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Monsoons and habitat influence trophic pathways and the importance of terrestrial-marine linkages for estuary sharks

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Abstract. Tropical estuaries often receive enhanced fluxes of terrestrial derived organic matter and phytoplankton during the wet season, and such monsoonal events may significantly influence the trophic dynamics of these systems. This study examined spatio-temporal terrestrial-marine linkages in a tropical estuary, the Pearl River Estuary (PRE), southern China, by investigating trophic pathways leading to estuary sharks. We investigated spatial (inshore vs. offshore) and seasonal (wet vs. dry season) variation in the relative importance of terrestrial- and marine-derived carbon, so as to assess the contribution of detrital pathways to the pelagic spadenose shark, *Scoliodon laticaudus*; ontogentic changes in shark diets were also documented. Stable isotope analyses (SIA) and fatty acid (FA) profiling indicated that spadenose sharks assimilated both marine and terrestrial carbon via consumption of zooplantivorous fish and shrimps. Detrital carbon sources were more important to juvenile and pre-mature sharks at inshore locations, especially during the wet season when river discharge increased and terrestrial detritus was more abundant. Ontogenetic dietary shifts were evident: juvenile and pre-mature sharks had significantly higher levels of bacterial (detrital) FA than adults which contained more animal-derived FA. Inshore sharks, with more depleted $\delta^{13}C$ signatures, relied more on terrestrial carbon than sharks offshore. Comparison of spadenose shark FA profiles with those of the sympatric, white-spotted bamboo shark (*Chiloscyllium plagiosum*)—a benthic predator that acquires detrital carbon via consumption of polychaetes and crustaceans—revealed that they made greater use of detrital carbon sources. However, spadenose sharks in the inner estuary assimilated higher proportions of terrestrial detritus (44–56%) than bamboo sharks (31–45%). The importance of terrestrial detritus for both shark species demonstrated the important contribution of terrestrial detritus to both pelagic and benthic food webs in the PRE. Terrestrial-marine linkages are therefore of great significance, particularly during the wet season, in this estuarine system, which serves as feeding and nursery grounds for both shark species, and trophic subsidies from land are likely to be important for marine predators in other tropical estuaries.

Key words: *Chiloscyllium plagiosum;* compound-specific stable isotopes; detritus; fatty acids; food webs; marine predators; mixing model; monsoonal climate; Pearl River Estuary; *Scoliodon laticaudus;* trophic subsidy; zooplankton.

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Introduction

Estuaries are highly productive ecosystems and are globally significant in terms of their ecological and economic functions, including production of fishery resources (Day 1989, Blaber 1997, Kennish 2004). Fish and fishery production are largely determined by estuarine trophic dynamics (i.e., primary productivity and distribution and availability of carbon sources), which vary according to the specific physico-chemical, and geographic settings of particular estuaries, as well as the biodiversity they host (Salen-Picard et al. 2002, Darnaude et al. 2004, Duarte and García 2004). Furthermore, any understanding of resource distributions within and between estuaries is dependent on knowledge of the underlying trophic dynamics in these ecosystems (Kennish 2004).

Estuaries are temporally and spatially complex, heterogeneous systems where in situ autochthonous production is supplemented by a mixture of exogenous carbon subsidies from both marine and terrestrial sources (Mann 1988, Colombini and Chelazzi 2003, Van den Meersche et al. 2009). Such subsidies may include dissolved or particulate organic matter, including leaf litter from rivers and mangroves (Paterson and Whitfield 1997, Mfilinge et al. 2005), as well as marine-derived organic matter from offshore plankton and adjacent habitats such as seagrass meadows and kelp forests (Boschker et al. 2000, Norderhaug et al. 2003, Bouillon et al. 2004). The complex dynamics of trophic subsidies and relative importance of terrestrial and marine carbon sources in most estuarine habitats are, however, case-specific (Chanton and Lewis 2002, Connolly et al. 2005, Heck et al. 2008), varying at multiple temporal and spatial scales, and according to the feeding ecologies of specific marine assemblages (Darnaude et al. 2004, Mcleod and Wing 2009, Schlacher et al. 2009).

The great majority of investigations of organic matter sources and energy flow in estuaries (including most studies cited above) have been conducted in temperate regions (Peterson and Fry 1987, Boschker et al. 2005, Van den Meersche et al. 2009). There, local primary production is often less than community respiration (Pace et al. 2004, del Giorgio and Pace 2008), and subsidies of allochthonous organic matter from terrestrial sources often supplement local autochthonous sources such as phytoplankton (Hoffman et al. 2007, 2008, Van den Meersche et al. 2009, Babler et al. 2011). In contrast to temperate regions, however, the origins of the organic matter sustaining food webs and secondary production in most tropical estuaries are largely unknown (Blaber 2000, Barros et al. 2010, Wai et al. 2011*a*).

Tropical estuaries in regions experiencing monsoonal climates are influenced by highly seasonal river flows and are often characterized by enhanced fluxes of terrestrial derived organic matter, phytoplankton blooms and subsequent phyto-detritus deposition during the wet season (Eyre and Balls 1999, Eyre and Ferguson 2006, Murrell et al. 2007). We postulate that such monsoonal events significantly influence the trophic dynamics in tropical estuarine systems. To test this, we examined terrestrial-marine linkages in the tropical Pearl River Estuary (PRE), in Guangdong Province southern China, and seasonal differences in the trophic pathways leading to estuary sharks.

Conditions in the PRE and adjacent marine waters are strongly affected by seasonal flow fluctuations of the Pearl River (Zhu Jiang) which is the second largest river in China and the 13th largest river in the world in terms of its annual water discharge. Its average annual discharge is $\sim 330 \times 10^9$ m^3 yr^{-1} with a sediment load of $\sim 80 \times 10^9$ kg yr^{-1} (Ip et al. 2004), but over 80% of this discharge and 95% of sediment transport occurs during the wet summer monsoon (Kot and Hu 1995). The PRE is an important sink for sediments and nutrients originating from the terrestrial landscape (Hu et al. 2006*a*, *b*, Zhang et al. 2009). As in most estuarine systems worldwide, the PRE has been increasingly impacted by anthropogenic activities in recent decades; sedimentary records of carbon distribution have shown a long-term increase in deposition of terrestrial organic matter since the 1940s (Hu et al. 2006*a*, 2008), some of which may be due to deforestation and land-use change (Huang et al. 2003). Due to the monsoonal climate of southern China, the relative importance of autotrophic and detrital pathways (i.e., recycling of marine and terrestrial materials) in the estuary can be expected to vary temporally (wet summer southwest monsoon vs. dry winter northeast monsoon) and spatially (inshore vs. offshore).

During the wet season, both allochthonous terrestrial detritus and autochthonous marine detritus are available, due to the seasonal die-back and decomposition of macroalgae (especially *Sargassum* spp.) when water temperatures rise from May through June at the beginning of summer (Kaehler and Williams 1996, Huang et al. 2004). Phytoplankton production peaks during the wet season (Huang et al. 2004, Lan et al. 2009) and also contributes to the detrital pool (Tan et al. 2004). Mass-balance models suggest that detritus from various sources is important in supporting food webs in the PRE and adjacent waters (Li and Lee 1998, Duan et al. 2009), and although consumers at lower trophic levels generally depend on autotrophic carbon (Lee 2000, Chen et al. 2008, Wai et al. 2008, 2011*b*), they make use of a mixture of allochthonous and autochthonous energy sources during the wet summer monsoon, when availability of detritus derived from terrestrial litter, macroalgae and phytoplankton peaks (Wai et al. 2008).

The importance of terrestrial carbon to higher trophic levels has recently been demonstrated for the white-spotted bamboo shark *Chiloscyllium plagiosum* (Hemiscylliidae), a common benthic predator in the PRE, and allochthonous energy was particularly important for juvenile *C. plagiosum* within the inner estuary nursery grounds (Wai et al. 2011*a*). The spadenose shark, *Scoliodon laticaudus* (Carcharhinidae), is sympatric with bamboo sharks in the PRE, and occurs widely in the coastal waters of the Indo-West Pacific (White and Sommerville 2010). Little is known of its trophic ecology (Lam and Sadovy de Mitcheson 2010) and, although its functional morphology suggests that it is a pelagic predator (see Cortés et al. 2008), both pelagic and benthic prey have been found in stomachs of *S. laticaudus* from Indian coastal waters (Devadoss 1989, Mathew and Devaraj 1997). The diet of these two shark species, may, therefore, overlap in the PRE.

We studied the feeding ecology of spadenose sharks by first describing the major prey items of this shark using stomach content analyses, and subsequently applying assimilation-based methods to trace the carbon sources and trophic pathways utilized by the spadenose sharks. A combination of three assimilation-based methods (stable isotope analyses (SIA), fatty acid profiling (FAP), and FA-specific SIA (FASIA)) were used to investigate the relative importance of terrestrial (i.e., leaf litter) and marine (i.e., macroalgae and phytoplankton) carbon sources and to elucidate the utilization of detritus derived from these sources, as has been revealed for the bamboo shark (see Wai et al. 2011*a*). Given the substantial seasonal input of terrestrial organic matter from the PRE basin during the wet, summer monsoon, we predicted that the carbon utilization of prey and spadenose sharks would vary according to season (wet vs. dry season) and location (inner vs. outer estuary). We specifically hypothesized that detrital food sources and especially the contribution of allochthonous carbon sources for the spadenose sharks would be more important during the wet season than in the winter, dry season, especially in the inner PRE where the supply of terrestrial organic matter is higher (Zhang et al. 2009, Wai et al. 2011*a*). We also assessed the potential importance of the estuary as a nursery ground for the spadenose shark, by comparing sharks at different life stages (from embryonic pups to adults) to test for ontogenetic shifts in food sources and thus in changes in the relative dependence upon detrital pathways. Finally, these data were used to investigate the relative importance of terrestrial and marine detrital carbon to pelagic and benthic food chains in the PRE, by comparing the diets of, and trophic pathways utilized by, pelagic spadenose sharks and benthic bamboo sharks.

Methods

Study site and sample collection

The coastal waters around Hong Kong (22°22′ N and 114°09′ E; Fig. 1) are strongly influenced by its monsoonal climate. Mean seawater temperatures (27–28°C in summer vs. 16–17°C in winter) and monthly total rainfall (159.2–1346.1 mm vs. 0.3–120.7 mm) are much higher during the wet, summer monsoon (June to September) than the dry, winter monsoon (December to March, Hong Kong Observatory 2008–2010; http://www.hko.gov.hk/cis/climat_e.htm). Based on the collective results of a series of water and sediment chemistry studies (see review by Wai et al. 2011*a*), the PRE can be divided into an inner and outer estuary. The inner estuary is strongly influenced by seasonal river runoff and fluctuations in the supply of allochthonous terrestrial

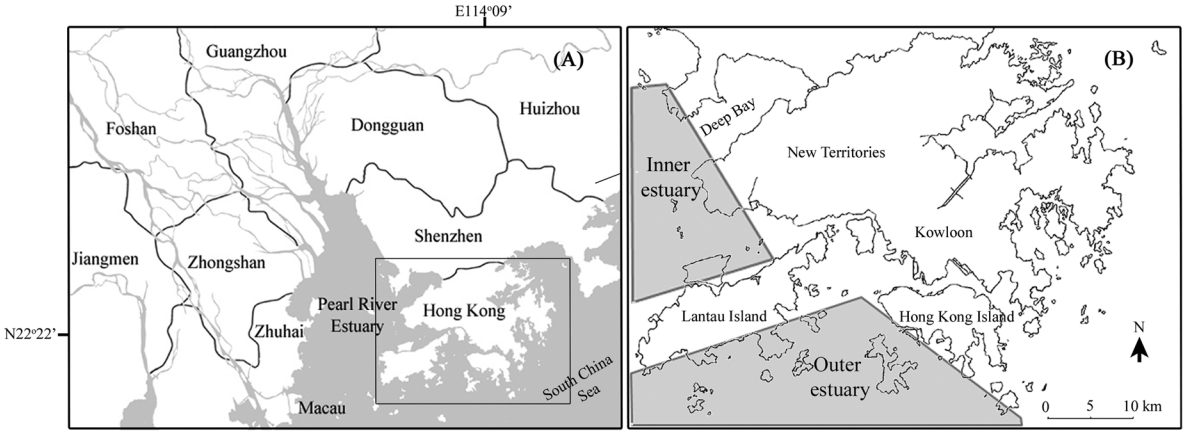

Fig. 1. Maps of (A) Pearl River Delta, southern China and (B) Hong Kong showing the foraging areas of spadenose sharks in the inner and outer Pearl River Estuary within coastal waters to the south and west of Hong Kong.

carbon, while the relative abundance of autochthonous carbon sources is higher in the outer estuary (Wai et al. 2011*a*).

Spadenose sharks were obtained from local fish markets in Hong Kong. The sharks were caught by stern trawling, beam trawling and purse seining, and their capture location was confirmed with reputable fishermen or market dealers who were familiar with our requirements and studies. Subsequently, sharks were separated according to whether they were collected from the inner or outer PRE (Fig. 1). Sampling was conducted in summer (June to September) and winter seasons (December to March) between 2008 and 2010. Sharks were further divided into five size classes to investigate ontogenetic variation: (1) pups (embryonic phase, collected from the uterus of pregnant females; 13–15 cm total length (TL)), (2) young-of-the-year (YOY, age 0+ shark; ~20 cm TL), (3) juvenile (25–35 cm TL), (4) pre-mature (36–45 cm TL) and (5) adult (>45 cm TL) sharks. Samples of potential prey items were also collected in the inner and outer estuary by trawling, purse seining, plankton net (>500 μm) and sediment grabs (Fig. 1; see Lui et al. 2007 for sample locations). In addition to spadenose sharks, bamboo sharks were also collected from the same locations during the same sampling period (see Wai et al. 2011*a*). Samples were frozen at −20°C prior to analysis.

All sharks were measured (TL ± 0.1 cm and BW ± 0.1 g) before dissection. Liver weight was measured to determine liver somatic index (LSI) as follows: LSI = (liver weight/total body weight) × 100% (Hussey et al. 2009). Shark prey items were determined from stomach content analyses (Hyslop 1980, Cortés 1997); all ingested prey items were identified to the lowest possible taxonomic level and wet weighed (±0.001 g). The relative importance of specific prey was expressed as % weight of total biomass of all prey (see results in Appendices A and B). Multivariate analyses, using PRIMER 6 and PERMANOVA+ (PRIMER-E; Anderson et al. 2008), were used to determine seasonal and spatial variation in dietary composition as represented by % weight of different prey items (Appendices A and B). Ontogenetic variation in stomach contents was not investigated due to the small number of juvenile sharks that had identifiable prey items in their stomachs.

Stable isotope analysis (SIA)

Dorsal muscle tissues of sharks and their fish prey, and whole muscle tissues of other prey items, were used for SIA. Except for the sharks, where single individuals were used as replicates, 3–4 individuals of each prey species or items were pooled as replicates for analysis. Animal tissues were oven-dried (for 72 h at 45°C) and ground to a fine powder. Animal samples were not acid treated, as this procedure has been shown to be inappropriate for taxa with low carbonate contents (Ng et al. 2007). The isotopic

ratios of dried samples ($R = {}^{13}C:{}^{12}C$ or ${}^{15}N:{}^{14}N$) were analyzed using a PDZ Europa 20–20 isotope ratio mass spectrometer (Sercon) at the UC Davis Stable Isotope Facility (Department of Plant Sciences, University of California, Davis), and reported in standard delta (δ) notation ($\delta^{13}C$ or $\delta^{15}N$), defined as parts per thousand (‰) deviation from a standard (Vienna Pee Dee belemnite for $\delta^{13}C$ and atmospheric nitrogen for $\delta^{15}N$): $\delta^{13}C$ or $\delta^{15}N = ((R_{sample}:R_{standard}) - 1) \times 1000$ (Peterson and Fry 1987). The analytical precision (as standard error for repeated measurements of the internal standards, $n = 10$) for the measurement was 0.02‰ for $\delta^{13}C$ and 0.17‰ for $\delta^{15}N$. Molar carbon to nitrogen (C:N) ratios were also calculated for each sample. Randomly selected subsets of sharks ($n = 10$) and prey samples ($n = 10$) were lipid-extracted and the relationship between changes in $\delta^{13}C$, % lipid (see below) and C:N ratio established, and used to correct for the lipid effect on $\delta^{13}C$ using the modeling methods described in Post et al. (2007).

