal variability that depended on the time elapsed since digestion of the last meal (McMeans et al., 2012). These results further emphasises that caution is required when interpreting FAs to infer aspects of a species trophic ecology and that comparative or monitoring studies should carefully consider the tissue analysed.

4.2. Dietary inferences based on FA trophic tracers

Essential FAs, such as C20:5n3 and C22:6n3, can only be synthesized by primary producers or bacteria, and are stored conservatively in the tissues of predators along the food chain, and can thus provide information on the items consumed by sharks (Kelly and Scheibling, 2012; Meyer et al., 2019; Stowasser et al., 2009). The presence of C20:5n3 indicates a diatom-based food web and serves as a trophic tracer for first-order carnivores, being maintained at relatively high levels in cephalopods (Dunstan et al., 1988; Kelly et al., 2009). In the present study, we detected statistical lower levels of C20:5n3 in muscle, plasma, and liver of *A. superciliosus* compared to all the other assessed shark species which all showed similar levels. There are a number of studies from around the world that have demonstrated the prevalence of cephalopods in the dies of sharks, and particularly for pelagic sharks (Smale and Cliff., 1998; Galván-Magaña et al., 2013; Rosas-Luis et al., 2016). The difference in *A. superciliosus*, seems to correspond well to a comparative dietary study, based on gut analysis, of many of the sharks assessed here that showed that fishes were a more important food source for *A. superciliosus* than cephalopods in the Mexican and Ecuadorian Pacific Ocean (Galván-Magaña et al., 2013). It has also been noted that *A. superciliosus* likely feeds in deeper and colder waters than the other assessed shark species (Musyl et al., 2011). In any case, it does seem as though *A. superciliosus* has a reduced connection to diatom-based food webs, which is also confirmed by the statistically lowest levels of other known diatom FA tracers (C16:1n7 and C14:0; Dalsgaard et al., 2003).

Another important trophic tracer is C22:6n3 (DHA) which characterises dinoflagellate based food webs and has been shown to indicate the degree of carnivory (Dalsgaard et al., 2003; Meyer et al., 2019). In the present study, we detected higher levels of C22:6n3 in muscle of the two thresher sharks (*A. superciliosus* and *A. pelagicus*) indicating that they may utilize dinoflagellate-dominated nutritional pathways and that they have a comparatively high trophic status in these pathways. The higher trophic position of these two species was further confirmed by ratios of DHA to EPA (Alderete-Macal et al., 2020; Varela et al., 2019) and are in line with stable isotope derived trophic position estimates for the assessed shark species (Méndez-Da Silveira et al., 2020).

We detected a high proportion of MUFAs, notably that of C18:1n9, in muscle of all the assessed shark species which is a strong trophic tracer of mesopelagic fish and cephalopods (Phillips et al., 2001; Meyer et al., 2019). It has also previously been reported that organisms inhabiting deep-sea regions are richer (up to 50% of total FAs) in C18:1n9 compared with specimens from either shallow warm or cold waters, reflecting an adaptive response to the high-pressure environment of deep waters (Hazel and Eugene Williams, 1990). We also detected significant differences amongst the five shark species with respect to the levels of C22:0 + C24:0, which can, as least in part, reflect a more coastal diet with greater contributions of terrestrial plants (Budge et al., 2001). Our observations of lower amounts of C22:0 + C24:0 in muscle of *P. glauca* is thus consistent with the exclusively oceanic life history of this species (Li et al., 2016). As reported, using stable carbon isotope values as a proxy for inshore versus offshore foraging habitat, lower $\delta^{13}C$ values of blue sharks would correspond to foraging in more oceanic regions, while silky sharks, with highest amounts of plasma C22:0 + C24:0, would feed more inshore (Rabehagasoa et al., 2012).

