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$\delta^{15}N$ and $\delta^{13}C$ diet–tissue discrimination factors for large sharks under semi-controlled conditions☆

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ABSTRACT

Stable isotopes ($\delta^{15}N$ and $\delta^{13}C$) are being widely applied in ecological research but there has been a call for ecologists to determine species- and tissue-specific diet discrimination factors ($\Delta^{13}C$ and $\Delta^{15}N$) for their study animals. For large sharks stable isotopes may provide an important tool to elucidate aspects of their ecological roles in marine systems, but laboratory based controlled feeding experiments are impractical. By utilizing commercial aquaria, we estimated $\Delta^{15}N$ and $\Delta^{13}C$ of muscle, liver, vertebral cartilage and a number of organs of three large sand tiger (*Carcharias taurus*) and one large lemon shark (*Negaprion brevirostris*) under a controlled feeding regime. For all sharks mean ± SD for $\Delta^{15}N$ and $\Delta^{13}C$ in lipid extracted muscle using lipid extracted prey data were 2.29‰ ± 0.22 and 0.90‰ ± 0.33, respectively. The use of non-lipid extracted muscle and prey resulted in very similar $\Delta^{15}N$ and $\Delta^{13}C$ values but mixing of lipid and non-lipid extracted data produced variable estimates. Values of $\Delta^{15}N$ and $\Delta^{13}C$ in lipid extracted liver and prey were 1.50‰ ± 0.54 and 0.22‰ ± 1.18, respectively. Non-lipid extracted diet discrimination factors in liver were highly influenced by lipid content and studies that examine stable isotopes in shark liver, and likely any high lipid tissue, should strive to remove lipid effects through standardising C:N ratios, prior to isotope analysis. Mean vertebral cartilage $\Delta^{15}N$ and $\Delta^{13}C$ values were 1.45‰ ± 0.61 and 3.75‰ ± 0.44, respectively. Organ $\Delta^{15}N$ and $\Delta^{13}C$ values were more variable among individual sharks but heart tissue was consistently enriched by ~1–2.5‰. Minimal variability in muscle and liver $\delta^{15}N$ and $\delta^{13}C$ sampled at different intervals along the length of individual sharks and between liver lobes suggests that stable isotope values are consistent within tissues of individual animals. To our knowledge, these are the first reported diet–tissue discrimination factors for large sharks under semi-controlled conditions, and are lower than those reported for teleost fish.

1. Introduction

The application of naturally occurring isotopes of nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) to address ecological questions has grown exponentially over the last 20 years (Martinez del Rio and Wolf, 2005; Martinez del Rio et al., 2009; Wolf et al., 2009). An important reason for this advancement is the ability to undertake minor invasive sampling of animals, such as blood, feathers and muscle biopsy (Hobson et al., 1993; Kurle and Worthy, 2001) to study endangered or difficult-to-study species (Hobson, 1999). This has led to ground breaking insights into the diet, trophic and movement ecology of organisms which were previously not well understood (e.g. Koch et al., 1995; Cherel and Hobson, 2005; Caut et al., 2008a).

More recently, our understanding of species' isotopic diet profiles has improved through the development of stable isotope mixing models such as IsoSource (Phillips and Gregg, 2003) and MixSIR (Semmens and Moore, 2008). These multi-source models have enabled ecologists to quantify complex diets of species, in many cases without sacrificing the animal and without the need for laborious stomach content analysis. Isotope mixing models are based on potential contributions of different isotopic sources (i.e., prey) to an isotopic mixture (i.e., the predator), but require species- and tissue-specific knowledge of the diet discrimination factor ($\Delta^{15}N = \delta^{15}N_{consumer} - \delta^{15}N_{prey}$ and $\Delta^{13}C = \delta^{13}C_{consumer} - \delta^{13}C_{prey}$) of the study species. Mean $\Delta^{15}N$ and $\Delta^{13}C$ of ~3.4‰ and ~1.0‰, respectively, have been determined to be appropriate for general use in ecological isotopic frameworks (DeNiro and Epstein, 1978; Fry et al., 1984; Minagawa and Wada, 1984; Post, 2002). These values have been adopted and widely applied in the literature (see review by Caut et al., 2009; Martinez del Rio et al., 2009). Several authors, however, have questioned both trophic level calculations and the precision of mixing models, based on concerns over the inclusion of

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Table 1
Details of the four large sharks sampled from Deep Sea World (DSW), The Deep (TD) and The Blue Planet (BP) aquaria.