Fatty acid profiling (FAP)

Lipids were extracted from aliquots of the samples following the 2:1 (volume : volume, V:V) chloroform-methanol method modified from Bligh and Dyer (1959), and the lipid content (%) of each sample was determined. Fatty acids of total lipids were transesterified to methyl esters with 6% (V:V) sulphuric acid (H_2SO_4) in methanol, and all prepared fatty acid methyl esters (FAMEs) were stored in nitrogen at −20°C before analysis. FAMEs were analyzed using gas chromatography equipped with mass spectrometry (GC-MS, Hewlett Packard 6890 series; see Wai et al. 2011*a* for operation conditions). FAMEs were identified by GC-MS, and also by comparison of GC peaks with the retention times of authentic standards (Supelco, USA and Alltech, Ireland). Each fatty acid was expressed as a relative percentage of the total fatty acids identified in a sample and designated by short-hand nomenclature X:YnZ, where X is the number of carbon atoms, Y is the number of double bonds, and Z is the position of the ultimate double bond from the terminal methyl group. FA biomarkers used to identify food sources were based on the taxonomically specific FA biomarkers documented in the reviews by Meziane et al. (1997), Kharlamenko et al. (2001), and Mfilinge et al. (2005) and a local FA database (T.-C. Wai, *unpublished data*).

Compound-specific fatty acid SIA (FASIA)

Aliquots of FAME samples were subject to FASIA at UC Davis Stable Isotope Facility. Stable isotope ratios of $\delta^{13}C$ in FAMEs were analyzed using a Thermo gas chromatography combustion isotope ratio mass spectrometer (GCC-IRMS) system composed of a Trace GC Ultra gas chromatograph coupled to a Delta Plus Advantage IRMS through a GCC-III interface (Thermo Electron; see Wai et al. 2011*a* for operation conditions). $\delta^{13}C$ values were corrected using working standards composed of several FAMEs calibrated against standard reference materials. $\delta^{13}C$ values of FAMEs were converted to fatty acids by correcting for the one carbon methyl group addition during derivatization (Boschker and Middelburg 2002). FASI values for detritus from primary sources, terrestrial, macroalgal (*Sargassum hemiphyllum*) and phytoplankton (i.e., particulate organic matter <125 μm) sources were obtained from a parallel study (Wai et al. 2011*a*) and used in Bayesian mixing models (see below).

Stable isotope mixing models

Bayesian mixing models using Stable Isotope Analysis in R (SIAR version 4.0, Parnell et al. 2008), which takes into account consumer and source isotopic variability (Parnell et al. 2010), were used to quantify the contribution of various potential food sources to the diet of juvenile, premature and adult sharks. The food sources used in each calculation were selected according to stomach content analyses and the consequent formation of mixing polygons (indicated by dashed lines in Fig. 2; Post 2002) was based on the stable isotopic (SI) values of the sharks and their prey (Phillips et al. 2005). To investigate spatial variation in food source utilization, potential prey items sampled in the inner and outer estuary were used to calculate their contribution to sharks sampled from each of these areas. Before calculations, $\delta^{13}C$ and $\delta^{15}N$ values of the consumers were corrected for isotope fractionations of 1‰ and 2.5‰, respectively (Vander Zanden and Rasmussen 2001, McCutchan et al. 2003, Wai et al. 2011*a*) and $\delta^{13}C$ values corrected for lipid effect using modeling methods (see above). Relative contributions of

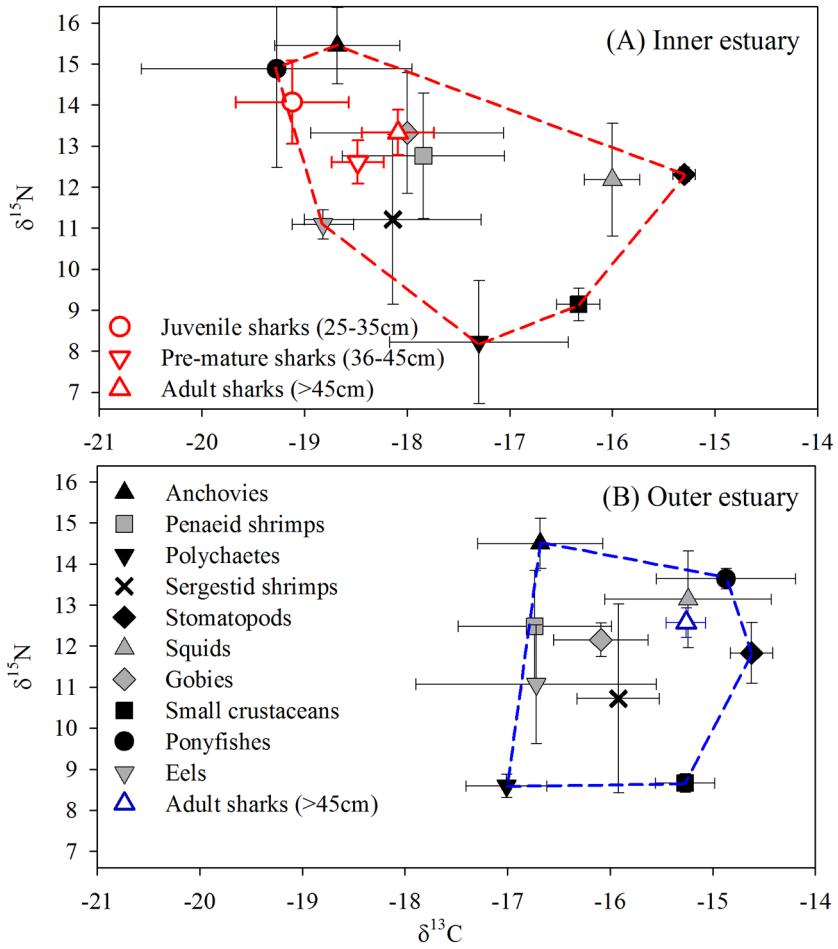

Fig. 2. Mean $\delta^{13}C$ and $\delta^{15}N$ values (‰, ± SD) of potential food sources of spadenose sharks from (A) the inner estuary and (B) the outer Pearl River Estuary (both seasons combined). Note that $\delta^{13}C$ and $\delta^{15}N$ values of sharks were back calculated for one lower trophic level to compute mixing models (see Methods). Dashed lines connecting potential food sources highlight mixing polygons; shark isotopic signatures should be surrounded by potential food sources in the mixing polygon, and hence their isotopic values were included in mixing models. The relative contribution of each food (%) to juvenile (J), pre-mature (P) and adult (A) sharks is shown in Table 5.

each food source were reported as mean and 95% credibility intervals (Parnell et al. 2010).

SIAR was also used to quantify the relative contribution of terrestrial, macroalgal and phytoplankton detritus to juveniles and adults of both spadenose and bamboo sharks in the inner estuary, based on the $\delta^{13}C$ values of four bacterial FAs (i.e., i, a15:0, i17:0, and 18:1n7; taken from Wai et al. 2011*a*). Given that the degree of diet-consumer fractionations of specific FA compounds is still not well understood (Boschker and Middelburg 2002, Londry et al. 2004), the $\delta^{13}C$ values of bacterial FA were not corrected for fractionation. The $\delta^{13}C$ values of individual bacterial FA of each potential detrital (carbon) source were directly used in the mixed models for calculation, while no back calculation (of a mean −3‰ carbon fractionation; Williams et al. 2009) was needed to estimate the bulk $\delta^{13}C$ values of whole bacteria in each source. As isotopic depletion mainly occurs only via metabolic fractionation of source carbon during bacterial FA synthesis by microbes (Boschker et al. 2005), and bacterial FA cannot be biosynthesized by most marine invertebrates and fish including sharks (Budge et al. 2002), direct

Table 1. Mean (± SD) of $\delta^{13}C$, $\delta^{15}N$, molar C:N ratio, liver somatic index (LSI) and lipid content of muscle tissue of spadenose shark size classes in different locations (inner and outer estuary) in (A) wet and (B) dry seasons. † LSI of shark pups was not determined. YOY, young-of-the-year. N = number of replicates of each group.

	Inner estuary					Outer estuary	
Variable	Pups	YOY	Juveniles	Pre-mature	Adults	Pre-mature	Adults
A) Wet season							
N	18	13	24	36	23	14	31
$\delta^{13}C$ (‰)	−16.21 ± 0.29	−16.31 ± 0.35	−18.12 ± 0.53	−17.51 ± 0.25	−16.98 ± 0.28	−14.29 ± 0.19	−14.26 ± 0.19
$\delta^{15}N$ (‰)	15.01 ± 0.36	15.49 ± 0.40	16.57 ± 1.01	15.04 ± 0.50	15.56 ± 0.33	14.87 ± 0.17	15.22 ± 0.43
C:N ratio	3.54 ± 0.06	3.65 ± 0.08	3.48 ± 0.10	3.42 ± 0.05	3.42 ± 0.13	3.48 ± 0.09	3.42 ± 0.12
LSI†	...	7.07 ± 3.09	4.54 ± 0.83	4.09 ± 1.26	5.36 ± 1.31	5.23 ± 1.24	4.21 ± 1.39
% Lipid	5.72 ± 0.43	3.96 ± 0.84	2.91 ± 0.40	2.60 ± 0.61	2.32 ± 0.62	3.01 ± 0.69	2.86 ± 0.88
B) Dry season							
N	...	...	...	19	20	3	16
$\delta^{13}C$ (‰)	...	...	...	−17.36 ± 0.36	−17.03 ± 0.31	−14.29 ± 0.14	−14.22 ± 0.21
$\delta^{15}N$ (‰)	...	...	...	15.72 ± 0.59	16.16 ± 0.57	14.82 ± 0.05	14.98 ± 0.23
C:N ratio	...	...	...	3.24 ± 0.04	3.26 ± 0.06	3.36 ± 0.03	3.35 ± 0.04
LSI†	...	...	...	4.45 ± 0.79	4.98 ± 1.26	6.22 ± 0.97	5.51 ± 1.03
% Lipid	...	...	...	4.45 ± 2.30	3.05 ± 1.63	2.49 ± 0.28	2.53 ± 0.43

assimilation of bacterial FA by consumers without carbon fractionation was assumed in this study (see also Wai et al. 2011*a*).

Statistical analyses

Ontogenetic differences of stable isotopic values ($\delta^{13}C$ and $\delta^{15}N$), and also condition indices of sharks such as molar C:N ratios, % lipid content, and LSI were compared using General Linear Models (GLM), followed by Student-Newman-Keuls (SNK) tests for multiple comparisons. Five size classes of sharks (Table 1) collected from the inner estuary during the wet summer were used. As preliminary studies showed no sex difference in SI signatures or FAP of spadenose sharks (T.-C. Wai, *unpublished data*), sex variation was not included as a factor in any analysis. Seasonal (wet summer vs. dry winter), spatial (inner vs. outer estuary) and size (pre-mature vs. adult) differences in stable isotopic values ($\delta^{13}C$ and $\delta^{15}N$), and also condition indexes (C:N ratios, % lipid content, and LSI) were compared. As no small sharks (<35 cm) were available from the outer estuary, only two size classes were used in this test. Prior to analysis, raw data were tested for homogeneity of variances (Levene's test). If data showed heterogeneous variances, GLM was performed on untransformed data but results were interpreted with a conservative significance level ($\alpha = 0.01$). Spatial differences in the food sources (Appendix C) from the inner and outer estuary were tested using Student's *t*-tests.

In addition to univariate analyses, multivariate analyses were performed to determine (1) ontogenetic, and (2) seasonal, spatial and ontogenetic variations in FA profiles (i.e., relative % weight of 34 FAs) (see Appendices F and G for a list of FA variables and biomarkers). To visualize multivariate patterns, principle coordinates analyses (PCoA) were performed on FAP data using PRIMER 6 and PERMANOVA+ (PRIMER-E; Anderson et al. 2008). One-way Permutational Analysis of Variance (PERMANOVA) was used to investigate observed ontogenetic variation while a three-way PERMANOVA was used to investigate seasonal, spatial and ontogenetic patterns (as GLM above), with test statistics computed after 9999 permutations. Multivariate analyses applied on FAP data were based on Bray-Curtis dissimilarity matrices calculated from square-root transformed data. The FA biomarkers that contributed most to the observed group separations were identified based on the Spearman's correlation coefficient (r_s) of the relationship between their values and those of the PCo axes. Only FA biomarkers with $r_s > 0.4$ (or less than −0.4) along any of the first three PCo axes were chosen for further univariate analyses and shown in the PCo plots. Ontogenetic, seasonal and spatial differences in FA biomarkers were compared using GLM as described above. According to the PCoA (see Results), the ontogenetic shifts in shark SI values, FAP and diet revealed two developmental stages: an early life phase from pups through young-of-the-year to juvenile, and a pre-mature to adult phase.

Relationships between individual FA biomarkers (% weight) and shark size (TL) were further analyzed by separate Spearman's correlation analyses for each of these two phases.

Species variation in both FA profiles (i.e., relative % weight of 34 FAs; see Appendix H) and FA-stable carbon isotopic (FASI) values (i.e., $\delta^{13}C$ values of 25 FA; Appendix I) of the juveniles and adults of both spadenose and bamboo sharks were investigated. Multivariate analyses applied to FASI data were based on untransformed, Euclidean distance matrices. Two-way PERMANOVA was used to investigate the observed species and size variation in PCo plots and subsequently individual biomarkers which contributed most to the observed species and size difference were further analyzed by GLM.

Results

Potential prey of sharks

Among the 526 spadenose sharks sampled, only 179 individuals (~34%) had identifiable stomach contents. Frequent prey items of the spadenose sharks were pelagic teleosts such as anchovies and sardines; demersal teleosts such as gobies, ponyfishes, eels and croakers (see Appendices A and B for a species list), and invertebrate prey represented mainly by penaeid and sergestid shrimps, as well as stomatopods, polychaetes and squids. Small amounts of macroalgae and terrestrial plant material were occassionally present (Appendices A and B).

Shark dietary composition showed significant seasonal (pseudo-$F_{1, 175} = 4.04$, $P < 0.01$) and location (pseudo-$F_{1, 175} = 2.60$, $P < 0.05$) differences but no interaction (pseudo-$F_{1, 175} = 0.94$, $P > 0.05$, 2-way PERMANOVA). Pelagic teleosts (particularly anchovies) and sergestid shrimps (*Acetes* sp.) were dominant prey items in both seasons (Appendices A and B). The dominant species of anchovies, however, varied with season; with the biomass of *Thyssa* sp. being higher during the dry season, whilst *Stolephorus insularis* biomass was higher in the wet season (see GLM results in Appendix A). Dermersal teleosts, particularly ponyfishes, were the dominant prey in the dry season (>wet; Appendix B). Gobies were only found in shark stomachs in the inner estuary. The biomass of eels was low but they were consistently found in shark stomach contents in both locations during both seasons (Appendices A and B). The total biomass of pelagic teleosts in the shark diet was higher in the inner than outer estuary, while the biomass of crustaceans (particularly sergestid shrimps) was relatively lower in the inner estuary (Appendices A and B).