Habitat segregation by sex appears common among oceanic sharks likely owing to, among other factors, differences in age, body size, behaviour, and nutritional and reproductive requirements (Wearmouth and Sims, 2008; Hernández-Aguilar et al., 2016). In the present study, dietary-derived FAs of three tissues of *C. falciformis*, *S. zygaena* and *A. superciliosus* suggest that both sexes feed on similar resources and share foraging areas, in line with the stable isotope results from the same species and study area (Li et al., 2016). However, C22:6n3, C18:1n9 and C22:0 + C24:0 in liver of *A. pelagicus* and C22:6n3 and C20:5n3 in liver and muscle of *P. glauca* all showed significant differences between males and females, suggesting sexually different feeding habitats that are unrelated to life stage. The populations of *P. glauca* in the North Atlantic is known to exhibit spatial segregation of the sexes, the males tending to frequent coastal waters more intensively than the females, which are completely oceanic (Vandeperre et al., 2014). This may influence the level of C20:5n3 and C22:6n3 of *P. glauca*. Similarly, known gender differences in feeding zones and foraging behaviors of *A. pelagicus*, where larger females forage over larger zones while males stay closer to the bottom (Polo-Silva et al., 2013), likely explain gender differences in the trophic tracers C18:1n9 and C22:6n3. The only species to show some degree of habitat segregation by life history stage was for *S. zygaena*, with higher MUFA in the muscle and lower PUFA in the plasma of immature individuals. Dietary studies have indicated that immature *S. zygaena* feed primarily within the pelagic zone of shallow coastal

habitats while adults have a more dispersed foraging area over deep reefs at the edge of the continental shelf (Smale, 1991; Dicken et al., 2018).

4.3. Trophic niche metrics suggest resource partitioning among oceanic sharks

Trophic niche width is the most tractable and frequent studied measurement of niche space as it reflects the competitiveness of organisms with respect to the utilization of dietary resources and habitats (Gong et al., 2020; Lin et al., 2020; Pedro et al., 2020). Based on muscle tissue FA profiles, we established that *P. glauca* and *C. falciformis* have larger FA niche widths compared to other species analysed, which was considered to be indicative of their more generalized diets. Gut content analyses have revealed high diet diversity in *P. glauca* with indications that it feeds on cephalopods, teleost fishes, mammalian carrion, crustaceans, and even seabirds (Markaida and Soca-Nishizaki, 2010; Preti et al., 2012). In the case of *C. falciformis*, although we didn't find evidence to sex and development-derived differences in tissue FA profiles, general synopses of the biology and ecology of silky sharks have described them as opportunistic predators that feed on fishes, molluscs and crustaceans (Flores-Martínez et al., 2017; Duffy et al., 2015), which may be the reason of their broader FA niche widths.

Based on muscle FA profiles, the species with the relatively narrowest FA niche width was *A. pelagicus* and *S. zygaena* (Table 5) suggesting that they may have more specialized diets. This finding confers with gut content analysis that has established that both these species, in regions of the Mexican and Ecuadorian Pacific, preys predominantly on abundant *D. gigas*, and lanternfish, *Benthosema panamense* (Galván-Magaña et al., 2013; Polo-Silva et al., 2013). However, the most recently reported analysis of gut contents indicated that *A. pelagicus* also hunts on other species of squid and fish (Calle-Morán and Galván-Magaña, 2020). In contrast the species with the largest muscle FA niche width, suggesting the broadest diet composition, was *C. faliciformis* and *P. glauaca*, which interestingly were the two species that had the narrowest FA niche widths according to plasma FA profiles. Plasma serves as a medium to transport multifarious endogenous and exogenous FAs via lipoproteins to meet short and long-term physiological requirements (Ballantyne, 1997; Metcalf and Gemmell, 2005) and consequently results in more variable FA measurements than the long-term assimilations of FA profiles in the muscle (Bierwagen et al., 2019). Our multi-tissue result therefore suggests that there are large shifts in diet selection and the degree of diet generalisation within individuals over time, with all sharks capable of consuming a diversity of prey at least over the short term (Scharf et al., 2000).

An overlap of trophic niches can reflect the extent of interspecific similarity with respect to resource utilization, and consequently the likelihood of competitive relationships among species (Páez-Rosas et al., 2018; Gong et al., 2020; Lin et al., 2020; Pedro et al., 2020). In this study, we detected a high degree of overlap between the muscle FA niches of *C. falciformis* and *S. zygaena* thereby indicating a high probability of intense competition between these two congeners for similar resources. Both species were characterised by high levels of C20:4n6, C18:0 and C20:5n3 which could indicate consumption of mesopelagic or deeper dwelling fish (Meyer et al., 2019) and migrating crustaceans (Kelly et al., 2009). Both these species are considered to active in tropical and warm seas and primarily occupy and feed in the pelagic or epipelagic zones, mostly in the top 50 m of the sea surface and shows relatively little variability in this regard (Musyl et al., 2011; Galván-Magaña et al., 2013). Interestingly *C. falaformis* had a notably larger niche width than *S. zygaena* and could thus be considered a more generalist predator. There very limited evidence of overlap in muscle FA niches of other sharks is likely associated with the very distinct depth distributions of these species, as noted to some degree by Musyl et al. (2011), which leads to FA niche partitioning (Polo-Silva et al., 2013).