Aquaria	Species	Common name	Dissection date	Reason for euthanasia	Total length (cm)	Sex	Maturity status	Estimated age (yr)[a]
DSW	*Carcharias taurus*	Sand tiger	27/06/2006	Spinal disorder[b]	198.0	M	Mature–adult	7–8
TD	*Carcharias taurus*	Sand tiger	05/06/2008	Spinal disorder[b]	241.6	F	Mature–adult	10–11
BP	*Carcharias taurus*	Sand tiger	07/05/2009	Spinal disorder[b]	261.0	M	Mature–adult	12–13
BP	*Negaprion brevirostris*	Lemon shark	01/03/2007	Neurological condition	199.0	M	Sub-adult	9–10

[a] Sharks were introduced to aquaria at age 1–2 yrs. Age is estimated on the period of time maintained in captivity prior to euthanasia plus 1–2 yrs.
[b] Spinal deformities are reported in wild sharks (Hoenig and Walsh, 1983; Bansemer and Bennett, 2009) and are not known to affect feeding regime or overall animal condition (Heupel et al., 1999).

suitable diet discrimination factors for the species and/or tissue type in question (Gannes et al., 1997; Robbins et al., 2005; Caut et al., 2008b, 2009; Martinez del Rio et al., 2009). This is in light of multiple studies that have identified large differences in diet–tissue discrimination factors between taxon and species, (Vanderklift and Ponsard, 2003; Caut et al., 2009), between tissues (Pinnegar and Polunin, 1999; MacNeil et al., 2006) with diet quality (McCutchan et al., 2003; Robbins et al., 2005) and with environment and feeding rate (Barnes et al., 2007). To refine the accuracy and precision of conclusions drawn from stable isotopes, there have been repeated calls for ecologists to determine diet–tissue discrimination factors for their study species through controlled laboratory experiments (Gannes et al., 1997; Caut et al., 2008b; Wolf et al., 2009). Moreover, if stable isotopes are to be considered as informative metrics for management and conservation, confidence in their interpretation is required.

Sharks are generally large, highly migratory predators that are typically difficult-to-study species in their natural environment. Correctly applied, stable isotope methods may provide a versatile ecological tool to complement and further our understanding of this important (Heithaus et al., 2008) and threatened (Baum et al., 2003) group of marine vertebrates. To date, only a few studies have applied stable isotopes to sharks to investigate trophic level (Fisk et al., 2002; Estrada et al., 2003, 2006), diet and diet switching (Domi et al., 2005; MacNeil et al., 2005) and isotope turnover rates (MacNeil et al., 2006). In studies where a diet discrimination factor was used, however, the reported values of ~3.4‰ and ~1.0‰ (Δ^{15}N and Δ^{13}C respectively) were assumed. Using this surrogate value, Fisk et al. (2002) reported inconsistencies between trophic level estimated by stable isotopes and contaminant tracers in the Greenland shark (*Somniosus microcephalus*). In contrast, Estrada et al. (2003) found that trophic level estimates for the blue (*Prionace glauca*), shortfin mako (*Isurus oxyrinchus*), thresher (*Alopias vulpinus*) and basking shark (*Cetorhinus maximus*) were in agreement with those calculated using standardised diet compositions (Cortes, 1999). Observed discrepancies, in conjunction with the call from the literature, accentuate the need to establish baseline diet–tissue discrimination factors for large sharks.

For many organisms, controlled rigorous experimental designs enable researchers to estimate Δ^{15}N and Δ^{13}C values in multiple tissues (e.g. Pinnegar and Polunin, 1999; Caut et al., 2008c). For sharks, the complications of maintaining large individuals in captivity under laboratory conditions make this approach impractical and for some species impossible. Here we opportunistically sampled large sharks (sand tiger[1], *Carcharias taurus* and lemon shark, *Negaprion brevirostris*) held in commercial aquaria to estimate diet–tissue discrimination factors for white muscle and liver tissue, vertebral cartilage and a selection of internal organs. Detailed feeding records and samples of diet items for stable isotope analysis were maintained by the aquaria allowing us to generate an accurate estimate of the feeding history of the individual sharks.

2. Materials and methods

2.1. Experimental sharks

Monitoring of four large sharks (three sand tiger and one lemon shark) held at three public aquaria [The Deep, Hull (TD); The Blue Planet, Ellesmere Port (BP) and Deep Sea World, North Queensferry (DSW)] was undertaken. All sharks were obtained from the wild as juvenile animals, maintained in captivity for extended periods and were euthanized due to medical conditions; details of the sharks sampled are included in Table 1.

2.2. Feeding history and environmental conditions

The feeding history of the three sharks, including the (i) feeding dates, (ii) prey species, and (iii) and mass of each prey item fed to individual sharks were recorded at all aquaria for the 12 month period prior to euthanasia. For each shark, the percent gravimetric weights of each prey item to total diet (for the 12 month period) were then calculated. Feeding records demonstrated that sharks fed normally until the point of euthanasia. Archived data on feeding regimes were also accessed to determine that all four sharks had been held on constant diets for a minimum of 2 years prior to this point. To determine mean annual tank temperatures, data loggers were installed in each tank (Tinytag, Gemini Data Loggers Ltd, Chichester, UK) and programmed to log data at 45 min intervals.

2.3. Tissue sampling, preparation and analysis

Various tissue types were sampled from each shark: (1) 5 g white muscle tissue samples was excised from the dorsal muscle block at 20 cm intervals between the posterior section of the gills and anterior to the caudal fin; (2) 5 g liver tissue samples were excised from the upper/mid and lower region of both liver lobes; (3) 5 g of other organs were sampled where practical and: (4) vertebral centra were excised from anterior to the dorsal fin[2].