Variation in stable isotope signatures of food sources

The $\delta^{13}C$ values of anchovies, ponyfishes, gobies, eels, sergestid and penaeid shrimps, stomatopods, and other small crustaceans were more depleted in the inner estuary (*t*-tests, df = 8 to 15, all P values < 0.05), while anchovies showed significantly more enriched $\delta^{15}N$ values (*t*-test, $t = 2.24$, df = 15, $P < 0.05$) in the inner estuary (Fig. 2; Appendix C).

Variation in shark isotopic signatures

The $\delta^{13}C$ and $\delta^{15}N$ values of the sharks varied significantly between the five size classes (Table 1, Appendix D). The $\delta^{13}C$ values of the juvenile sharks were the most depleted among the five size classes (Table 1, Appendix D), followed by pre-mature and adult sharks. The $\delta^{13}C$ values of the embryonic pups and YOY were the most enriched and similar to adults (Table 1, Appendix D). The pup and YOY sharks had significantly higher C:N ratios and lipid contents than the juvenile, pre-mature and adult sharks, and all these larger sharks had significantly lower liver somatic indices (LSI) than the YOY sharks (Table 1; Appendix D).

In the early life phases (i.e., pups to YOY to juveniles, 13–35 cm TL), shark $\delta^{15}N$ increased significantly with TL (Spearman's correlation coefficients, $r_s = 0.620$); whereas the values of $\delta^{13}C$ ($r_s = -0.754$), C:N ratio ($r_s = -0.318$), LSI ($r_s = -0.525$) and lipid content ($r_s = -0.855$) decreased significantly with TL; the $\delta^{13}C$ values of individual sharks were negatively correlated with $\delta^{15}N$ ($r_s = -0.534$; for all cases: $N = 37$–55, $P < 0.01$).

During pre-mature to mature phases, the $\delta^{13}C$ values increased significantly with TL ($r_s = 0.770$), while the values of $\delta^{15}N$ ($r_s = -0.261$), C:N ratio ($r_s = -0.232$) and lipid content ($r_s = -0.498$) were negatively correlated with TL; the $\delta^{13}C$ values of individual sharks were negatively correlated with $\delta^{15}N$ ($r_s = -0.343$; for all cases: $N = 77$ to 83, $P < 0.001$). Both pre-mature and adult sharks

Table 2. Student-Newman-Keuls (SNK) tests for significant location × size term in general linear models (GLMs) of stable isotope variables, condition index and selected fatty acid (FA) variables and biomarkers (see Appendix E for GLMs and details). Abbreviation: LSI (liver somatic index).

Variable	Inner (I)	Outer (O)	Pre-mature (P)	Adult (A)
$\delta^{13}C$	P < A	P = A	I < O	I < O
LSI	P < A	P > A	I < O	I = O
i15:0#	P > A	P > A	I > O	I = O
a15:0	P > A	P > A	I > O	I > O
i17:0	P > A	P > A	I > O	I = O
18:3n3#	P > A	P > A	I > O	I > O
22:6n3#	P < A	P < A	I < O	I = O

had significantly more depleted $\delta^{13}C$ values and lower C:N ratios in the inner estuary (Tables 1 and 2; Appendix E). Although the seasonal difference was small, the C:N ratio of both pre-mature and adult sharks was significantly higher in the wet summer than dry winter. However, there were no consistent seasonal differences in $\delta^{15}N$, LSI or % lipid (Tables 1, 3 and 4; Appendix E).

Estimates of the relative contribution of the prey groups to juvenile, pre-mature and adult sharks derived from SIAR Bayesian mixing models showed that the three size classes in the inner estuary generally shared similar prey items despite the differences evident from Fig. 2 (see Table 5 for the estimates of the % relative contribution). In the inner estuary, anchovies and ponyfishes were the major food sources for juvenile, pre-mature and adult sharks, followed by eels, gobies, penaeid shrimps and sergestid shrimps (Fig. 2A; Table 5). Other prey items included polychaetes, stomatopods, squids and small crustaceans, which jointly made a small contribution to the diets of the sharks across all size ranges (Fig. 2A; Table 5). In contrast, adults in the outer estuary gained carbon mainly from stomatopods, followed by ponyfishes, anchovies and squids, with only minor contributions from other sources (Fig. 2B; Table 5).

Table 3. Student-Newman-Keuls (SNK) tests for significant season × location term in general linear models (GLMs) of stable isotope variables, condition index and selected fatty acid (FA) variables and biomarkers (see Appendix E for GLMs and details). Abbreviations: LSI (liver somatic index), SFA (saturated fatty acids), MUFA (monounsaturated fatty acids), PUFA (polyunsaturated fatty acids), BaFA (Bacterial FA), Zoo FA (zooplankton FA).

Variable	Wet (W)	Dry (D)	Inner (I)	Outer (O)
$\delta^{15}N$	I = O	I = O	W < D	W = D
Lipid (%)	I = O	I > O	W = D	W = D
LSI	I = O	I = O	W = D	W < D
Total SFA#	I = O	I > O	W = D	W > D
Total MUFA#	I > O	I < O	W > D	W < D
Total PUFA	I = O	I > O	W < D	W = D
i15:0#	I > O	I = O	W > D	W = D
i17:0	I > O	I = O	W > D	W = D
a17:0	I > O	I = O	W > D	W = D
18:1n7#	I = O	I < O	W > D	W = D
Total BaFA#	I = O	I < O	W > D	W = D
20:1n9	I > O	I < O	W > D	W < D
Total Zoo FA	I > O	I < O	W > D	W < D
20:4n6#	I < O	I = O	W < D	W = D

Table 4. Student-Newman-Keuls (SNK) tests for season × size interaction term in general linear models (GLMs) of stable isotope variables, condition index and selected fatty acid (FA) variables and biomarkers (see Appendix E for GLMs and details). Abbreviations: MUFA (monounsaturated fatty acids), PUFA (polyunsaturated fatty acids) and BaFA (Bacterial FA).

Variable	Wet (W)	Dry (D)	Pre-mature (P)	Adult (A)
Total MUFA#	P > A	P = A	W > D	W = D
Total PUFA	P < A	P = A	W < D	W = D
i15:0#	P > A	P = A	W > D	W = D
a15:0	P > A	P = A	W > D	W = D
i17:0	P > A	P > A	W > D	W = D
18:1n7#	P = A	P < A	W > D	W = D
Total BaFA#	P > A	P > A	W > D	W > D
18:3n3#	P > A	P > A	W > D	W = D
22:6n3#	P < A	P < A	W < D	W = D

Variation in FAP of sharks

Fatty acid profiles (FAP) of the five shark size classes, based on 34 FAs (see Appendices F and G), were clearly separated in the PCoA plots (Fig. 3A, B); and significant size differences were detected by PERMANOVA (pseudo-$F_{4,\ 107} = 148.56$, $P < 0.001$). The separation (i.e., dissimilarity) between pups and adults was the largest, with other size classes at intermediate positions (Fig. 3A), indicating a gradual, ontogenetic shift in FAP along the life phases. However, the separations between pups, YOY and juveniles (the early life phases) were primarily observed along the horizontal dimension (i.e., PCo1) whereas the separations between juvenile to mature sharks mainly occurred along the vertical dimension (i.e., PCo2;

Table 5. Relative contribution of food items (mean, 5–95% credibility intervals [CI]) for juvenile, pre-mature and adult spadenose sharks.

	Inner estuary						Outer estuary	
	Juvenile		Pre-mature		Adult		Adult	
Prey item	Mean	CI	Mean	CI	Mean	CI	Mean	CI
Anchovies	25.3	7–43	20.5	10–30	31.7	20–44	11.0	4–18
Gobies	6.3	0–17	6.6	0–15	11.4	0–24	6.9	0–15
Ponyfishes	40.8	25–56	11.4	3–19	12.9	2–22	21.9	13–31
Eels	10.4	0–21	37.7	27–48	9.4	0–18	2.0	0–5
Squids	1.8	0–5	1.9	0–5	6.0	0–13	11.0	2–20
Polychaetes	2.2	0–6	4.2	0–10	3.5	0–9	1.4	0–4
Sergestid shrimps	5.2	0–14	7.5	0–15	6.4	0–15	3.4	0–8
Penaeid shrimps	4.8	0–13	6.4	0–15	10.2	0–22	3.5	0–9
Stomatopods	1.5	0–4	1.4	0–4	4.9	0–11	36.3	26–46
Small crustaceans	1.6	0–5	2.3	0–6	3.6	0–9	2.6	0–6

Note: See Appendix A for a species list.

Fig. 3A).

Based on the correlation of FAs to PCoA axes (Fig. 3B), seven FA biomarkers (see Appendix D) with seemingly different origins were further analyzed by univariate analyses using GLMs. The percentage of all these biomarkers was ontogenetically variable in sharks in the inner estuary during the wet season (Appendix D). Shark pups were characterized by significantly higher abundances of SFA, MUFA, diatom FA (only 16:1n9), bacterial FA (only a17:0 and 18:1n7), zooplankton FA (20:1n9 and 22:1n9), and LCSFA, but lower percentages of bacterial FA (a15:0 and i17:0) and PUFA including 22:6n3, 20:4n6, diatom FA (20:5n3), and essential FA (18:2n6 and 18:3n3) than adult sharks (Fig. 3A, B; Table 6; Appendix D).

In the early life phases (i.e., pups to YOY to juveniles), juveniles had significantly higher abundances of PUFA, diatom FA (20:5n3), bacterial FA (i, a15:0 and i17:0), EFA (18:2n6 and 18:3n3), and plant and animal-derived PUFA (20:4n6 and 22:6n3) than the pups (Fig. 3A, B; Table 6; Appendix D). All the FA biomarkers of the sharks increased significantly with size ($r_s =$ 0.530–0.844, $N = 54$, $P < 0.001$).

During the later life phase, adults had significantly higher levels of PUFA, EFA (18:2n6), 20:4n6 and 22:6n3 but lower abundances of MUFA, diatom FA (16:1 and 20:5n3), bacterial FA (i and a15:0, i and a17:0), EFA (18:3n3), zooplankton FA (20:1n9 and 22:1n9) and LCSFA, than the juveniles (Appendix D; Table 6). This ontogenetic change in FA variables was further

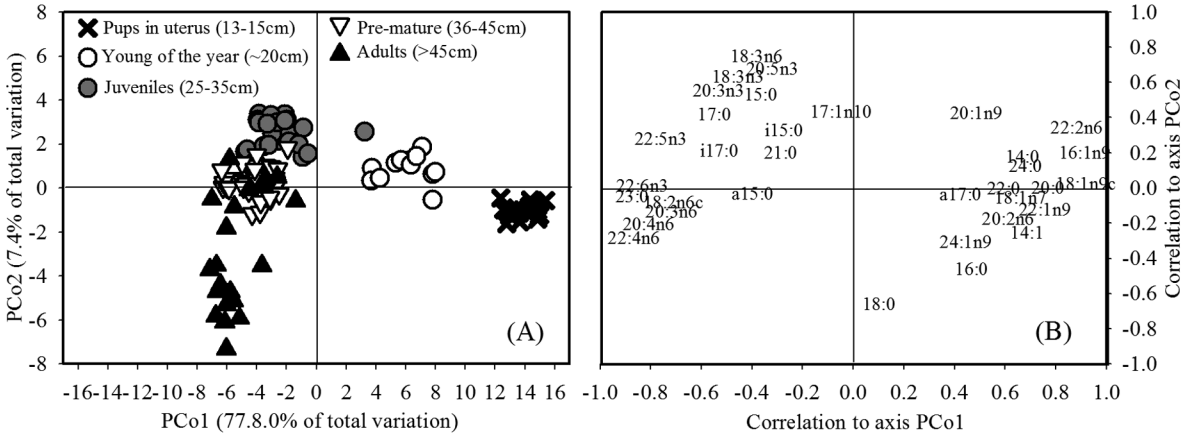

Fig. 3. (A) PCoA plots of FA composition (34 variables) in lipids of five spadenose shark size classes in the inner Pearl River Estuary during wet season. (B) Discriminating FA variables (i.e., correlation of variables to PCO axes).

Table 6. Mean fatty acid (FA) % weight (± SD) of selected FA variables and biomarkers in total lipids of spadenose shark size classes in different locations (inner and outer estuary) in wet season.

	Inner estuary					Outer estuary	
Variable	Pups	YOY	Juveniles	Pre-mature	Adults	Pre-mature	Adults
N	18	12	24	36	22	14	31
SFA (Total)	37.70 ± 0.85	30.01 ± 1.49	29.73 ± 1.50	29.91 ± 1.17	30.01 ± 1.08	31.44 ± 0.94	32.17 ± 2.19
MUFA (Total)	35.07 ± 0.86	29.48 ± 1.77	23.73 ± 2.47	23.54 ± 1.71	20.60 ± 2.36	22.44 ± 1.84	19.45 ± 2.44
PUFA (Total)	17.65 ± 1.28	33.84 ± 2.86	42.68 ± 2.65	43.22 ± 2.21	46.82 ± 2.67	42.86 ± 2.19	46.05 ± 2.39
Diatom FA (Total)	10.35 ± 0.60	9.94 ± 0.94	7.11 ± 0.87	5.68 ± 0.84	5.24 ± 1.48	6.03 ± 0.77	4.58 ± 1.39
Σ16:1	8.87 ± 0.55	6.11 ± 1.05	2.87 ± 0.52	2.18 ± 0.39	1.81 ± 0.71	2.41 ± 0.31	1.67 ± 0.7
20:5n3	1.48 ± 0.27	3.83 ± 0.70	4.23 ± 0.56	3.50 ± 0.59	3.43 ± 0.98	3.62 ± 0.69	2.92 ± 0.89
BaFA (Total)	9.55 ± 0.58	7.81 ± 0.67	8.18 ± 0.70	8.58 ± 0.54	7.73 ± 0.67	8.31 ± 0.35	7.57 ± 0.59
i15:0	0.19 ± 0.03	0.11 ± 0.02	0.26 ± 0.04	0.29 ± 0.04	0.16 ± 0.04	0.23 ± 0.04	0.15 ± 0.04
a15:0	0.06 ± 0.01	0.04 ± 0.01	0.07 ± 0.02	0.09 ± 0.02	0.06 ± 0.01	0.06 ± 0.01	0.05 ± 0.01
i17:0	0.22 ± 0.04	0.18 ± 0.03	0.45 ± 0.07	0.55 ± 0.07	0.37 ± 0.06	0.40 ± 0.05	0.32 ± 0.06
a17:0	0.24 ± 0.03	0.23 ± 0.03	0.21 ± 0.07	0.23 ± 0.05	0.17 ± 0.02	0.17 ± 0.05	0.14 ± 0.03
18:1n7	8.84 ± 0.63	7.26 ± 0.68	7.20 ± 0.69	7.43 ± 0.56	6.97 ± 0.67	7.45 ± 0.38	6.90 ± 0.53
EFA (Total)	0.55 ± 0.11	0.64 ± 0.12	1.71 ± 0.61	2.59 ± 0.54	2.03 ± 1.05	0.75 ± 0.22	1.02 ± 0.90
18:2n6	0.51 ± 0.10	0.58 ± 0.12	1.31 ± 0.64	2.32 ± 0.53	1.87 ± 1.04	0.60 ± 0.20	0.91 ± 0.89
18:3n3	0.05 ± 0.02	0.06 ± 0.01	0.40 ± 0.11	0.28 ± 0.04	0.16 ± 0.04	0.14 ± 0.03	0.11 ± 0.03
Dino FA (22:6n3)	5.85 ± 0.72	11.36 ± 1.25	17.79 ± 2.01	17.40 ± 1.33	20.13 ± 1.49	18.90 ± 0.89	20.91 ± 1.66
Zoo FA (Total)	3.61 ± 0.21	3.80 ± 0.60	3.47 ± 0.72	3.23 ± 0.54	2.53 ± 0.53	2.77 ± 0.50	2.10 ± 0.60
20:1n9	2.66 ± 0.16	3.10 ± 0.53	2.92 ± 0.64	2.76 ± 0.50	2.09 ± 0.49	2.34 ± 0.46	1.73 ± 0.58
22:1n9	0.95 ± 0.08	0.70 ± 0.12	0.54 ± 0.13	0.46 ± 0.09	0.44 ± 0.08	0.42 ± 0.06	0.37 ± 0.08
20:4n6	2.49 ± 0.32	3.93 ± 0.31	4.17 ± 0.52	4.94 ± 0.44	5.50 ± 0.58	5.62 ± 0.48	7.05 ± 1.36
LCSFA (Total)	1.24 ± 0.09	0.82 ± 0.16	0.89 ± 0.16	0.78 ± 0.12	0.70 ± 0.08	0.80 ± 0.10	0.83 ± 0.18

Notes: Abbreviations are as follows: SFA (saturated fatty acids), MUFA (monounsaturated fatty acids), PUFA (polyunsaturated fatty acids), BaFA (Bacterial FA), EFA (essential FA), Dino FA (dinoflagellate FA), Zoo FA (zooplankton FA), and LCSFA (long carbon chain SFA). N = sample size of each group.

confirmed by significant correlations with TL of the sharks: FAs PUFA, 18:2n6, 20:4n6 and 22:6n3 were positively correlated with TL, whereas FAs MUFA diatom FA (16:1 and 20:5n3), bacterial FA (i15:0, i and a17:0), 18:3n3, zooplankton FA (20:1n9 and 22:1n9) and LCSFA were negatively correlated with TL (for all cases: $r_s = -0.33$ to -0.78, $N = 58$, $P < 0.01$).