Our niche metric analysis, based on both muscle and liver tissue, suggested a high degree of dietary segregation by *P. glauca*, with overlap coefficients below 10% (Table 6). This is consistent with the findings of a previous study showing trophic niche partitioning between *P. glauca* and other sharks in the central-easter Pacific, which the authors attributed to the strictly oceanic life history of *P. glauca* (Li et al., 2016). According to Rabehagasoa et al. (2012), significant $\delta^{13}C$ differences between *P. glauca* and *C. falciformis* suggested niche partitioning between these species with *C. falciformis* having a more inshore foraging habitat than the other. Research looking at also would indicate that *P. glauca* have preference for feeding in warm mesopelagic waters and spend >95% of their time in temperatures ranging from 9.4 to 27 °C (Musyl et al., 2011). However, our findings for plasma FAs, would tend to indicate a much higher degree of resource partitioning between these sharks, at least in the short term.

5. Conclusion

This study expands our limited knowledge of the trophic ecology of pelagic and oceanic sharks and provides useful guidance for future studies wanting to use FAs to infer dietary resource partitioning between sharks. Through a comprehensive multi-tissue approach, we showed that FA profiles and their trends within and among species are unique for liver, plasma and muscle tissues which greatly impacts trophic conclusions. Similar to other FA studies of sharks, we concluded that the liver tissue would be unsuitable for FA studies on the trophic ecology of pelagic sharks given that it is a site of extensive FA modification, and thus high variability of dietary FA profiles. The FA profiles of plasma and muscle were considered to provide reasonable insights regarding short- and long-term dietary intake, respectively. We found evidence to support some degree of sex segregation in dietary derived FA for *A. pelagicus* and *P. glauca* and maturation differences in *S. zygaena*. We detected that there are large differences in the FA profiles that contribute to subtle differences in FA niche widths between species. We also found that there was a large degree of trophic niche overlap between *C. falciformis* and *S. zygaena* and dietary segregation of *P. glauca* which are in line with known similarities or differences in feeding habitats. These results on the trophic niche partitioning of oceanic pelagic sharks provides greater understanding into the way that these coexisting species may compete for available dietary resources and respond to environment change. To gain a more comprehensive multidimensional understanding of the feeding ecology of pelagic sharks, FA profile analyses should be used in combination with complementary approaches, such as stable isotope and molecular metabarcoding analyses, which would further increase our understanding of the key mechanisms underlying the coexistence of species within apex predator communities.

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CRediT authorship contribution statement

Min Xu: Investigation, Data curation, Writing – original draft, preparation. **Heidi R. Pethybridge:** Writing – review & editing, Project administration. **Yunkai Li:** Conceptualization, Supervision, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix

Table A1
Reproductive indices used for staging the maturity condition of sharks (Walker, 2005)

Organ	Index	Description	Binary maturity condition
Uterus	$U = 1$	uteri uniformly thin and white tubular structures; small ovaries and with no yolked ova	immature
	$U = 2$	uterus thin, tubular structure that is partly enlarged posteriorly; small yolked ova developing in ovary	immature
	$U = 3$	uterus uniformly enlarged tubular structure; yolked ova developing in ovary	mature
	$U = 4$	uterus enlarged with in utero eggs or embryos macroscopically visible: pregnant	mature
	$U = 5$	uterus enlarged, flaccid and distended tubular structure: postpartum	mature
Clasper	$C = 1$	pliable with no calcification	immature
	$C = 2$	partly calcified	immature
	$C = 3$	rigid and fully calcified	mature

Table A2
Summary statistical results (F and p-values) of five shark species from the tropical Eastern Pacific according to one-way ANOVAs. Values in bold are significant ($P < 0.05$).