Muscle tissue of prey items were sampled from each bulk food order acquired by individual aquaria, between March 2006 and March 2007 (for TD and BP, prey samples were collected until May 2008 and May 2009, respectively). For teleost prey, muscle was excised from the dorsal/flank muscle section anterior to the first dorsal fin; for cephalopods, muscle tissue from both mantle and tentacles were sampled. Total mass (g) and fork length (FL)/ mantle length (ML) were recorded for all sampled prey. All shark and prey samples were immediately frozen and stored at −20 °C prior to analysis. For certain occasional prey items (e.g. hake, *Merluccius merluccius*), samples were not available and stable isotope values were sourced from the literature.

[1] *Carcharias taurus* is referred to as 'sand tiger shark' in Europe and North America, 'raggies' in South Africa and 'grey nurse shark' in Australia.

[2] Vertebral centra were excised anterior to the deformity in the spine, where this condition occurred – see Table 1.

Tissues sampled from both shark and prey were freeze–dried and homogenised in an SPEX CertiPrep 8000-D ball milling unit (SPEX CertiPrep; Metuchen, NJ, USA). Vertebral centra were cleaned to remove connective tissue and then oven dried at 40 °C for 24 h. The outer edges of the corpus calcareum of each centra, i.e. the most recent growth band, were drilled using a 0.4 mm diameter steel carbide burr (Minerva Dental Ltd) attached to a hand-held dental drill.

For shark and prey muscle tissue and shark internal organs, lipid extraction were undertaken by twice agitating the dried powdered tissue in a 2:1 chloroform–methanol solution for 24 h according to MacNeil et al. (2005). The tissue and solvent were then filtered through 25 mm GF/F filters and the resulting residue/filter paper dried at 60 °C for 48 h to evaporate off remaining solvent. For shark liver tissue, which has a high lipid content (Jayasinghe et al., 2003), lipid extraction were undertaken using a 2:1 chloroform–methanol soxhlet extraction for a period of 16 h and samples dried as above. Between 400–600 µg of both non-lipid extracted (BULK) and lipid extracted (LE) dried tissue per sample were weighed into tin capsules and both stable carbon and nitrogen isotope ratios and total percent carbon (C) and percent nitrogen (N) were determined by a continuous flow isotope ration mass spectrometer (IRMS, Finnigan MAT Delta V, Thermo Finnigan, San Jose, CA, USA) equipped with an elemental analyzer (Costech, Valenica, CA, USA).

Stable isotope abundances are expressed in delta (δ) values as the deviation from standards in parts per thousand (‰) from the following equation:

$$\delta^{15}\mathrm{N\,or}\,\delta^{13}\mathrm{C} = \left(\left(\frac{R_{\mathrm{sample}}}{R_{\mathrm{standard}}}\right) - 1\right) \times 1000 \quad (1)$$

where R is the ratio $^{15}N/^{14}N$ or $^{13}C/^{12}C$. The standard reference material was Pee Dee Belemnite carbonate for CO_2 and atmospheric nitrogen N_2. The analytical precision based on the standard deviation of three standards (82 standards analysed) for $\delta^{15}N$ ranged from 0.13‰ to 0.15‰ and for $\delta^{13}C$ ranged from 0.03‰ to 0.07‰ during the analysis of these samples. The analysis of NIST standards (sucrose and ammonium sulphate; $n = 3$ for each) during the analysis of samples generated values that were within 0.01‰ and 0.07‰ of certified values for $\delta^{15}N$ and $\delta^{13}C$, respectively.

2.4. Lipid extraction effects

Paired t-tests were performed to examine the effects of lipid extraction on $\delta^{13}C$, C, $\delta^{15}N$ and N of the muscle tissue of prey items fed to sharks. We then calculated the mean difference between BULK and LE $\delta^{13}C$, C, $\delta^{15}N$, N and C:N ratio of (i) shark muscle, (ii) shark liver and (iii) muscle tissue of prey items fed to sharks to examine directional trends of the lipid extraction process on the above defined parameters of the three sets of tissues.

2.5. Diet–tissue discrimination factor calculation

To enable the calculation of diet–tissue discrimination factors, the isotopic composition of the total diet of each shark over the 12 month period prior to euthanasia were calculated. Accepting that prey items vary in proximate composition, i.e. the proportion of BULK N and BULK C, it was first necessary to constrain the fractional contributions of N, C and biomass (i.e. the % gravimetric contribution; B) to 1. If $f_{X,B}$, $f_{Y,B}$ and $f_{Z,B}$ equal the fractions of assimilated biomass of prey items X, Y and Z and $f_{X,N}$, $f_{Y,N}$, $f_{Z,N}$, $f_{X,C}$, $f_{Y,C}$ and $f_{Z,C}$ represent the fractions of assimilated N and C of individual prey items, then:

$$f_{X,B} + f_{Y,B} + f_{Z,B} = 1 \quad (2)$$

$$f_{X,N} + f_{Y,N} + f_{Z,N} = 1 \quad (3)$$

$$f_{X,C} + f_{Y,C} + f_{Z,C} = 1 \quad (4)$$

According to Phillips and Koch (2002), we then assumed that the contribution of a prey item to the consumer is proportional to the assimilated biomass multiplied by the elemental (N and C) concentration of that prey. So if $[N]_X$, $[N]_Y$, $[N]_Z$, $[C]_X$, $[C]_Y$ and $[C]_Z$ are equal to the contributions of N and C in prey items X,Y and Z, then for nitrogen:

$$f_{X,N} = \frac{f_{X,B}[N]_X}{f_{X,B}[N]_X + f_{Y,B}[N]_Y + f_{Z,B}[N]_Z} \quad (5)$$

$$f_{Y,N} = \frac{f_{Y,B}[N]_Y}{f_{X,B}[N]_X + f_{Y,B}[N]_Y + f_{Z,B}[N]_Z} \quad (6)$$

$$f_{Z,N} = \frac{f_{Z,B}[N]_Z}{f_{X,B}[N]_X + f_{Y,B}[N]_Y + f_{Z,B}[N]_Z} \quad (7)$$

We repeated the above calculations for carbon, $f_{X,C}$, $f_{Y,C}$, $f_{Z,C}$ by substituting $[C]_X$, $[C]_Y$ and $[C]_Z$ for $[N]_X$, $[N]_Y$ and $[N]_Z$. The isotopic signatures ($\delta^{15}N$ and $\delta^{13}C$) of total diet ($\delta^{15}N$ Diet^T and $\delta^{13}C$ Diet^T), corrected for C and N concentration, were then calculated as:

$$\delta^{15}\mathrm{N\ Diet}^T = f_{X,N}\delta^{15}N_X + f_{Y,N}\delta^{15}N_Y + f_{Z,N}\delta^{15}N_Z; \text{ and} \quad (8)$$

$$\delta^{13}\mathrm{C\ Diet}^T = f_{X,C}\delta^{13}C_X + f_{Y,C}\delta^{13}C_Y + f_{Z,C}\delta^{13}C_Z \quad (9)$$

where $\delta^{15}N_X$, $\delta^{15}N_Y$, $\delta^{15}N_Z$, $\delta^{13}C_X$, $\delta^{13}C_Y$ and $\delta^{13}C_Z$ are the $\delta^{15}N$ and $\delta^{13}C$ values of prey items X, Y and Z. We calculated both BULK diet ($DIET^T_{BULK}$) and LE diet ($DIET^T_{LE}$).

Diet discrimination factors ($\Delta^{15}N$ and $\Delta^{13}C$) for each shark BULK and LE white muscle tissue, BULK and LE liver tissue, vertebral cartilage and LE organs were calculated as:

$$\Delta^{15}N = (\delta^{15}N_{tissue} - \delta^{15}N\,Diet^T) \quad (10)$$

$$\Delta^{13}C = (\delta^{13}C_{tissue} - \delta^{13}C\,Diet^T) \quad (11)$$

where $\delta^{15}N_{tissue}$ and $\delta^{13}C_{tissue}$ is the nitrogen and carbon isotope value of the BULK or LE shark muscle tissue, BULK or LE liver tissue, vertebral cartilage and LE organ of an individual shark. Data are presented as mean ± SD.

3. Results

Mean annual tank temperatures were 16.27 °C ± 1.97, 24.37 °C ± 0.96, and 24.29 °C ± 1.39 for DSW, TD and BP, respectively. The diet of the TD sand tiger, BP sand tiger and BP lemon shark consisted predominantly of single prey items, haddock (*Melanogrammus aeglefinus*), trevally (*Pseudocaranx dentex*) and octopus (*Eledone cirrhosa*), respectively, while the DSW sand tiger diet was composed of trevally, saithe (*Pollachius virens*) and mackerel (*Scomber scombrus*) in descending order of importance (Fig. 1).

Lipid extracted and bulk muscle tissue $\delta^{15}N$, N, $\delta^{13}C$, C and C:N ratios of all prey items fed to sharks are presented in Table 2. For $\delta^{15}N$ and $\delta^{13}C$ there was minimal variance among prey samples, with the exception of TD haddock, BP mullet and BP octopus (SD > 1; Table 2). The proximate composition of fish prey items was similar based on C and N values; cephalopod prey was depleted in both C and N relative to fish (Table 2). As a result of lipid extraction, $\delta^{13}C$ values of most prey items were enriched and C depleted relative to BULK values (Table 3; Fig. 2a). For $\delta^{15}N$, LE prey samples were enriched by 0.47 ± 0.10‰ (mean ± SD) when compared to BULK. Total N of most prey items significantly increased following lipid extraction (Table 3; Fig. 2a).