There were significant seasonal, spatial and size variations in FA composition of pre-mature and adult sharks (PERMANOVA; Se × Si: pseudo-$F_{1,\ 153} = 4.71$, $P < 0.01$; Se × Lo: pseudo-$F_{1,\ 153} = 9.91$, $P < 0.001$). Patterns of group separations were clearly shown in PCo plots (Fig. 4A, B). The significant season × location and season × size interactions indicated that spatial and seasonal differences were inconsistent among size classes (Appendix E; Tables 6 and 7; Fig. 4A, B).

The size differences in FA composition significantly varied with season and location (Appendix E). Pre-mature sharks had significantly higher abundances of bacterial FA (i15:0, a15:0 and i17:0) and EFA (18:3n3) but lower levels of PUFA and 22:6n3 than adults in both the inner and outer estuary but only during the wet season (Appendix E; Tables 2, 4, 6 and 7). The abundance of bacterial FA (i15:0, a15:0 and i17:0) and EFA (18:3n3) of pre-mature sharks were higher in the inner estuary whereas adults did not show a clear spatial pattern (Appendix E; Tables 2, 6 and 7). Bacterial FAs were the major discriminant variables for seasonal and spatial variations (Fig. 4A, B). During the wet season, both pre-mature and mature sharks had enriched levels of MUFA, bacterial FA (i15:0, i17:0 and a17:0), zooplankton FA (20:1n9) but lower levels of 20:4n6 in the inner than outer estuary (Appendix E; Tables 4, 6 and 7). During the dry season, pre-mature sharks in the inner estuary had significantly higher levels of 20:5n3 than sharks in other seasons or locations (Appendix E; Tables 6 and 7). In the inner estuary, both size classes had significantly higher abundances of MUFA, bacterial FA (i15:0, i17:0 and a17:0), zooplankton FA (20:1n9) but lower levels of 20:4n6 in the wet season than in the dry season (SNK tests: Se × Lo; Appendix E; Tables 3, 6 and 7). All sharks exhibited higher levels of LCSFA during the wet season irrespective of location (Appendix E). No consistent seasonal or spatial differences in other FA biomarkers were found

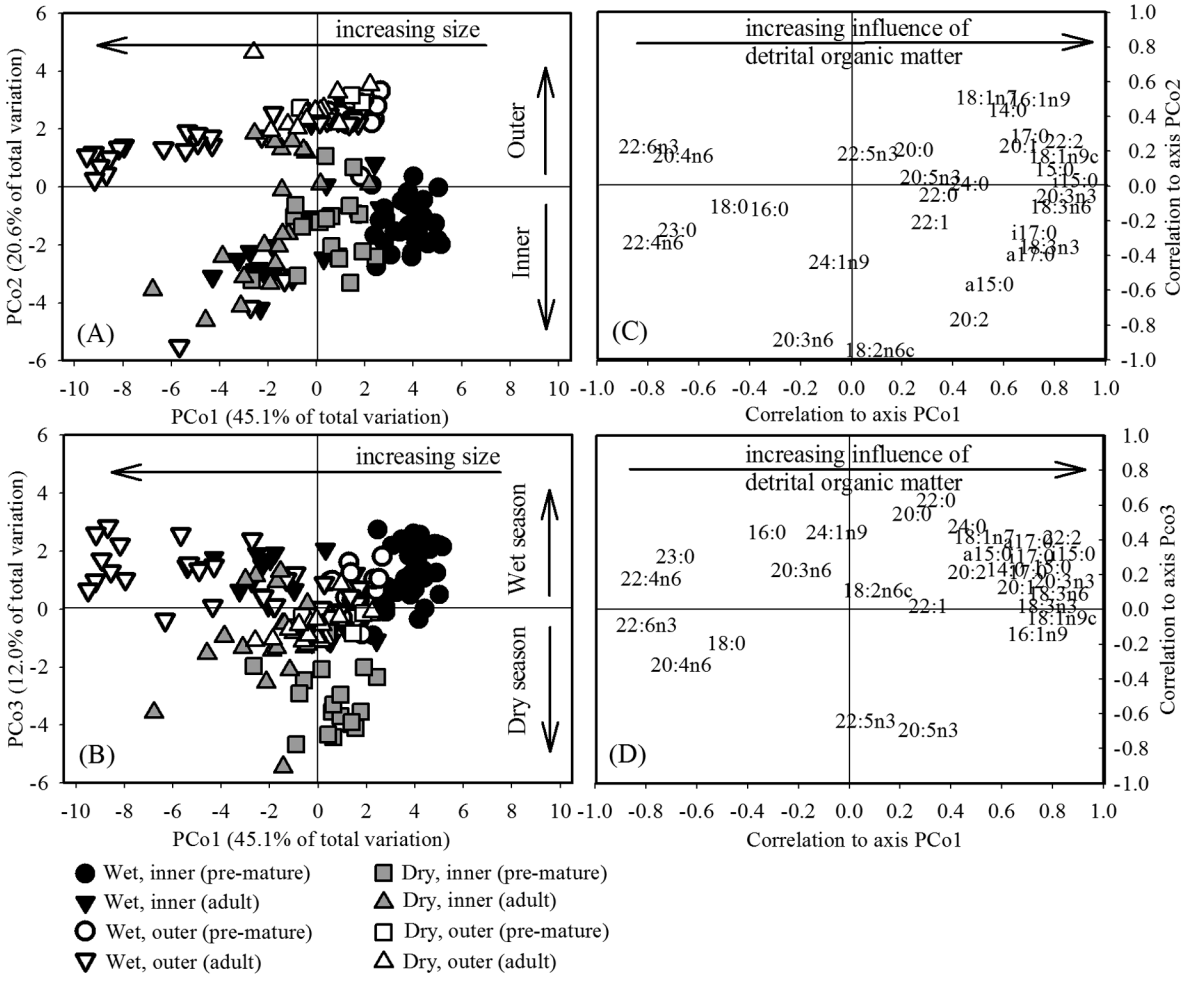

Fig. 4. PCoA plots of FA composition (34 variables) in lipids of pre-mature and adult spadenose sharks in different seasons and locations. (A) PCO axes 1 and 2. (B) PCO axes 1 and 3. (C) and (D) Discriminating FA variables.

(Appendix E).

Comparing SI values, FAP and FASIA between pelagic and benthic sharks

Almost all bulk $\delta^{13}C$, $\delta^{15}N$ values, C:N ratio, FA-specific $\delta^{13}C$ values of sharks were variable with species and size (Table 8). Spadenose sharks had significantly higher values of $\delta^{15}N$ and C:N ratio, but more depleted $\delta^{13}C$ values than the bamboo sharks, and this pattern was consistent with size (Table 8A).

FAP (PERMANVOA: pseudo-$F_{1,42} = 15.87$, $P < 0.001$) and FA-specific $\delta^{13}C$ values (PERMANVOA: pseudo-$F_{1,42} = 7.26$, $P < 0.001$) showed significant interactions between species and size (Fig. 5). Pairwise-comparisons revealed that both species showed significant size differences, and there were significant species differences for both juveniles and adults in both FAP and FA $\delta^{13}C$ values. For FAP, the bacterial FA (i and a15:0, i and a 17:0) biomarkers were the discriminant FA variables which best explained the species and size class separations (Fig. 5). Spadenose sharks were characterized by a significantly lower abundance of bacterial FA (i and a15:0, i and a 17:0) when compared with the bamboo sharks (Fig. 5A, B; Table 8B). Species differences in bacterial FA 18:1n7 were not, however, consistent with size; juvenile spadenose sharks had significantly lower levels of 18:1n7 and total bacterial

Table 7. Mean fatty acid (FA) % weight (± SD) of selected FA variables and biomarkers in total lipids of spadenose shark size classes in different locations (inner and outer estuary) in dry season.

	Inner estuary		Outer estuary	
Variable	Pre-mature	Adults	Pre-mature	Adults
N	19	20	3	16
SFA (Total)	30.24 ± 1.77	29.96 ± 2.08	28.12 ± 1.19	28.97 ± 1.22
MUFA (Total)	19.54 ± 1.26	18.76 ± 2.20	23.32 ± 1.74	22.29 ± 1.66
PUFA (Total)	47.59 ± 2.28	49.06 ± 1.96	45.55 ± 2.80	46.01 ± 2.59
Diatom FA (Total)	6.86 ± 0.85	5.21 ± 1.05	5.99 ± 0.91	5.63 ± 0.85
Σ16:1	1.86 ± 0.31	1.57 ± 0.25	2.33 ± 0.86	2.04 ± 0.41
20:5n3	5.00 ± 0.72	3.64 ± 0.96	3.66 ± 0.05	3.59 ± 0.52
BaFA (Total)	5.44 ± 0.84	6.32 ± 1.39	8.01 ± 0.51	7.98 ± 0.53
i15:0	0.16 ± 0.03	0.14 ± 0.03	0.15 ± 0.02	0.16 ± 0.03
a15:0	0.06 ± 0.01	0.06 ± 0.01	0.04 ± 0.00	0.05 ± 0.01
i17:0	0.38 ± 0.06	0.32 ± 0.05	0.31 ± 0.05	0.33 ± 0.06
a17:0	0.16 ± 0.03	0.14 ± 0.02	0.16 ± 0.02	0.15 ± 0.03
18:1n7	4.67 ± 0.83	5.66 ± 1.34	7.34 ± 0.55	7.29 ± 0.53
EFA (Total)	2.49 ± 0.76	2.00 ± 0.88	0.61 ± 0.05	0.62 ± 0.09
18:2n6	2.29 ± 0.77	1.85 ± 0.91	0.49 ± 0.04	0.51 ± 0.07
18:3n3	0.20 ± 0.03	0.15 ± 0.03	0.12 ± 0.01	0.11 ± 0.03
Dino FA (22:6n3)	18.52 ± 1.62	20.89 ± 1.47	21.18 ± 1.72	20.26 ± 0.89
Zoo FA (Total)	2.60 ± 0.50	2.38 ± 0.63	3.77 ± 0.30	3.20 ± 0.59
20:1n9	2.09 ± 0.46	1.93 ± 0.59	3.31 ± 0.33	2.78 ± 0.59
22:1n9	0.52 ± 0.07	0.45 ± 0.06	0.46 ± 0.04	0.43 ± 0.09
20:4n6	6.29 ± 0.88	5.89 ± 1.12	5.70 ± 0.52	6.35 ± 0.57
LCSFA (Total)	0.62 ± 0.05	0.67 ± 0.10	0.59 ± 0.05	0.64 ± 0.07

Notes: See Table 6 for abbreviations.

FA than the adults and both size classes of the bamboo sharks (Fig. 5A, B; Table 8B). The $\delta^{13}C$ values of almost all spadenose shark FAs, including the bacterial FA biomarkrs (except 22:6n3 and 20:4n6), were significantly more depleted than in the bamboo sharks (Fig. 5C, D; Table 8C). Bayesian mixing models (SIAR) showed that the detritus utilized by juvenile and adult spadenose sharks in the inner estuary was mainly of terrestrial origins, followed by phytoplankton and smaller proportions of macroalgal detritus (Fig. 6A, B). In contrast, adult bamboo sharks derived much of their detrital food from phytoplankton, and smaller proportions from terrestrial material and macroalgae; juvenile bamboo sharks mainly assimilated terrestrial detritus, followed by phytoplankton and smaller proportions of macroalgal detritus (Fig. 6C, D).

Discussion

Higher trophic level consumers in estuaries assimilate carbon subsidies from both terrestrial and marine sources (e.g., Darnaude 2005); the relative importance of terrestrial carbon, however, varies according to the features of individual estuaries and the feeding ecology of the species of interest (Heck et al. 2008, Connolly et al. 2009, Schlacher et al. 2009). Pelagic spadenose shark and benthic bamboo sharks both had generalized diets in the PRE, and consumed a wide range of prey including both pelagic and benthic teleosts, polychaetes and crustaceans (see also Wai et al. 2011*a*). They relied mainly on autochthonous marine sources, as shown by $\delta^{13}C$ signatures within the range of their food sources (−16.7‰ to −19.9‰; see Wai et al. 2011*a*), but the combined use of complementary FAP and FA-specific $\delta^{13}C$ revealed spatial, seasonal, ontogenetic and interspecific dietary differences that were mainly attributable to changes in detrital carbon utilization, largely reflecting the input of allochthonous detritus from the land during the wet season.

Food source utilization of the spadenose shark and significance of detrital pathways

Stomach content analyses showed that anchovies, ponyfishes and sergestid shrimps were the dominant food sources of spadenose sharks. Although inferences made when interpreting results of SIAR Baysian mixing models should

Table 8. *F*-ratio of general linear models to investigate variation in (A) $\delta^{13}C$, $\delta^{15}N$ and C:N ratio, (B) % weight of selected fatty acid (FA) biomarkers, and (C) compound-specific FA stable carbon isotopic (FASI) value (‰) of selected FA variables and biomarkers, between two size classes of spadenose sharks (SS) and bamboo sharks (BM), in the inner estuary.