Fatty Acids	Liver		Plasma		Muscle	
	F	P	F	P	F	P
C14:0	29.73	**0.00**	22.48	**0.00**	2.14	0.08
C16:0	3.64	**0.01**	15.75	**0.00**	20.33	**0.00**
C17:0	11.56	**0.00**	15.85	**0.00**	5.54	**0.00**
C18:0	3.06	**0.02**	19.98	**0.00**	33.02	**0.00**
C20:0	4.62	**0.00**	14.34	**0.00**	5.83	**0.00**
C22:0	1.84	0.13	15.79	**0.00**	3.51	**0.01**
C23:0	3.15	**0.02**	5.14	**0.00**	0.82	0.51
C24:0	2.67	**0.04**	14.46	**0.00**	2.92	**0.02**
∑SFA	2.17	0.08	14.13	**0.00**	2.34	0.06
C16:1n7	9.10	**0.00**	10.48	**0.00**	7.05	**0.00**
C18:1n9	4.11	**0.00**	20.12	**0.00**	5.46	**0.00**
C20:1	22.69	**0.00**	21.85	**0.00**	0.99	0.42
C22:1n9	50.23	**0.00**	8.82	**0.00**	3.69	**0.01**
C24:1n9	13.70	**0.00**	8.56	**0.00**	19.71	**0.00**
∑MUFA	7.94	**0.00**	7.87	**0.00**	7.99	**0.00**
C18:2n6	6.14	**0.00**	5.40	**0.00**	1.63	0.17
C20:2	12.71	**0.00**	1.74	0.15	4.30	**0.00**
C22:2n6	5.30	**0.00**	4.45	**0.00**	3.05	**0.02**
C20:3n3	3.32	**0.01**	2.26	0.07	10.56	**0.00**
C20:3n6	3.88	**0.01**	1.85	0.12	1.97	0.10
C20:4n6	7.32	**0.00**	9.76	**0.00**	18.76	**0.00**
C20:5n3	16.85	**0.00**	4.94	**0.00**	6.02	**0.00**
C22:6n3	4.00	**0.00**	28.00	**0.00**	23.14	**0.00**
∑PUFA	1.53	0.20	17.96	**0.00**	7.16	**0.00**
C22:0 + C24:0	2.92	**0.02**	28.33	**0.00**	20.86	**0.00**
DHA/EPA	20.88	**0.00**	20.60	**0.00**	26.28	**0.00**