There was minimal variance in $\delta^{15}N$ and $\delta^{13}C$ of BULK and LE muscle tissue with sampling location along the length of each shark

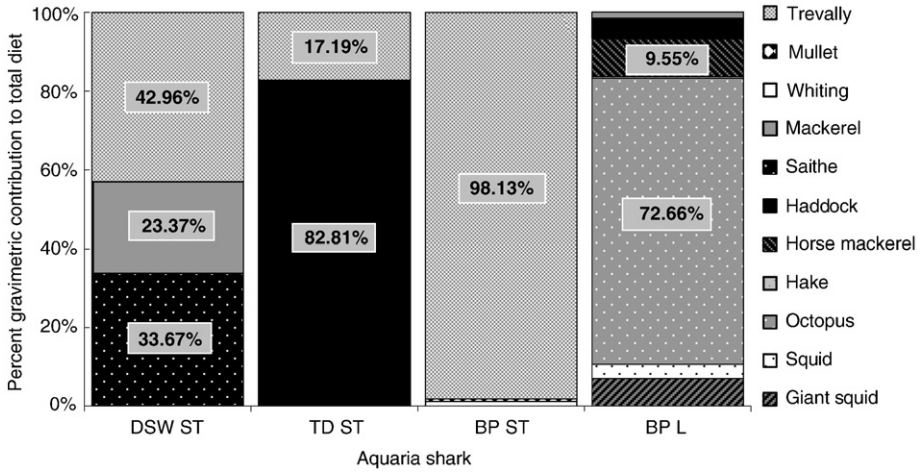

Fig. 1. Percent gravimetric contribution of prey items to total diet of the Deep Sea World (DSW) sand tiger (*Carcharias taurus*), The Deep (TD) sand tiger, The Blue Planet (BP) sand tiger and The Blue Planet (BP) lemon shark (*Negaprion brevirostris*) for the 12 month period prior to euthanasia. Note hake (*Merluccius merluccius*) constituted 0.40% to total diet of the BP lemon shark; whiting (*Merlangius merlangus*) and mullet (*Liza ramada*) constituted 1.20% and 0.67% to total diet of the BP sand tiger, respectively.

(δ^{15}N and δ^{13}C SD range: 0.14–0.30 and 0.24–0.78, respectively; Table 4). Lipid extraction resulted in an increase in δ^{15}N and a corresponding decrease in N of shark muscle tissue, while δ^{13}C, C and C:N ratio increased (Table 4; Fig. 2b).

Equally, there was minimal variance in δ^{15}N and δ^{13}C of bulk and lipid extracted liver tissue sampled from upper, mid and lower sections of each lobe of the three sharks where data were available (δ^{15}N and δ^{13}C SD range: 0.06–0.22 and 0.17–0.91, respectively; Table 4). Neither δ^{15}N nor δ^{13}C differed between right or left liver lobe of the TD sand tiger (δ^{15}N: $T_3 = 1.0$, $p = 0.42$; δ^{13}C: $T_3 = 0.98$, $p = 0.43$), BP sand tiger (δ^{15}N: $T_3 = -0.18$, $p = 0.87$; δ^{13}C: $T_3 = -1.77$, $p = 0.22$) or BP lemon shark (δ^{15}N: $T_3 = 0.24$, $p = 0.83$; δ^{13}C:

Table 2
Mean (± SD) lipid extracted (LE) and non-lipid extracted (BULK) stable nitrogen and carbon (δ^{15}N and δ^{13}C), total percent nitrogen and carbon (N and C), and C:N ratio in tissues of shark prey items sampled from Deep Sea World (DSW), The Deep (TD) and The Blue Planet (BP) aquaria and calculated δ^{15}N and δ^{13}C total diet isotopic signatures for the four sharks (see text).