				Spadenose sharks		Bamboo sharks	
Variable	Species	Size	Species × Size	Juveniles	Adults	Juveniles	Adults
N				6	16	12	12
(A) Isotopic data							
$\delta^{13}C$ (‰)	**222.98*** **	**9.80** *	**5.95***	$-17.88^{C} \pm 0.56$	$-17.24^{B} \pm 0.34$	$-15.89^{A} \pm 0.23$	$-15.81^{A} \pm 0.39$
$\delta^{15}N$ (‰)#	**191.10*** **	1.13	1.29	15.65 ± 0.89	15.63 ± 0.35	10.19 ± 0.78	10.99 ± 2.00
C:N ratio#	**179.44*** **	1.12	**29.06*** **	$3.44^{A} \pm 0.05$	$3.27^{B} \pm 0.07$	$2.92^{D} \pm 0.03$	$3.04^{C} \pm 0.15$
(B) FAMEs							
SFA (total)	**70.49*** **	0.49	**10.45*** **	$29.40^{B} \pm 1.46$	$31.56^{A} \pm 1.86$	$26.56^{C} \pm 1.23$	$25.17^{C} \pm 2.10$
MUFA (total)	0.08	0.00	**72.54*** **	$25.73^{A} \pm 2.07$	$20.25^{B} \pm 2.30$	$20.04^{B} \pm 1.08$	$25.58^{A} \pm 2.41$
PUFA (total)	**20.94*** **	**0.06*** **	**28.45*** **	$43.89^{C} \pm 1.96$	$47.50^{C} \pm 2.35$	$50.92^{A} \pm 1.40$	$46.96^{B} \pm 2.86$
Diatom FA (total)#	**154.76*** **	**55.88*** **	**55.25*** **	$6.10^{C} \pm 0.63$	$6.07^{C} \pm 1.44$	$17.68^{A} \pm 2.13$	$8.99^{B} \pm 2.36$
Σ16:1	**181.56*** **	**8.69** *	3.01	2.35 ± 0.27	1.69 ± 0.29	4.02 ± 0.60	3.85 ± 0.52
20:5n3#	**118.53*** **	**64.59*** **	**87.09*** **	$3.74^{B} \pm 0.47$	$4.38^{B} \pm 1.28$	$13.66^{A} \pm 1.62$	$5.14^{B} \pm 2.08$
BaFA (total)#	**29.06*** **	6.05*	**31.51*** **	$8.46^{A} \pm 0.39$	$5.56^{B} \pm 1.30$	$8.38^{A} \pm 0.77$	$9.51^{A} \pm 1.41$
i15:0#	**290.47*** **	3.82*	6.21*	0.25 ± 0.04	0.14 ± 0.02	0.62 ± 0.08	0.63 ± 0.13
a15:0#	**147.98*** **	3.66	0.13	0.07 ± 0.02	0.06 ± 0.01	0.15 ± 0.02	0.14 ± 0.03
i17:0#	**55.58*** **	1.59	0.00	0.47 ± 0.08	0.34 ± 0.06	1.23 ± 0.57	1.10 ± 0.25
a17:0#	**65.20*** **	2.06	0.07	0.19 ± 0.03	0.15 ± 0.03	0.48 ± 0.19	0.42 ± 0.08
18:1n7#	1.35	3.70	**34.68*** **	$7.48^{A} \pm 0.41$	$4.87^{C} \pm 1.24$	$5.90^{B} \pm 0.37$	$7.22^{A} \pm 1.41$
EFA (total)#	5.79*	7.05*	0.04	1.98 ± 0.52	2.67 ± 0.74	2.60 ± 0.34	3.41 ± 1.44
18:2n6#	**7.52** **	**9.33** **	0.00	1.64 ± 0.57	2.51 ± 0.75	2.43 ± 0.33	3.27 ± 1.43
18:3n3#	**49.51*** **	**68.09*** **	**29.37*** **	$0.35^{A} \pm 0.07$	$0.16^{B} \pm 0.04$	$0.18^{B} \pm 0.03$	$0.14^{B} \pm 0.04$
Dino FA#	**54.20*** **	0.74	0.50	18.95 ± 1.19	19.05 ± 1.63	14.21 ± 1.02	15.13 ± 2.84
Zoo FA (total)#	**69.74*** **	0.01	**56.98*** **	$3.53^{A} \pm 0.90$	$2.21^{B} \pm 0.48$	$0.79^{C} \pm 0.18$	$2.07^{B} \pm 0.66$
20:1n9#	**88.10*** **	0.68	**61.92*** **	$3.05^{A} \pm 0.79$	$1.78^{B} \pm 0.44$	$0.52^{C} \pm 0.14$	$1.56^{B} \pm 0.51$
22:1n9#	1.93	3.90	**9.17** **	$0.48^{A} \pm 0.18$	$0.43^{A} \pm 0.07$	$0.26^{B} \pm 0.05$	$0.51^{A} \pm 0.27$
20:4n6	**8.50** **	**26.95*** **	1.08	4.49 ± 0.69	6.06 ± 0.98	5.49 ± 0.72	6.53 ± 0.63
LCSFA (total)	**15.32*** **	**46.15*** **	**10.28** **	$0.77^{B} \pm 0.12$	$0.63^{D} \pm 0.07$	$1.04^{A} \pm 0.15$	$0.66^{C} \pm 0.15$
(C) FASIA data							
SFA (average)	**143.40*** **	**45.52*** **	**4.53** *	$-25.37^{D} \pm 0.57$	$-23.31^{C} \pm 0.77$	$-22.10^{B} \pm 0.79$	$-21.03^{A} \pm 0.70$
MUFA (average)	**87.59*** **	3.00	0.51	-25.92 ± 0.58	-25.33 ± 0.74	-23.55 ± 0.92	-23.31 ± 0.60
PUFA (average)#	**94.39*** **	2.75	**13.64** **	$-25.93^{D} \pm 0.39$	$-25.37^{C} \pm 0.70$	$-22.62^{A} \pm 1.38$	$-23.72^{B} \pm 0.52$
Diatom FA (average)#	**36.55*** **	**11.37** **	5.70*	-25.23 ± 0.47	-24.95 ± 0.69	-24.20 ± 1.38	-22.59 ± 0.60
16:1#	**10.53** **	3.48	2.90	-25.57 ± 0.51	-27.41 ± 2.03	-24.78 ± 1.86	-24.86 ± 1.02
20:5n3#	**11.20** **	**30.84*** **	0.80	-24.89 ± 0.63	-22.50 ± 2.23	-23.63 ± 1.36	-20.32 ± 1.16
BaFA (average)	**91.33*** **	2.94	1.81	-25.28 ± 0.72	-25.32 ± 0.85	-24.33 ± 0.70	-23.28 ± 0.77
i15:0	1.87	**6.34** *	0.69	-26.76 ± 1.01	-26.13 ± 1.51	-26.56 ± 0.53	-25.30 ± 1.29
a15:0	**17.21*** **	0.36	**25.95*** **	$-21.94^{B} \pm 1.50$	$-24.76^{C} \pm 1.22$	$-22.41^{B} \pm 1.51$	$-20.19^{A} \pm 2.03$
i17:0#	**9.34** **	1.71	0.50	-26.67 ± 1.95	-26.37 ± 2.06	-25.50 ± 0.83	-24.49 ± 1.17
a17:0	**38.98*** **	5.54*	**10.99** **	$-25.76^{C} \pm 0.80$	$-24.04^{B} \pm 0.99$	$-22.86^{A} \pm 1.09$	$-23.15^{A} \pm 0.85$
18:1n7#	**91.05*** **	0.05	0.64	$-25.73^{B} \pm 1.42$	$-25.09^{B} \pm 1.07$	$-19.24^{A} \pm 2.87$	$-19.60^{A} \pm 2.12$
EFA (18:2n6)	0.50	**9.06** **	0.36	-25.76 ± 1.44	-27.60 ± 1.88	-25.70 ± 1.82	-26.94 ± 1.02
Dino FA#	2.58	0.29	3.73	-26.54 ± 0.83	-25.47 ± 0.66	-26.40 ± 2.07	-27.00 ± 1.42
Zoo FA (20:1n9)#	**32.74*** **	5.34*	4.66*	-26.27 ± 1.21	-23.57 ± 1.32	-21.51 ± 3.27	-21.41 ± 0.61
20:4n6#	2.82	3.56	0.35	-27.33 ± 0.65	-28.71 ± 2.06	-26.72 ± 2.15	-27.44 ± 1.17
LCSFA (average)	**94.25*** **	**23.35*** **	4.05	-25.83 ± 0.94	-23.33 ± 1.43	-21.54 ± 0.94	-20.51 ± 1.04

Notes: Degrees of freedom (df) for the factors Species, Size, the interaction term, and the residual are 1, 1, 1 and 42, respectively. Where the Species × Size interaction was significant, mean values (±SD) and results of SNK tests are shown, with inter-group differences designated by superscripts A, B, C and D; significant differences between size classes are indicated by bold *F*-ratios: *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$. # indicates data failed Levene's test for homogeneity of variances (see text for details). See Table 6 for abbreviations.

be made with caution, they have utility in revealing trophic pathways (Phillips and Gregg 2003, Bond and Diamond 2011, see review in Wai et al. 2008, 2011*a*). In this instance, SIAR models showed that pelagic fishes, anchovies and ponyfishes were the major prey of the spadenose shark in the inner estuary, while adult sharks in the outer estuary had a wider diet, consuming larger prey such as benthic crustaceans (i.e., stomatopods) in addition to anchovies and ponyfishes. This pattern was also evident from the stomach content analyses. Both anchovies

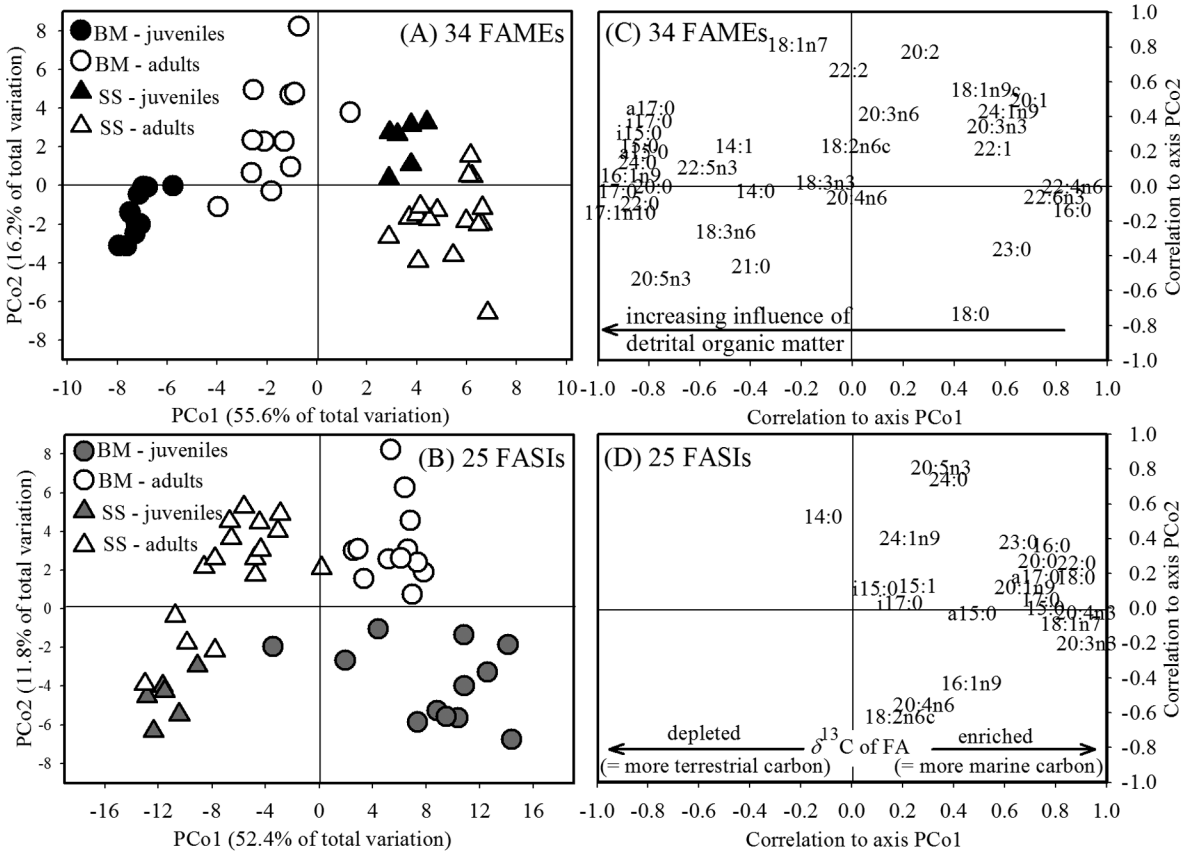

Fig. 5. PCoA plots of (A) FA composition (34 variables) and (B) 25 compound-specific FA $\delta^{13}C$ values in lipids of juveniles and adults of spadenose shark (SS) and bamboo shark (BM) in different seasons and locations. (C) and (D) Discriminating FA variables.

and ponyfishes are zooplanktivores (Froese and Pauly 2011), a fact supported by the similar $\delta^{13}C$ values between these fishes (−18.7‰ to −19.27‰ in the inner estuary and −14.8‰ to −16.7‰ in the outer estuary) and zooplankton (−18.7‰ and −16.4‰; T.-C. Wai, *unpublished data*). The importance of sergestid shrimps was not, however, supported by SIAR models indicating that sergestid shrimps might not be major target food items, but inadvertently ingested when the sharks fed on fishes that ate sergestid shrimps and zooplankton. However, while it is clear that the $\delta^{13}C$ value is good biomarker for spatial variation in carbon source utilization, seasonal shifts in diet, here and in other studies, could not be revealed by SIAR models or traced by bulk SI signatures alone (Wai et al. 2008, Lau et al. 2009; T.-C. Wai, *unpublished data*).

FAP is a valuable dietary tracer complementary to SIA, and it provided further confirmation of the spatial, seasonal and ontogenetic dietary shifts of spadenose sharks. The major FA variables which explained the observed ontogenetic and spatial variation were the enhanced levels of bacterial and zooplankton FA biomarkers in juvenile and pre-mature sharks. Seasonal differences in pre-mature sharks were reflected in higher levels of these biomarkers and also LCSFA (as a biomarker of terrestrial carbon) during the wet season, and were probably related to the increased rainfall and run-off of terrestrial detritus as well as phytoplankton and zooplankton production in summer (Wai et al. 2008, Chen et al. 2009). Shark assimilation of terrestrial-derived carbon was probably through consumption of zooplanktivores, which assimilated carbon, via comsumption of zooplankton, from suspended particulate organic matter composed

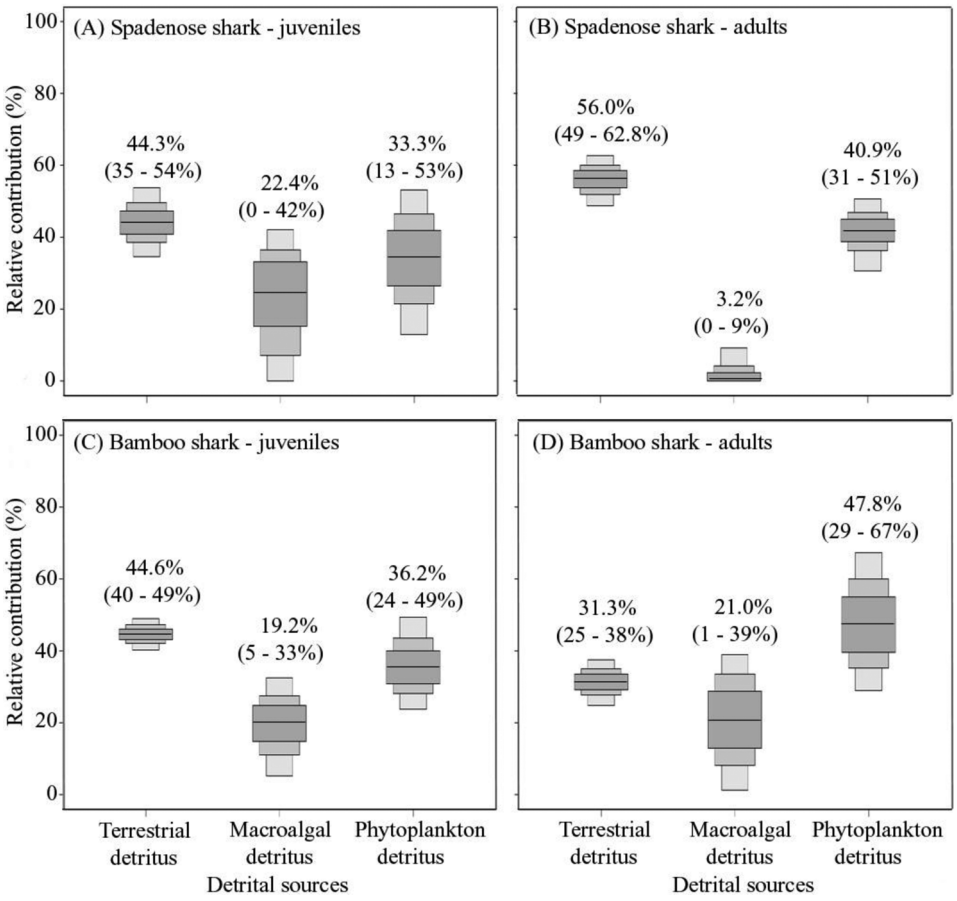

Fig. 6. SIAR results based on $\delta^{13}C$ values of BaFAs (i, a15:0, i17:0 and 18:1n7). Box plots of mean relative % contribution (±50%, 75% and 95% credibility intervals) of bacterial (detrital) sources to (A) juveniles and (B) adults of spadenose sharks, and (C) juveniles and (D) adults of bamboo sharks in the inner Pearl River Estuary. Mean values of % contribution (5–95% credibility intervals) of each detrital source to the sharks are shown also.

of phytoplankton, heterotrophic protists and detrital organic matter (including terrestrial-derived carbon). Both pre-mature and adult sharks had higher levels of zooplankton FA biomarkers in the outer estuary during the dry season attributable to enhanced abundance of zooplankton (e.g., copepods) which are carried into the PRE by the China Coastal Current during the northeast monsoon period (Hwang and Wong 2005).