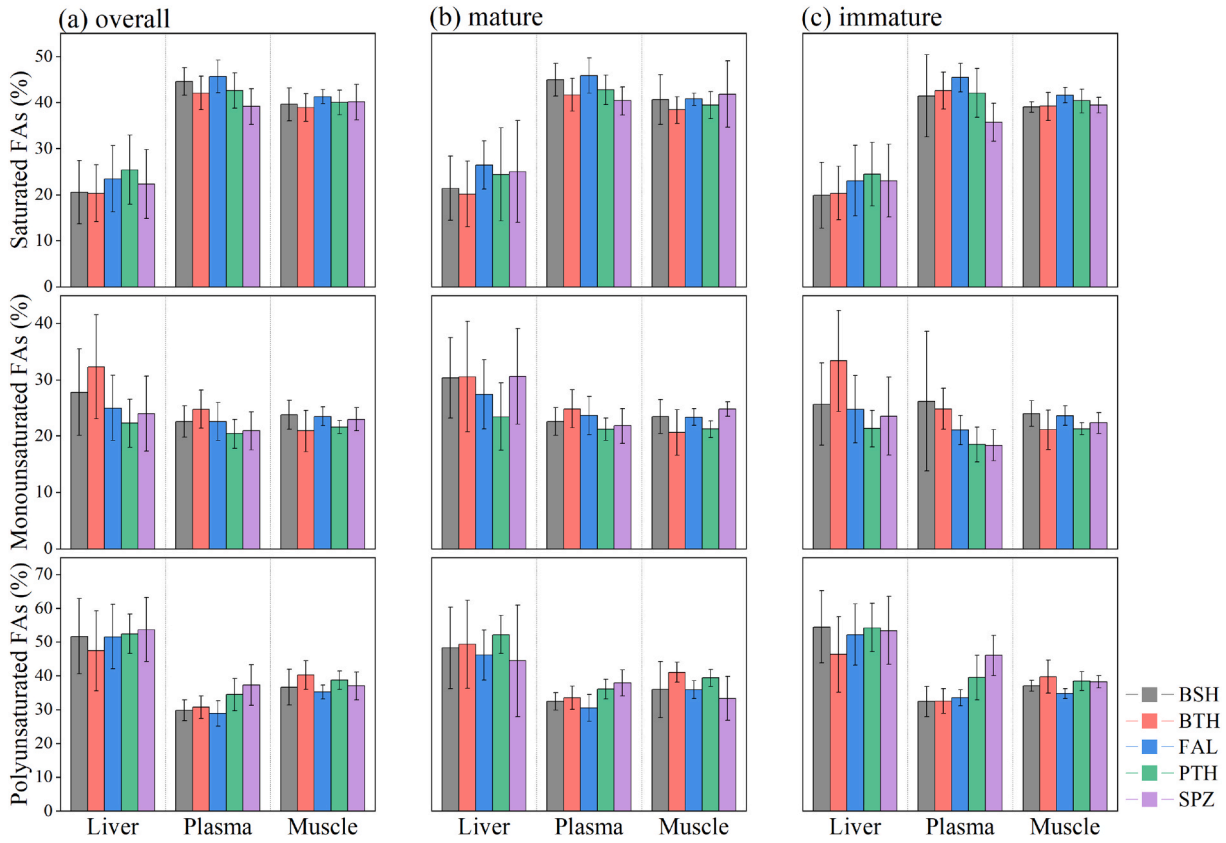

Fig. A1. Comparison of the relative means (±standard deviation) of saturated, monounsaturated, and polyunsaturated fatty acid (FA) profiles based on the liver, plasma, and muscle tissues taken from five shark species *Prionace glauca* (BSH), *Alopias superciliosus* (BTH), *Carcharhinus falciformis* (FAL), *Alopias pelagicus* (PTH) and *Sphyrna zygaena* (SPZ) from the tropical eastern Pacific Ocean.

Table A3
Summary statistical results (p-values) of the effect of maturation (P1) and sex (P2) on fatty acid trophic tracers of five shark species from the tropical Eastern Pacific according to non-parametric Kruskal-Wallis tests. Values in bold are significant ($P < 0.05$). BSH: blue shark (Prionace glauca), BTH: bigeye thresher shark (Alopias superciliosus), FAL: silky shark (Carcharhinus falciformis), PTH: pelagic thresher (Alopias pelagicus), SPZ: scalloped hammerhead shark (Sphyrna zygaena).

Tissues	Species	C22:6n3		C20:5n3		C20:4n6		C18:1n9		C22:0 + C24:0		DHA/EPA	
		P1	P2	P1	P2	P1	P2	P1	P2	P1	P2	P1	P2
Liver	BSH	0.36	**0.04**	0.23	**0.01**	0.59	1.00	0.98	0.06	0.31	0.98	0.23	0.01
	BTH	0.92	0.66	0.74	0.23	0.34	0.33	0.74	0.18	0.63	0.86	0.50	0.16
	FAL	**0.00**	0.73	0.25	0.70	0.27	0.92	0.35	0.77	0.00	0.88	0.74	0.63
	PTH	0.30	**0.00**	0.88	0.23	0.50	0.47	0.88	0.01	0.46	0.00	0.33	0.94
	SPZ	0.08	0.71	0.06	0.13	0.03	0.85	0.17	0.36	0.10	0.54	0.02	0.50
Plasma	BSH	0.75	**0.02**	0.67	0.36	0.79	0.15	0.82	0.73	0.93	0.66	0.89	0.04
	BTH	0.18	0.48	0.94	0.22	0.07	0.72	0.07	0.17	0.18	0.35	0.29	0.51
	FAL	0.10	0.73	0.90	0.05	0.14	0.37	0.19	0.85	0.16	0.07	0.08	0.36
	PTH	0.32	0.52	0.41	0.72	0.62	0.43	0.32	0.43	0.93	0.10	0.68	0.43
	SPZ	**0.00**	0.12	0.27	0.81	0.02	0.58	0.04	0.16	0.00	0.62	0.01	0.58
Muscle	BSH	0.83	**0.01**	0.15	**0.05**	0.24	0.59	0.06	0.74	0.93	0.11	0.80	0.08
	BTH	0.31	0.29	0.33	0.89	0.88	0.16	0.18	0.08	0.08	0.55	0.20	0.40
	FAL	0.90	0.25	0.06	0.19	0.00	0.19	0.76	0.22	0.58	0.25	0.45	0.17
	PTH	0.94	0.29	0.71	0.67	0.60	0.32	0.94	0.26	0.71	0.32	1.00	0.37
	SPZ	**0.00**	1.00	0.74	0.64	0.13	0.35	0.74	0.50	0.00	0.09	0.02	0.68