Prey item	*N*	Mass (g)	Fork length (cm)	δ^{15}N	N	δ^{13}C	C	C:N
DSW — sand tiger (C. taurus)								
LE saithe (*P. virens*)	15	2310.0 ± 1703.0	52.20 ± 7.04	11.97 ± 0.74	14.15 ± 0.11	−19.11 ± 0.79	44.81 ± 0.30	3.17 ± 0.03
BULK saithe (*P. virens*)				11.52 ± 0.71	13.93 ± 0.11	−19.34 ± 0.84	44.87 ± 0.43	3.22 ± 0.02
LE mackerel (*S. scombrus*)	6	432.0 ± 287.0	30.16 ± 2.14	13.13 ± 0.16	14.12 ± 0.12	−18.04 ± 0.11	45.01 ± 0.26	3.19 ± 0.04
BULK mackerel (*S. scombrus*)				12.56 ± 0.10	12.88 ± 0.78	−18.68 ± 0.52	46.85 ± 1.18	3.65 ± 0.33
LE trevally (*P. dentex*)	9	772.2 ± 258.7	33.78 ± 4.79	14.41 ± 0.48	14.33 ± 0.11	−16.82 ± 0.24	45.34 ± 0.26	3.16 ± 0.03
BULK trevally (*P. dentex*)				13.77 ± 0.40	13.85 ± 0.44	−17.11 ± 0.43	45.91 ± 1.28	3.32 ± 0.20
Calculated total diet (LE|BULK)				13.27|12.71		−17.89|−18.25		
TD — sand tiger (C. taurus)								
LE haddock (*M. aeglefinus*)	26	978.3 ± 119.5	45.62 ± 2.47	13.37 ± 1.06	14.17 ± 0.20	−16.97 ± 0.76	44.76 ± 0.40	3.16 ± 0.06
BULK haddock (*M. aeglefinus*)				12.95 ± 1.09	14.11 ± 0.23	−17.30 ± 0.72	45.08 ± 0.42	3.20 ± 0.05
LE trevally (*P. dentex*)	7	1246.9 ± 130.7	39.71 ± 1.76	13.16 ± 0.22	14.27 ± 0.18	−16.50 ± 0.15	44.99 ± 0.30	3.15 ± 0.03
BULK trevally (*P. dentex*)				12.67 ± 0.23	14.12 ± 0.13	−16.69 ± 0.13	45.61 ± 0.56	3.23 ± 0.06
Calculated total diet (LE|BULK)				13.32|12.89		−16.87|−17.17		
BP — sand tiger (C. taurus)								
LE trevally (*P. dentex*)	18	768.8 ± 169.3	32.32 ± 2.27	14.34 ± 0.64	14.17 ± 0.19	−16.94 ± 0.62	44.68 ± 0.48	3.15 ± 0.05
BULK trevally (*P. dentex*)				13.93 ± 0.63	14.45 ± 0.10	−16.93 ± 0.55	45.94 ± 0.38	3.18 ± 0.04
LE grey mullet (*L. ramada*)	7	907.0 ± 447.0	37.62 ± 2.21	16.62 ± 3.17	13.81 ± 0.13	−14.48 ± 1.25	45.09 ± 0.80	3.27 ± 0.08
BULK grey mullet (*L. ramada*)				16.28 ± 3.20	13.54 ± 0.30	−14.65 ± 1.21	46.85 ± 1.07	3.46 ± 0.14
LE whiting (*M. merlangus*)	5	560 ± 84.0	32.03 ± 3.18	16.28 ± 0.75	13.63 ± 0.15	−16.61 ± 0.29	43.54 ± 0.36	3.19 ± 0.01
BULK whiting (*M. merlangus*)				15.79 ± 0.78	14.16 ± 0.07	−16.57 ± 0.26	44.92 ± 0.20	3.17 ± 0.01
Calculated total diet (LE|BULK)				14.40|13.99		−16.90|−16.89		
BP — lemon shark (N. brevirostris)								
LE giant squid (Teuthoida)	3	662.5 ± 53.0	30 ± 1.4	14.46 ± 0.42	12.14 ± 0.06	−15.36 ± 0.13	37.82 ± 0.14	3.11 ± 0.00
BULK giant squid (Teuthoida)				13.91 ± 0.53	11.17 ± 0.39	−17.05 ± 0.24	41.35 ± 0.19	3.70 ± 0.15
LE octopus (*E. cirrhosa*)	14	433.9 ± 100.8	12.09 ± 2.08	10.11 ± 1.02	12.67 ± 0.41	−17.15 ± 0.48	40.72 ± 1.52	3.22 ± 0.16
BULK octopus (*E. cirrhosa*)				9.51 ± 1.14	12.03 ± 0.59	−17.85 ± 0.48	42.07 ± 1.65	3.51 ± 0.28
LE squid (*L. opalescens*)	5	40.42 ± 11.22	10.76 ± 1.54	13.57 ± 0.74	12.86 ± 0.42	−17.00 ± 0.59	41.12 ± 0.67	3.20 ± 0.14
BULK squid (*L. opalescens*)				13.08 ± 0.49	12.15 ± 0.16	−17.83 ± 0.51	42.74 ± 0.35	3.52 ± 0.03
hake (*M. merluccius*)			9–15[a]	11.84[a]		−18.42[a]		
horse mackerel (*T.trachurus*)				12.33[a]		−18.14[a]		
Calculated total diet (LE|BULK)				11.05|10.50		−17.05|−17.81		

Mean mass (g) and fork length (cm) of prey samples are provided.
[a] Values obtained from Le Loc'h and Hily (2005).

Table 3
The effects of lipid extraction on $\delta^{13}C$, total percent carbon (C), $\delta^{15}N$ and total percent nitrogen (N) values of prey items fed to sharks.