Ontogenetic dietary shifts are common in elasmobranchs (see review by Wetherbee and Cortés 2004), and can be related to changes in morphology or behaviour associated with growth, as well as shifts in habitat use and increase in foraging range that will affect diversity and abundance of prey encountered (reviewed by Grubbs 2010). FAP results indicated a clear ontogenetic diet shift in spadenose sharks and early life phase (13–35cm TL) and also more mature phase (>35cm TL) could be identified. In the early life phase, teleosts such as anchovies and ponyfishes were the major prey items for juvenile sharks, both of which had more depleted $\delta^{13}C$ and enriched $\delta^{15}N$ values than other prey, thereby accounting for relative $\delta^{13}C$ depletion and δ15N enrichment of smaller sharks. Increasing utilization of detrital carbon as sharks grew through this early developmental phase was shown by the positive correlation between bacterial FA and shark size, probably via consumption of $\delta^{13}C$ depleted zooplanktivores

(i.e., anchovies and ponyfishes; Froese and Pauly 2011) as revealed by the SIAR models.

In the later phase, isotopic signatures of larger sharks indicated an ontogenetic dietary shift to food sources with more $\delta^{13}C$ enriched but $\delta^{15}N$ depleted values such as benthic fishes and invertebrates, and may have resulted from exploitation of foraging grounds further offshore. Detrital carbon sources also became less important for adult sharks than younger individuals in the inner and outer PRE during both seasons. As maneuverability increases with size, larger sharks are usually able to expand their foraging range and handle larger and faster prey compared to juveniles (Grubbs 2010). The dominance of large shark diets by teleosts also explains the enhanced levels of polyunsaturated FA (e.g., 22:6n3) with shark size, as fishes often contain high levels of polyunsaturated FA (Budge et al. 2002). Stomach content analyses, SIA and FAP results all suggest that adult spadenose sharks forage in both pelagic and benthic habitats, and hence encounter and capture a diverse array of prey.

Potential relationship between spadenose shark feeding ecology and reproductive biology

While variations in fatty acid composition can be indicative of ontogenetic dietary shifts, the contrasting FA composition between embryonic pups and adults may also highlight an important relationship between shark dietary shifts and reproductive biology. Spadenose sharks are placental viviparous elasmobranchs, characterized by fast growth to mature size (>45cm) at 2–3 years old; and can produce many (11–13) pups with a relatively short gestation period (~4 months; Wourms and Demski 1993, Mathew and Devaraj 1997). By giving birth to morphologically well-developed and fully functional young, spadenose shark pups can survive independently after birth (Wourms and Demski 1993). The lipid content of the pups was the highest among all the sharks' life phases and decreased with shark size. Lipid is, therefore, likely the major energy reserve for new born individuals before they can obtain nutrition by feeding in the sea. FA compositions of embryonic pups at the later phases of gestation were significantly different from the adults, while other size classes represented intermediate/transition phases between the pups and adults. The low abundance of polyunsaturated FA (PUFA) such as 20:4n6, 20:5n3 and 22:6n3 in the shark pups indicate that, during gestation, the pups may be supplied with lipids which have low levels of PUFA or the pups may have utilized most of the PUFA for growth and development. FA data show that after birth these PUFA can be readily obtained from the diet and concentrations increased with body size. Producing pups with high lipid content but low PUFA levels may be a consequence of placental viviparity, allowing mother sharks to produce more offspring by minimizing investment of essential fatty acids in each pup (see also Musick and Ellis 2005).

Relative importance of carbon from terrestrial and marine sources to pelagic and benthic sharks

For spadenose and bamboo sharks, spatial differences and ontogenetic shifts in carbon utilization reflect changes in the relative importance of different sources of detritus. Our previous study (Wai et al. 2011*a*) demonstrated an enhanced assimilation of detrital, especially terrestrial carbon, by juvenile bamboo sharks in the inner PRE than by adults at any location. While bamboo sharks assimilate detrital carbon via consumption of polychaetes and small crustaceans along benthic trophic pathways, spadenose sharks mainly fed on zooplanktivores (anchovies, ponyfishes and sergestid shrimps) and assimilated detrital carbon via pelagic trophic pathways. In the inner PRE, bamboo shark diets are characterized by a higher bacterial (detrital) FA than spadenose sharks studied herein, indicating a higher dependence on detrital carbon by benthic bamboo sharks. The relatively depleted $\delta^{13}C$ values of almost all FA variables in the spadenose sharks indicated that this pelagic species assimilated more terrestrial detritus. The same conclusion was also suggested by the more depleted bulk and FA-specific $\delta^{13}C$ values (of almost all FA variables) of spadenose shark tissues. Furthermore, SIAR models revealed that a large proportion of detrital carbon assimilated by both juvenile and adult spadenose sharks in the inner PRE (44% and 56%, respectively) was derived from allochthonous, terrestrial sources with considerably less utilization of

autochthonous detritus from phytoplankton (33% and 41%) and macroalgae (22% and 3%, respectively). Bamboo sharks, however, showed size variation in detritus utilization: adults assimilated more autochthonous (70% vs. 30%) than allochthonous carbon; juveniles were more similar to spadenose sharks with detritus from allochthonous sources constituting 45% of assimilated carbon. Despite uncertainty over fractionation of FA-specific $\delta^{13}C$ used in the SIAR models (Bouillon and Boschker 2006, Mcleod and Wing 2007, see review by Wai et al. 2011*a*), the interspecific differences in terrestrial carbon use and its importance to sharks in the inner PRE were substantial and were also confirmed by results of FAP and bulk $\delta^{13}C$ values.

Body condition and nutrition measurements of the spadenose shark (the present study) and the bamboo shark (Wai et al. 2011*a*) did not show significant spatial variation, despite differences in the food sources assimilated in the inner and outer PRE. The overall carbon distribution in the PRE may nevertheless indirectly influence shark survival and reproduction of the sharks by promoting secondary production of both pelagic (e.g., zooplankton and sergestid shrimps) and benthic (e.g., polychaetes) prey (see also Salen-Picard et al. 2002, Dunton et al. 2006, Mcleod and Wing 2009). For instance, production of zooplankton and sergestid shrimps in monsoonal estuaries is usually coupled with distribution of particulate organic matter and production of phytoplankton upon the onset of the wet season (Chiou et al. 2000, Chen et al. 2009).

Conclusion

This study revealed the relative importance of terrestrial carbon and detrital pathways for two predators in the PRE, the pelagic spadenose shark and the benthic bamboo shark. Although autochthonous marine carbon sources (benthic macroalgae and phytoplankton) generally form the base of both pelagic and benthic food chains in the estuary, and were important for sharks further offshore, FA and FA-specific $\delta^{13}C$ biomarkers indicated spatial variation in detrital carbon distribution and associated spatial differences in utilization of terrestrial detritus by sharks in the estuary, especially during the wet season when terrestrial run-off is high (~95% of total; Kot and Hu 1995). These allochthonous trophic subsidies were especially important to immature sharks of both species. These findings highlight the importance of the PRE, especially inshore areas, as a shark feeding ground and a nursery for immature individuals. Zooplanktivorous fish and sergestid shrimps appear to be the major route of transfer of energy from detritus to spadenose sharks (this study), while bamboo sharks assimilated detrital carbon via consumption of benthic polychaetes and small crustaceans (Wai et al. 2011*a*). Similar prey taxa are abundant in estuaries over the world (Wantiez et al. 1996, Rhodes 1998, Hajisamae et al. 2006), and may represent an important medium for transfer of allochthonous detrital carbon from the land to marine predators in other estuarine ecosystems.

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SUPPLEMENTAL MATERIAL

APPENDIX A

Table A1. Diet composition of spadenose sharks. *F*-ratio and power estimates of general linear models to investigate variation in % weight of shark prey items in different seasons (Se: wet vs. dry) and locations (Lo: inner vs. outer estuary).

Prey item	Genus/Species	*F*-ratio			Power estimate		
		Se	Lo	Se × Lo	Se	Lo	Se × Lo
Pelagic teleosts	Total %weight#	1.23	**6.92****	2.06	0.20	0.74	0.30
Bregmacerotidae	*Bregmaceros lanceolatus*#	2.05	5.43*	2.05	0.30	0.64	0.30
Carangidae	*Alepes vari*	...	...	...	...	...	...
	Decapterus sp.	...	...	...	...	...	...
Clupeidae	*Sardinella melanura*	...	...	...	...	...	...
Engraulidae	Total %weight of anchovies#	1.54	3.35	1.48	0.23	0.44	0.23
	Coilia sp.	...	...	...	...	...	...
	Thryssa sp.#	**8.07****	0.67	0.61	0.81	0.13	0.12
	Stolephorus insularis#	6.39*	2.25	1.01	0.71	0.32	0.17
Demersal teleosts	Total %weight#	**11.52****	0.19	1.57	0.92	0.07	0.24
Ambassidae	*Ambassis* sp.#	2.71	2.71	2.71	0.37	0.37	0.37
Gobiidae	Total %weight of gobies#	0.20	4.74*	0.20	0.07	0.58	0.07
	Parachaeturichthys polynema	...	...	...	...	...	...
	Trypauchen vagina#	0.40	4.76*	0.40	0.10	0.58	0.10
Leiognathidae	Total %weight of ponyfishes#	**7.36****	0.95	0.15	0.77	0.16	0.07
	Secutor ruconius#	**7.98****	0.19	0.12	0.80	0.07	0.06
	Leiognathus brevirostris#	0.03	2.19	0.03	0.05	0.31	0.05
Mugilidae	*Valamugil cunnesius*	...	...	...	...	...	...
Sciaenidae	*Collichthys lucidus*	...	...	...	...	...	...
Trichiuridae	*Trichiurus* sp.	...	...	...	...	...	...
Eels	#	0.78	0.06	0.63	0.14	0.06	0.12
Unidentified fish tissues		0.06	0.32	1.38	0.06	0.09	0.21
Cephalopoda							
Loligonidae	*Uroteuthis* sp.#	0.20	0.31	1.75	0.07	0.09	0.26
Crustaceans	Total %weight of crustaceans	1.90	**5.25***	0.08	0.28	0.63	0.06
Sergestid shrimps	*Acetes* sp.#	0.04	4.12*	1.45	0.05	0.52	0.22
Penaeid shrimps	#	4.75*	0.56	0.46	0.58	0.12	0.10
Stomatopods	#	0.48	1.68	0.05	0.11	0.25	0.06
Unidentified crab fragments	#	5.52*	5.52*	**7.55****	0.65	0.65	0.78
Annelids							
Polychaetes		...	...	...	...	...	...
Plant materials	#	1.76	0.03	0.04	0.26	0.05	0.05
Unidentified organic matter		0.00	0.00	1.11	0.05	0.05	0.18

Notes: Significant terms are indicated by bold *F*-ratio and * ($P < 0.05$) and ** ($P < 0.01$). # indicates data failed Levene's test for homogeneity of variances (see text for details).

APPENDIX B

Table B1. Diet composition of spadenose sharks. Mean % weight (± SD) of shark prey items in different seasons and locations. N = number of stomachs examined.

		Wet season		Dry season	
Prey items	Genus/Species	Inner estuary $N = 94$	Outer estuary $N = 47$	Inner estuary $N = 16$	Outer estuary $N = 22$
Pelagic teleosts	Total %weight	39.79 ± 46.19	29.98 ± 43.71	42.48 ± 46.86	9.09 ± 29.42
Bregmacerotidae	*Bregmaceros lanceolatus*	2.21 ± 14.38	0.00 ± 0.00	9.23 ± 26.97	0.00 ± 0.00
Carangidae	*Alepes vari*	0.35 ± 3.38	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Decapterus sp.	3.78 ± 18.18	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Clupeidae	*Sardinella melanura*	0.00 ± 0.00	1.40 ± 9.58	0.00 ± 0.00	0.00 ± 0.00
Engraulidae	Total %weight of anchovies	33.46 ± 44.01	28.58 ± 42.84	33.25 ± 46.08	9.09 ± 29.42
	Coilia sp.	1.06 ± 10.31	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Thryssa sp.	2.24 ± 11.89	2.13 ± 14.59	14.50 ± 32.84	9.09 ± 29.42
	Stolephorus insularis	30.15 ± 43.54	26.45 ± 41.68	18.75 ± 40.31	0.00 ± 0.00
Demersal teleosts	Total %weight	8.35 ± 26.98	3.98 ± 19.12	19.90 ± 35.74	29.02 ± 44.56
Ambassidae	*Ambassis* sp.	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	4.55 ± 21.32
Gobiidae	Total %weight of gobies	6.31 ± 24.29	0.00 ± 0.00	9.52 ± 27.43	0.00 ± 0.00
	Parachaeturichthys polynema	1.06 ± 10.31	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	Trypauchen vagina	5.24 ± 22.25	0.00 ± 0.00	9.52 ± 27.43	0.00 ± 0.00
Leiognathidae	Total %weight of ponyfishes	1.83 ± 12.67	3.98 ± 19.12	10.38 ± 27.12	15.42 ± 34.63
	Secutor ruconius	1.83 ± 12.67	2.13 ± 14.59	10.38 ± 27.12	13.11 ± 33.84
	Leiognathus brevirostris	0.00 ± 0.00	1.85 ± 12.68	0.00 ± 0.00	2.31 ± 10.82
Mugilidae	*Valamugil cunnesius*	0.21 ± 2.04	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
Sciaenidae	*Collichthys lucidus*	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	4.55 ± 21.32
Trichiuridae	*Trichiurus* sp.	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	4.51 ± 19.13
Eels		1.06 ± 10.31	4.26 ± 20.40	6.25 ± 25.00	4.55 ± 21.32
Unidentified fish tissues		1.78 ± 11.63	0.60 ± 4.14	0.00 ± 0.00	3.34 ± 15.68
Cephalopoda					
Loligonidae	*Uroteuthis* sp.	2.13 ± 14.51	0.28 ± 1.90	0.00 ± 0.00	4.55 ± 21.32
Crustaceans	Total %weight of crustaceans	39.38 ± 45.26	56.64 ± 47.22	25.10 ± 44.66	47.24 ± 47.30
Sergestid shrimps	*Acetes* sp.	11.94 ± 31.00	34.17 ± 44.17	18.85 ± 40.26	24.51 ± 39.55
Penaeid shrimps		18.25 ± 36.47	18.71 ± 36.82	0.00 ± 0.00	9.09 ± 29.42
Stomatopods		8.14 ± 25.73	3.77 ± 16.91	6.25 ± 25.00	0.00 ± 0.00
Unidentified crab fragments		1.06 ± 10.31	0.00 ± 0.00	0.00 ± 0.00	13.64 ± 35.13
Annelids					
Polychaetes		0.00 ± 0.00	0.00 ± 0.00	6.25 ± 25.00	0.00 ± 0.00
Plant materials		5.57 ± 22.63	4.26 ± 20.40	0.02 ± 0.08	0.11 ± 0.53
Unidentified organic matter		1.93 ± 13.23	0.00 ± 0.00	0.00 ± 0.00	2.11 ± 9.88

APPENDIX C

Table C1. Mean values (‰ ± SD) of $\delta^{13}C$ and $\delta^{15}N$ of spadenose shark prey items in the inner and outer Pearl River Estuary. N = sample sizes of each group. # Data are extracted from Wai et al. (2011*a*).