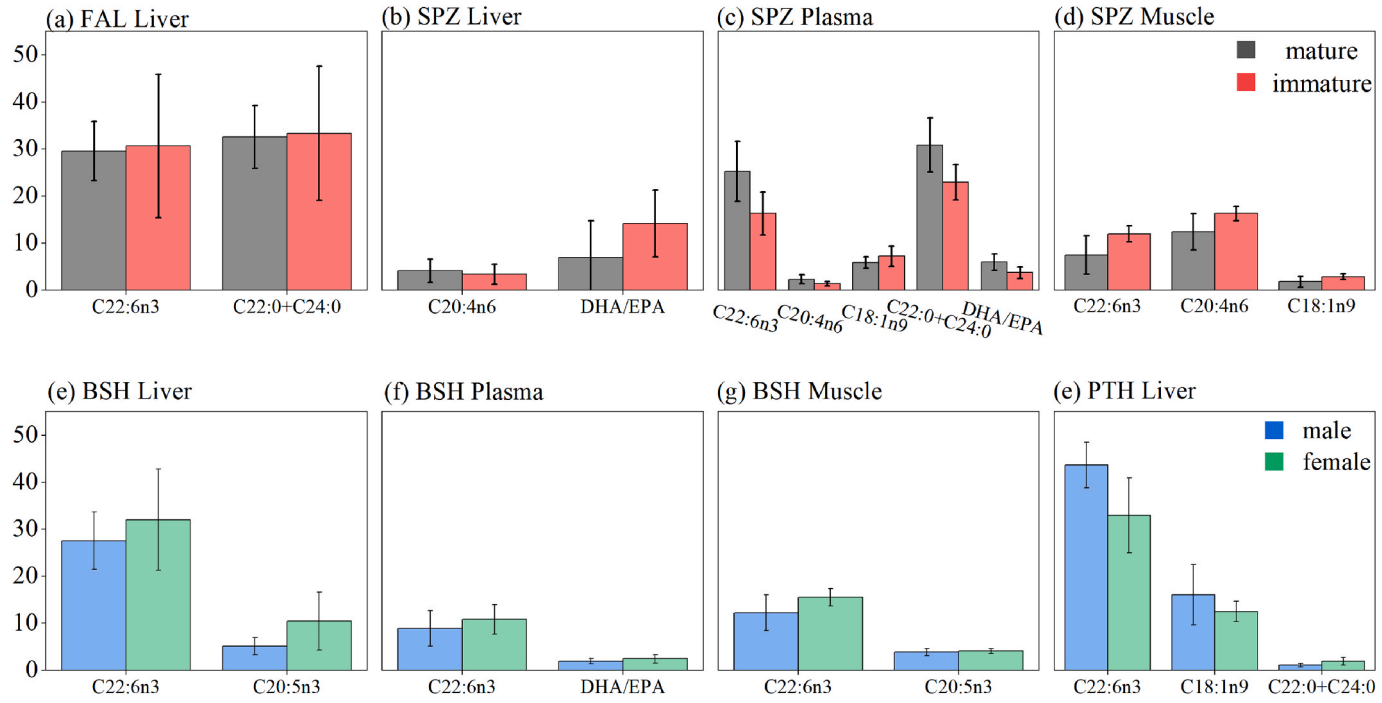

Fig. A2. Comparison of the relative means (±standard deviation) of dietary FAs having significant differences between maturation and sex based on the liver, plasma, and muscle tissues taken from five shark species *Prionace glauca* (BSH), *Alopias superciliosus* (BTH), *Carcharhinus falciformis* (FAL), *Alopias pelagicus* (PTH) and *Sphyrna zygaena* (SPZ) from the tropical eastern Pacific Ocean.

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