	n	$\delta^{13}C$		C		$\delta^{15}N$		N	
		t-value	*p*	*t*-value	*p*	*t*-value	*p*	*t*-value	*p*
DSW prey									
Saithe	15	**−7.82**	**<0.0001**	0.75	0.466	**−19.18**	**<0.0001**	**−5.96**	**<0.0001**
Mackerel	6	**−3.08**	**0.027**	**3.84**	**0.012**	**−7.95**	**0.001**	**−3.93**	**0.011**
Trevally	9	**−2.38**	**0.045**	1.49	0.174	**−12.64**	**<0.0001**	**−3.80**	**0.005**
TD prey									
Haddock	26	**−13.93**	**<0.0001**	**5.01**	**<0.0001**	**−14.71**	**<0.0001**	**−2.33**	**0.029**
Trevally	7	**−2.93**	**0.026**	**2.88**	**0.028**	**−8.98**	**<0.0001**	**−2.38**	0.055
BP ST prey									
Trevally	18	0.14	0.891	**8.09**	**<0.0001**	**−11.63**	**<0.0001**	**5.60**	**<0.0001**
Mullet	7	−1.36	0.223	**4.55**	**0.004**	**−17.99**	**<0.0001**	−2.26	0.065
Whiting	5	0.58	0.591	**6.15**	**0.004**	**−4.48**	**0.011**	**7.52**	**0.002**
BP L prey									
Giant squid	3	**−9.35**	**0.011**	**20.98**	**0.002**	**−38.94**	**0.001**	**−7.09**	**0.019**
Octopus	14	**−7.95**	**<0.0001**	**6.48**	**<0.0001**	**−9.77**	**<0.0001**	**−8.69**	**<0.0001**
Squid	5	**−4.88**	**0.008**	**5.98**	**0.004**	**−3.26**	**0.031**	**−3.46**	**0.026**

Paired *t*-tests were used to compare non-lipid extracted (BULK) and lipid extracted (LE) data. Bold values indicate significant *t*-test results.

$T_3 = 0.41$, $p = 0.72$). Lipid extraction of shark liver tissue resulted in an increase in total N and $\delta^{13}C$, a decrease in total C and C:N ratio and no change in $\delta^{15}N$ (Table 4; Fig. 2c).

Mean BULK and LE muscle tissue $\delta^{15}N$ of the DSW, TD and BP sand tigers were higher than the BP lemon shark; $\delta^{13}C$ values were similar between all sharks (Table 4). For the TD sand tiger, the LE liver $\delta^{15}N$ value was similar to LE muscle, while the LE liver $\delta^{15}N$ values of the BP sand tiger and BP lemon were depleted. For $\delta^{13}C$, LE liver values of the TD and BP sand tigers were similar to LE muscle, but the BP lemon $\delta^{13}C$ was depleted (Table 4). The $\delta^{15}N$ values of the outer edge of vertebral centra, i.e. vertebral cartilage, were lower than LE muscle tissue for all three sharks sampled; $\delta^{13}C$ values were enriched (Table 4). Values of $\delta^{15}N$ and $\delta^{13}C$ in most LE organs were similar or slightly depleted in $\delta^{15}N$ and enriched in $\delta^{13}C$ relative to LE muscle tissue (accepting a higher degree of variability in the BP lemon), but $\delta^{15}N$ LE heart tissue of both species was consistently enriched by ~1–2.5‰ (Table 4).

C:N ratios, total C and the difference between BULK and LE $\delta^{13}C$ values indicated that lipid extraction of internal organs was required and produced adequate results (C:N ~3.0; Table 4; Fig. 3b). For most LE organs, $\delta^{15}N$ either remained the same (within analytical error) or there was a marginal increase, with the exception of spleen tissue, which was consistently depleted in $\delta^{15}N$ in all three animals sampled (Fig. 3a).

Individual shark and mean $\Delta^{15}N$ and $\Delta^{13}C$ values for BULK and LE muscle and liver tissue, LE organs and vertebral cartilage calculated from both $DIET_{BULK}$ and $DIET_{LE}$ are presented in Tables 4 and 5, respectively. There was an observed difference in $\Delta^{15}N$ values of LE white muscle tissue among the two species of sharks fed on different diets; the $\Delta^{15}N$ of the BP lemon shark fed on cephalopods of 2.60‰ was higher than that of the DSW, TD and BP sand tigers fed on teleost prey (2.27‰, 2.15‰ and 2.14‰, respectively; Table 4). For $\Delta^{13}C$, the LE muscle tissue of the BP lemon was depleted relative to the three sand tiger sharks (Table 4). Accepting that $\Delta^{13}C$ values of the BP lemon shark LE organs were highly variable and negative discrimination factors were calculated from $DIET_{LE}$, this animal was excluded from the mean organ $\Delta^{13}C$ summary in Table 5.

4. Discussion

The mean $\Delta^{15}N$ values for LE shark muscle tissue, using LE prey data, of 2.29‰ ± 0.22 (all sharks) and 2.19‰ ± 0.07 (sand tiger sharks only), were lower than the widely applied values (3.4‰) of Minagawa and Wada (1984) and Post (2002) and lower, but more similar, to the mean values of 2.96‰ and ~2.5‰ reported for fish muscle by Vanderklift and Ponsard (2003) and Caut et al. (2009), respectively. Sharks are unusual in that they retain levels of urea and trimethylamine oxide (TMAO) in their tissues for osmoregulatory purposes (Olson, 1999). Fisk et al. (2002) suggested that as urea is a metabolic waste product, it would be isotopically light therefore rendering shark muscle tissue artificially low in $\delta^{15}N$ if not removed. For sharks, we observed an increase in $\delta^{15}N$ of ~0.5‰ in muscle tissue following lipid extraction. This effect on $\delta^{15}N$ was similar to both that of teleost prey items in this study (Table 3) and the difference between BULK and LE $\delta^{15}N$ muscle tissue previously reported by Sotiropoulos et al. (2004), Ingram et al. (2007) and Logan et al. (2008). Murray et al. (2006) and Ingram et al. (2007) suggested this increase in $\delta^{15}N$ may be due to the leaching of nitrogenous metabolites or waste, principally ammonia and ammonium, via the lipid extraction process. Christie (1993) stated that chloroform–methanol may act to remove urea, and thus the observed increase in $\delta^{15}N$ shark muscle tissue may be due to this effect. For shark muscle, we also found a large decrease in total N following lipid extraction, further providing evidence for the removal of nitrogenous waste products.