Prey item	Inner estuary			Outer estuary		
	N	$\delta^{13}C$	$\delta^{15}N$	N	$\delta^{13}C$	$\delta^{15}N$
Anchovies	11	−18.68 ± 0.61	15.45 ± 0.93	6	−16.68 ± 0.61	14.50 ± 0.61
Gobies	12	−18.00 ± 0.94	13.32 ± 1.47	6	−16.09 ± 0.46	12.15 ± 0.41
Ponyfishes	7	−19.27 ± 1.32	14.87 ± 0.68	5	−14.87 ± 0.68	13.64 ± 0.25
Eels	4	−18.82 ± 0.30	11.09 ± 0.36	6	−16.72 ± 1.17	11.07 ± 1.45
Squids	4	−16.00 ± 0.27	12.18 ± 1.37	9	−15.24 ± 0.81	13.14 ± 1.18
Polychaetes#	6	−17.30 ± 0.87	8.22 ± 1.50	6	−17.01 ± 0.39	8.92 ± 0.85
Sergestid shrimps	6	−18.14 ± 0.86	11.21 ± 2.07	6	−15.92 ± 0.40	10.72 ± 2.30
Penaeid shrimps#	6	−17.84 ± 0.79	12.76 ± 1.53	6	−16.73 ± 0.75	12.48 ± 1.36
Stomatopods	4	−15.30 ± 0.11	12.31 ± 0.18	6	−14.62 ± 0.21	11.83 ± 0.74
Other small crustaceans (overall mean)	8	−16.33 ± 0.21	9.14 ± 0.40	8	−15.27 ± 0.29	8.66 ± 0.22
Charybdis crabs#	2	−16.07 ± 0.21	8.70 ± 0.39	2	−15.40 ± 0.21	8.55 ± 0.21
Porcelain crabs#	2	−16.55 ± 0.52	9.45 ± 0.12	2	−15.20 ± 0.23	9.00 ± 0.11
Alpheid shrimps#	2	−16.48 ± 0.08	8.83 ± 0.23	2	−14.88 ± 0.12	8.65 ± 0.32
Unidentified shrimps#	2	−16.23 ± 0.11	9.56 ± 0.21	2	−15.60 ± 0.12	8.45 ± 0.15

APPENDIX D

Table D1. General linear models to investigate differences in spadenose shark size classes with respect to (1) $\delta^{13}C$, $\delta^{15}N$, C:N ratio, liver somatic index (LSI) and lipid content, and (2) % weight of selected fatty acid (FA) variables and biomarkers. Abbreviations are as follows: YOY (young-of-the-year), SFA (saturated fatty acids), MUFA (monounsaturated fatty acids), PUFA (polyunsaturated fatty acids), BaFA (Bacterial FA), EFA (essential FA), Dino FA (dinoflagellate FA), Zoo FA (zooplankton FA), and LCSFA (long carbon chain SFA).

Variable	Biomarker	MS	*F*-ratio	SNK tests
(1)				
$\delta^{13}C$		13.25	**108.44****	juvenile < pre-mature < adult < YOY = pup
$\delta^{15}N$		9.94	**28.05*****	pup = pre-mature < YOY = adult < juvenile
C:N		0.16	**21.46*****	adult = pre-mature = juvenile < pup < YOY
Lipid (%)		30.88	**92.82*****	$adult^{D}$ $pre\text{-}mature^{CD}$ = $juvenile^{C}$ < YOY^{B} < pup^{A}
LSI†		26.28	**12.56****	pre-mature = juvenile = adult < YOY
(2)				
SFA	Total	230.36	**153.42*****	pup > YOY = adult = pre-mature = juvenile
MUFA	Total	644.95	**169.53*****	pup > YOY = juvenile = pre-mature > adult
PUFA	Total	2704.8	**481.55*****	adult > pre-mature = juvenile > YOY > pup
Diatom FA	16:1	182.08	**491.03*****	pup > YOY > juvenile > pre-mature = adult
	20:5n3	21.31	**49.42*****	juvenile > YOY = pre-mature = adult > pup
	Total	109.08	**112.63*****	pup = YOY > juvenile > pre-mature = adult
BaFA	i15:0#	0.11	**88.43*****	pre-mature > juvenile > pup > adult > YOY
	a15:0	0.01	**35.71*****	pre-mature > juvenile = adult > YOY > pup
	i17:0	0.51	**144.32*****	pre-mature > juvenile > adult > pup > YOY
	a17:0	0.02	**7.30*****	pup = YOY = juvenile = pre-mature > adult
	18:1n7#	10.40	**25.71*****	pup > YOY = juvenile = pre-mature = adult
	Total#	10.00	**25.61*****	pup^{A} > YOY^{B} = $juvenile^{BC}$ $pre\text{-}mature^{C}$ = $adult^{C}$
Essential FA	18:2n6#	13.87	**34.82*****	pre-mature > adult > juvenile > YOY = pup
	18:3n3#	0.46	**130.03*****	juvenile = pre-mature > adult > pup > YOY
	Total#	16.99	**43.23*****	pre-mature > adult > juvenile > YOY = pup
Dino FA	22:6n3#	652.54	**306.65*****	adult > juvenile = pre-mature > YOY > pup
Zoo FA	20:1n9	2.88	**11.47*****	YOY^{A} = pup^{AB} $juvenile^{B}$ = $pre\text{-}mature^{B}$ > $adult^{C}$
	22:1n9	0.91	**89.81*****	pup > YOY > juvenile > pre-mature = adult
	Total	4.67	**15.25*****	pup^{A} = YOY^{AB} $juvenile^{B}$ = $pre\text{-}mature^{B}$ > $adult^{C}$
FA for macroalgae and protozoa	20:4n6#	26.61	**124.95*****	adult > pre-mature > juvenile = YOY > pup
LCSFA	Total#	0.87	**56.52*****	pup^{A} > $juvenile^{AB}$ YOY^{B} = $pre\text{-}mature^{BC}$ $adult^{C}$

Notes: For (1), degrees of freedom (df) for size and residual are 4 and 109, respectively (except the LSI residual which is 90); † LSI of pups was not available. For (2), df for factor size and residual are 4 and 107, respectively. Significant differences between size classes are indicated by bold *F*-ratio and $*P < 0.05$, $**P < 0.01$ and $***P < 0.001$. # indicates data failed Levene's test for homogeneity of variances (see text for details). Results of SNK tests are shown, with inter-size class differences designated by superscripts A, B, C and D when differences cannot be simply shown by "=", "<" or ">" symbols; see Tables 1 and 6 for means.

APPENDIX E

Table E1. *F*-ratio of general linear models to investigate variations of seasons (Se), locations (Lo) and size classes (Si) in (1) $\delta^{13}C$, $\delta^{15}N$, C:N ratio, liver somatic index (LSI) and lipid content of spadenose sharks; (2) % weight of selected fatty acid (FA) variables and biomarkers. Abbreviations are as follows: SFA (saturated fatty acids), MUFA (monounsaturated fatty acids), PUFA (polyunsaturated fatty acids), BaFA (Bacterial FA), EFA (essential FA), Dino FA (dinoflagellate FA), Zoo FA (zooplankton FA), and LCSFA (long carbon chain SFA).

Variable	Biomarker	Se	Lo	Si	Se × Lo	Se × Si	Lo × Si	Se × Lo × Si
(1)								
$\delta^{13}C$		0.34	**3061.98*** **	**20.22*** **	0.08	0.57	**12.39*** **	1.50
$\delta^{15}N$#		8.06*	**49.56*** **	**15.97*** **	**19.67*** **	0.40	1.33	0.15
C:N#		**58.68*** **	**14.92*** **	0.93	4.63*	1.13	1.64	0.37
Lipid (%)#		2.93	2.26	3.20	**12.40*** **	0.75	2.39	1.58
LSI		**5.28***	**5.31***	0.00	**5.03***	0.15	**11.88*** **	1.03
(2)								
SFA	Total#	**22.52*** **	0.20	1.13	**26.73*** **	0.04	1.79	0.14
MUFA	Total	1.67	**9.51****	**22.20*** **	**33.87*** **	**6.34***	0.03	0.01
PUFA	Total	**23.25*** **	**10.44****	**20.53*** **	**4.23***	**6.44***	0.54	0.09
Diatom FA	16:1#	0.50	**7.17****	**19.26*** **	4.95*	1.88	0.94	0.94
	20:5n3#	**14.43*** **	**7.76****	**11.93****	2.52	1.07	1.04	**9.03****
	Total#	5.78*	0.69	**19.04*** **	0.03	0.02	0.10	6.57*
BaFA	i15:0	**53.49*** **	4.09	**48.89*** **	**8.55*** **	**43.08****	**6.18***	0.45
	a15:0	**26.07*** **	**52.27*** **	**15.52*** **	1.67	**19.09*** **	**5.34***	1.69
	i17:0	**36.87*** **	**29.61*** **	**39.65*** **	**7.88****	**17.41*** **	**14.86*** **	0.11
	a17:0	**8.71****	**8.10****	**18.90*** **	**12.38****	2.53	1.92	0.54
	18:1n7#	**39.46*** **	**50.05*** **	**0.01*** **	**51.83*** **	**10.39****	3.44	2.43
	Total#	**51.99*** **	**38.01*** **	1.47	**57.87*** **	**15.62*** **	1.67	2.69
Essential FA	18:2n6#	0.87	**91.62*** **	0.82	0.62	0.21	4.04	0.24
	18:3n3#	**11.94****	**104.54*** **	**57.00*** **	5.42*	**11.23****	**15.15*** **	3.21
	Total#	1.22	**101.43*** **	1.63	0.45	0.08	4.84*	0.33
Dino FA	22:6n3#	**9.10****	**13.61*** **	**28.25*** **	0.04	**8.00*** **	**11.88*** **	4.85*
Zoo FA	20:1n9	**7.50****	**8.81****	**20.95*** **	**43.32*** **	1.91	0.55	1.00
	22:1n9	**5.39***	**8.24****	**7.81****	0.11	0.11	0.05	0.87
	Total	**8.45****	5.75*	**22.15*** **	**39.11*** **	1.59	0.45	0.66
FA†	20:4n6#	2.46	**8.62****	**9.80****	**10.97****	5.98*	7.26*	0.07
LCSFA	Total#	**38.65*** **	0.82	0.31	5.04*	2.30	1.22	1.24

Notes: Degrees of freedom (df) for Se, Lo, Si, Se × Lo, Se × Si, Lo × Si and Se × Lo × Si, and residual are 1, 1, 1, 1, 1, 1, 1 and 153, respectively. Significant differences are indicated by bold *F*-ratio and *$P < 0.05$, **$P < 0.01$ and ***$P < 0.001$. # indicates data failed Levene's test for homogeneity of variances (see text for details). †FA for macroalgae and protozoa.

Appendix F

Table F1. Mean fatty acid (FA) % weight (± SD) of 34 FA variables in total lipids of different spadenose shark size classes in different locations in wet season. N = sample size of each group.

	Inner estuary					Outer estuary	
FA variables	Pup	YOY	Juvenile	Pre-mature	Adult	Pre-mature	Adult
N	18	13	24	36	22	14	31
14:0	2.11 ± 0.13	1.58 ± 0.20	1.67 ± 0.18	1.50 ± 0.19	1.25 ± 0.34	1.72 ± 0.18	1.26 ± 0.35
14:1	0.08 ± 0.02	0.05 ± 0.02	0.01 ± 0.01	0.01 ± 0.00	0.02 ± 0.02	0.01 ± 0.00	0.01 ± 0.01
i15:0	0.19 ± 0.03	0.11 ± 0.02	0.26 ± 0.04	0.29 ± 0.04	0.16 ± 0.04	0.23 ± 0.04	0.15 ± 0.04
a15:0	0.06 ± 0.01	0.04 ± 0.01	0.07 ± 0.02	0.09 ± 0.02	0.06 ± 0.01	0.06 ± 0.01	0.05 ± 0.01
15:0	0.19 ± 0.03	0.15 ± 0.02	0.44 ± 0.05	0.39 ± 0.05	0.25 ± 0.06	0.37 ± 0.06	0.25 ± 0.06
16:0	21.99 ± 0.64	16.55 ± 1.32	15.17 ± 1.17	15.35 ± 0.58	15.70 ± 0.74	15.87 ± 0.54	16.93 ± 1.50
∑16:1	8.87 ± 0.55	6.11 ± 1.05	2.87 ± 0.52	2.18 ± 0.39	1.81 ± 0.71	2.41 ± 0.31	1.67 ± 0.57
i17:0	0.22 ± 0.04	0.18 ± 0.03	0.45 ± 0.07	0.55 ± 0.07	0.37 ± 0.06	0.40 ± 0.05	0.32 ± 0.06
a17:0	0.24 ± 0.03	0.23 ± 0.03	0.21 ± 0.07	0.23 ± 0.05	0.17 ± 0.02	0.17 ± 0.05	0.14 ± 0.03
17:0	0.18 ± 0.02	0.20 ± 0.03	0.89 ± 0.13	0.90 ± 0.11	0.64 ± 0.12	0.88 ± 0.10	0.66 ± 0.14
17:1n10	0.18 ± 0.11	0.20 ± 0.10	0.32 ± 0.10	0.24 ± 0.15	0.21 ± 0.12	0.22 ± 0.18	0.23 ± 0.10
18:0	11.98 ± 0.45	10.72 ± 0.43	10.67 ± 0.54	11.00 ± 0.62	11.47 ± 0.70	11.79 ± 0.60	12.24 ± 0.93
18:1n9c	20.74 ± 0.76	17.20 ± 1.42	11.72 ± 1.46	11.72 ± 0.86	9.91 ± 1.34	11.14 ± 1.08	9.34 ± 1.57
18:1n7	8.84 ± 0.63	7.26 ± 0.68	7.20 ± 0.69	7.43 ± 0.56	6.97 ± 0.67	7.45 ± 0.38	6.90 ± 0.53
18:2n6c	0.51 ± 0.10	0.58 ± 0.12	1.31 ± 0.64	2.32 ± 0.53	1.87 ± 1.04	0.60 ± 0.20	0.91 ± 0.89
18:3n6	0.09 ± 0.02	0.14 ± 0.03	0.24 ± 0.06	0.20 ± 0.04	0.14 ± 0.05	0.16 ± 0.04	0.10 ± 0.04
18:3n3	0.05 ± 0.02	0.06 ± 0.01	0.40 ± 0.11	0.28 ± 0.04	0.16 ± 0.04	0.14 ± 0.03	0.11 ± 0.03
20:0	0.38 ± 0.03	0.26 ± 0.05	0.20 ± 0.04	0.17 ± 0.03	0.15 ± 0.02	0.19 ± 0.02	0.17 ± 0.04
∑20:1	2.66 ± 0.16	3.10 ± 0.53	2.92 ± 0.64	2.76 ± 0.50	2.09 ± 0.49	2.34 ± 0.46	1.73 ± 0.58
20:2n6	3.11 ± 0.25	3.59 ± 0.57	1.74 ± 0.62	1.84 ± 0.29	1.42 ± 0.50	0.81 ± 0.17	0.84 ± 0.59
20:3n6	0.19 ± 0.05	0.32 ± 0.08	0.38 ± 0.09	0.48 ± 0.07	0.56 ± 0.20	0.31 ± 0.05	0.49 ± 0.25
20:4n6	2.49 ± 0.32	3.93 ± 0.30	4.17 ± 0.52	4.94 ± 0.44	5.50 ± 0.58	5.62 ± 0.48	7.05 ± 1.36
21:0	0.02 ± 0.01	0.01 ± 0.01	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.00	0.03 ± 0.00	0.03 ± 0.01
20:3n3	0.0 ± 0.01	0.03 ± 0.01	0.39 ± 0.12	0.31 ± 0.05	0.16 ± 0.07	0.18 ± 0.03	0.10 ± 0.04
20:5n3	1.48 ± 0.27	3.83 ± 0.70	4.23 ± 0.56	3.50 ± 0.59	3.43 ± 0.98	3.62 ± 0.69	2.92 ± 0.89
22:0	0.46 ± 0.03	0.26 ± 0.06	0.27 ± 0.07	0.24 ± 0.05	0.20 ± 0.03	0.25 ± 0.05	0.21 ± 0.06
∑22:1	0.95 ± 0.08	0.70 ± 0.12	0.54 ± 0.13	0.46 ± 0.09	0.44 ± 0.08	0.42 ± 0.06	0.37 ± 0.08
22:2n6	1.70 ± 0.29	2.81 ± 0.50	1.03 ± 0.39	0.64 ± 0.16	0.37 ± 0.13	0.49 ± 0.11	0.31 ± 0.18
22:4n6	0.43 ± 0.04	1.02 ± 0.29	1.29 ± 0.25	1.93 ± 0.25	3.24 ± 1.21	1.96 ± 0.26	4.18 ± 1.96
23:0	0.09 ± 0.01	0.12 ± 0.01	0.21 ± 0.02	0.21 ± 0.02	0.23 ± 0.04	0.20 ± 0.01	0.31 ± 0.11
22:5n3	1.75 ± 0.27	6.17 ± 1.55	9.70 ± 2.37	9.39 ± 1.01	9.84 ± 1.31	10.07 ± 0.87	8.12 ± 2.07
22:6n3	5.85 ± 0.72	11.36 ± 1.25	17.79 ± 2.02	17.40 ± 1.33	20.13 ± 1.49	18.90 ± 0.89	20.91 ± 1.66
24:0	0.29 ± 0.04	0.17 ± 0.04	0.18 ± 0.05	0.13 ± 0.03	0.10 ± 0.02	0.13 ± 0.03	0.10 ± 0.02
24:1n9	1.62 ± 0.16	0.97 ± 0.30	1.00 ± 0.30	0.91 ± 0.19	0.97 ± 0.21	0.85 ± 0.16	0.86 ± 0.21

Appendix G

Table G1. Mean fatty acid (FA) % weight (± SD) of 34 FA variables in total lipids of different spadenose shark size classes in different locations in dry season. N = sample size of each group.

	Inner estuary		Outer estuary	
FA variables	Pre-mature	Adult	Pre-mature	Adult
N	19	20	3	16
14:0	1.10 ± 0.23	1.15 ± 0.24	1.36 ± 0.23	1.23 ± 0.19
14:1	0.01 ± 0.01	0.01 ± 0.01	0.02 ± 0.02	0.01 ± 0.00
i15:0	0.16 ± 0.03	0.14 ± 0.03	0.15 ± 0.02	0.16 ± 0.03
a15:0	0.06 ± 0.01	0.06 ± 0.01	0.04 ± 0.00	0.05 ± 0.01
15:0	0.25 ± 0.05	0.21 ± 0.03	0.27 ± 0.01	0.27 ± 0.04
16:0	14.77 ± 1.15	15.47 ± 1.14	14.15 ± 0.75	14.49 ± 0.46
∑16:1	1.86 ± 0.31	1.57 ± 0.25	2.33 ± 0.86	2.04 ± 0.41
i17:0	0.38 ± 0.06	0.32 ± 0.05	0.31 ± 0.05	0.33 ± 0.06
a17:0	0.16 ± 0.03	0.14 ± 0.02	0.16 ± 0.02	0.15 ± 0.03
17:0	0.65 ± 0.08	0.54 ± 0.11	0.67 ± 0.08	0.70 ± 0.08
17:1n10	0.35 ± 0.07	0.20 ± 0.09	0.27 ± 0.23	0.23 ± 0.15
18:0	12.85 ± 0.87	11.91 ± 1.35	11.09 ± 0.48	11.65 ± 0.76
18:1n9c	11.08 ± 0.90	9.53 ± 0.91	11.22 ± 1.33	10.85 ± 0.99
18:1n7	4.67 ± 0.83	5.66 ± 1.34	7.34 ± 0.55	7.29 ± 0.53
18:2n6c	2.29 ± 0.77	1.85 ± 0.91	0.49 ± 0.04	0.51 ± 0.07
18:3n6	0.15 ± 0.03	0.12± 0.02	0.11 ± 0.01	0.12 ± 0.03
18:3n3	0.20 ± 0.03	0.15 ± 0.03	0.12 ± 0.01	0.11 ± 0.03
20:0	0.13 ± 0.02	0.14 ± 0.02	0.15 ± 0.02	0.15 ± 0.02
∑20:1	2.09 ± 0.46	1.93 ± 0.59	3.31 ± 0.33	2.78 ± 0.59
20:2n6	1.28 ± 0.28	1.17 ± 0.41	0.84 ± 0.11	0.71 ± 0.08
20:3n6	0.46 ± 0.08	0.52 ± 0.15	0.27 ± 0.02	0.32 ± 0.04
20:4n6	6.29 ± 0.88	5.89 ± 1.12	5.70 ± 0.52	6.35 ± 0.57
21:0	0.04 ± 0.01	0.03 ± 0.01	0.02 ± 0.00	0.02 ± 0.01
20:3n3	0.15 ± 0.04	0.12 ± 0.04	0.14 ± 0.02	0.11 ± 0.02
20:5n3	5.00 ± 0.72	3.64 ± 0.96	3.66 ± 0.05	3.59 ± 0.52
22:0	0.16 ± 0.02	0.17 ± 0.04	0.15 ± 0.03	0.17 ± 0.02
∑22:1	0.52 ± 0.07	0.45 ± 0.06	0.46 ± 0.04	0.43 ± 0.09
22:2n6	0.22 ± 0.11	0.27 ± 0.16	0.61 ± 0.15	0.42 ± 0.09
22:4n6	2.12 ± 0.54	3.45 ± 1.00	2.00 ± 0.25	2.23 ± 0.51
23:0	0.20 ± 0.02	0.24 ± 0.03	0.20 ± 0.01	0.21 ± 0.02
22:5n3	10.91 ± 1.21	10.99 ± 1.89	10.44 ± 0.63	11.30 ± 2.64
22:6n3	18.52 ± 1.62	20.89 ± 1.47	21.18 ± 1.72	20.26 ± 0.89
24:0	0.09 ± 0.01	0.09 ± 0.03	0.08 ± 0.01	0.08 ± 0.02
24:1n9	0.83 ± 0.12	0.98 ± 0.21	0.70 ± 0.15	0.71 ± 0.11

APPENDIX H

Table H1. Mean fatty acid (FA) % weight (± SD) of 34 FA variables in total lipids of juveniles and adults of spadenose sharks and bamboo sharks in the inner Pearl River Estuary in wet season. *N* = sample size of each group.

	Spadenose sharks		Bamboo sharks	
FA variable	Juveniles	Adults	Juveniles	Adults
N	6	16	12	12
14:0	1.50 ± 0.14	1.06 ± 0.23	1.63 ± 0.46	1.07 ± 0.26
14:1	0.01 ± 0.00	0.01 ± 0.01	0.03 ± 0.02	0.05 ± 0.04
i15:0	0.25 ± 0.04	0.14 ± 0.02	0.62 ± 0.08	0.63 ± 0.13
a15:0	0.07 ± 0.02	0.06 ± 0.01	0.15 ± 0.02	0.14 ± 0.03
15:0	0.40 ± 0.02	0.22 ± 0.03	0.45 ± 0.03	0.41 ± 0.05
16:0	15.34 ± 1.23	16.23 ± 1.13	11.29 ± 0.65	12.00 ± 1.44
∑16:1	2.35 ± 0.27	1.69 ± 0.29	4.02 ± 0.60	3.85 ± 0.52
i17:0	0.47 ± 0.08	0.34 ± 0.06	1.23 ± 0.57	1.10 ± 0.25
a17:0	0.19 ± 0.03	0.15 ± 0.03	0.48 ± 0.19	0.42 ± 0.08
17:0	0.87 ± 0.07	0.54 ± 0.08	1.26 ± 0.13	0.93 ± 0.09
17:1n10	0.28 ± 0.13	0.28 ± 0.09	1.04 ± 0.46	0.54 ± 0.06
18:0	10.52 ± 0.61	12.88 ± 1.16	10.90 ± 0.50	10.11 ± 0.94
18:1n9c	11.21 ± 0.91	10.30 ± 1.18	7.66 ± 0.43	10.94 ± 1.27
18:1n7	7.48 ± 0.41	4.87 ± 1.24	5.90 ± 0.37	7.22 ± 1.41
18:2n6c	1.64 ± 0.57	2.51 ± 0.75	2.43 ± 0.33	3.27 ± 1.43
18:3n6	0.21 ± 0.03	0.13 ± 0.04	0.24 ± 0.07	0.11 ± 0.04
18:3n3	0.35 ± 0.07	0.16 ± 0.04	0.18 ± 0.03	0.14 ± 0.04
20:0	0.17 ± 0.03	0.13 ± 0.02	0.25 ± 0.03	0.16 ± 0.02
∑20:1	3.05 ± 0.79	1.78 ± 0.44	0.52 ± 0.14	1.56 ± 0.51
20:2n6	1.67 ± 0.26	1.39 ± 0.44	1.14 ± 0.38	1.81 ± 0.69
20:3n6	0.38 ± 0.04	0.58 ± 0.13	0.46 ± 0.07	0.86 ± 0.16
20:4n6	4.49 ± 0.69	6.06 ± 0.98	5.49 ± 0.72	6.53 ± 0.63
21:0	0.03 ± 0.01	0.04 ± 0.01	0.05 ± 0.02	0.04 ± 0.03
20:3n3	0.36 ± 0.05	0.11 ± 0.03	0.04 ± 0.02	0.09 ± 0.03
20:5n3	3.74 ± 0.47	4.38 ± 1.28	13.66 ±1.62	5.14 ± 2.08
22:0	0.23 ± 0.05	0.16 ± 0.03	0.37 ± 0.05	0.23 ± 0.04
∑22:1	0.48 ± 0.18	0.43 ± 1.28	0.26 ± 0.05	0.51 ± 0.27
22:2n6	0.82 ± 0.29	0.18 ± 0.03	0.21 ± 0.07	0.20 ± 0.08
22:4n6	1.61 ± 0.36	3.10 ± 0.96	0.72 ± 0.14	1.49 ± 0.56
23:0	0.21 ± 0.03	0.22 ± 0.03	0.13 ± 0.04	0.09 ± 0.09
22:5n3	9.68 ± 0.82	9.83 ± 1.51	12.14 ± 2.26	12.18 ± 2.47
22:6n3	18.95 ± 1.19	19.05 ± 1.63	14.21 ± 1.02	15.13 ± 2.84
24:0	0.14 ± 0.03	0.08 ± 0.02	0.24 ± 0.05	0.14 ± 0.03
24:1n9	0.86 ± 0.23	0.89 ± 0.14	0.61 ± 0.09	0.91 ± 0.17

Appendix I

Table I1. Mean fatty acid stable carbon isotopic (FASI) value (‰ ± SD) of 25 FA variables in total lipids of juveniles and adults of spadenose sharks and bamboo sharks in the inner Pearl River Estuary. N = sample size of each group.

	Spadenose sharks		Bamboo sharks	
FA variable	Juveniles	Adults	Juveniles	Adults
N	6	16	12	12
14:0	−24.88 ± 0.32	−23.24 ± 1.07	−25.06 ± 1.04	−22.45 ± 1.09
i15:0	−26.76 ± 1.01	−26.13 ± 1.51	−26.56 ± 0.53	−25.30 ± 1.29
a15:0	−21.94 ± 1.05	−24.76 ± 1.22	−22.41 ± 1.51	−20.19 ± 2.03
15:1	−27.88 ± 1.87	−26.34 ± 2.08	−25.84 ± 0.81	−24.14 ± 2.07
15:0	−25.53 ± 0.67	−24.18 ± 2.34	−21.45 ± 2.12	−20.07 ± 2.23
16:1	−25.57 ± 0.51	−27.41 ± 2.03	−24.78 ± 1.86	−24.86 ± 1.02
16:0	−23.74 ± 0.14	−21.93 ± 0.39	−21.25 ± 1.01	−20.55 ± 0.79
i17:0	−26.67 ± 1.95	−26.37 ± 2.06	−25.50 ± 0.83	−24.49 ± 1.17
a17:0	−25.76 ± 0.80	−24.04 ± 0.99	−22.86 ± 1.09	−23.15 ± 0.85
17:0	−26.65 ± 1.14	−24.54 ± 1.28	−23.26 ± 0.86	−22.89 ± 1.02
18:2n6c	−25.76 ± 1.44	−27.60 ± 1.88	−25.70 ± 1.82	−26.94 ± 1.02
18:1n9c	−25.47 ± 1.51	−25.03 ± 0.88	−25.55 ± 1.63	−25.32 ± 0.85
18:1n7	−25.73 ± 1.42	−25.09 ± 1.07	−19.24 ± 2.87	−19.60 ± 2.12
18:0	−24.21 ± 0.22	−22.63 ± 0.42	−21.74 ± 0.95	−21.28 ± 0.59
20:4n6	−27.33 ± 0.65	−28.71 ± 2.06	−26.72 ± 2.15	−27.44 ± 1.17
20:5n3	−24.89 ± 0.63	−22.50 ± 2.23	−23.63 ± 1.36	−20.32 ± 1.16
20:3n6	−25.15 ± 0.48	−24.24 ± 2.28	−13.23 ± 3.13	−19.89 ± 1.72
20:4n3	−25.90 ± 0.27	−23.70 ± 2.43	−17.87 ± 2.28	−20.74 ± 1.20
20:1n9	−26.27 ± 1.21	−23.57 ± 1.32	−21.51 ± 3.27	−21.41 ± 0.61
20:0	−23.73 ± 0.86	−23.71 ± 1.83	−21.18 ± 1.24	−19.96 ± 1.54
22:6n3	−26.54 ± 0.83	−25.47 ± 0.66	−26.40 ± 2.07	−27.00 ± 1.42
22:0	−26.06 ± 0.78	−24.55 ± 1.23	−20.93 ± 1.19	−19.65 ± 1.55
23:0	−28.74 ± 3.04	−23.43 ± 2.13	−21.93 ± 0.95	−21.78 ± 0.98
24:1n9	−26.47 ± 0.36	−24.40 ± 0.84	−24.40 ± 1.22	−24.54 ± 1.18
24:0	−24.80 ± 0.64	−21.61 ± 1.83	−22.14 ± 0.91	−20.66 ± 1.42