For the teleost and cephalopod prey items, we found a significant increase in $\delta^{13}C$ and associated decrease in total C following lipid extraction as would be expected for tissue containing lipids. For shark muscle tissue, there was a marginal increase in $\delta^{13}C$, indicating low lipid content (Bone and Roberts, 1969), but in contrast to the prey tissue, C increased. The diametric directional trends of total C and N of shark and prey muscle indicate that the prey had proportionally higher lipid content than nitrogenous waste, in contrast to shark muscle. The issue of increasing $\delta^{15}N$ following lipid extraction and whether this correlates with urea/ammonia/ammonium removal, however, requires further investigation. Work undertaken by Moeri et al. (2003), may suggest that the urea signature is labelled in muscle tissue at the amino acid level.

White muscle $\Delta^{15}N$ values of the three sand tiger sharks were similar but lower than that of the lemon shark. These $\Delta^{15}N$ differences could be species-specific (Kurle, 2002), but might also be explained by diet. The BP lemon shark was fed on a predominantly cephalopod diet with lower $\delta^{15}N$ and total N values that contrast the teleost diet of the three sand tigers (Table 2). The significant negative relationship between $\Delta^{15}N$ values and $\delta^{15}N$ diet values reported by Felicetti et al. (2003), Caut et al. (2008b, 2009) and Overmyer et al. (2008) may therefore explain the enriched $\Delta^{15}N$ value recorded for the BP lemon shark.

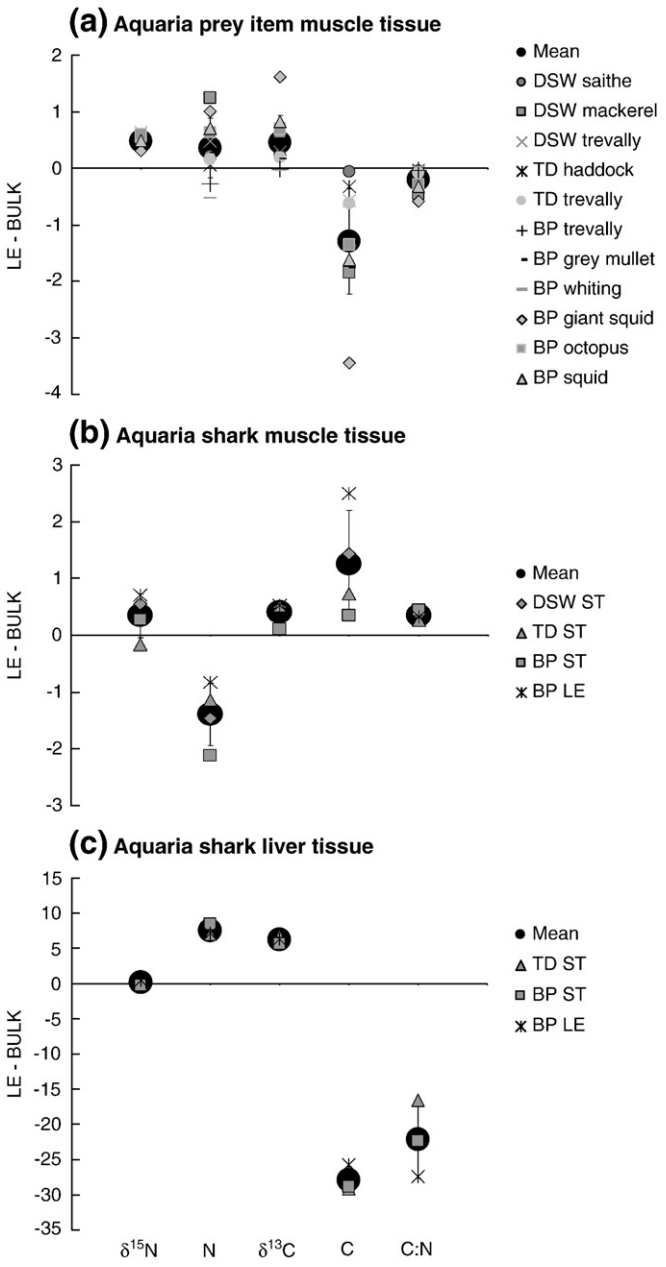

Fig. 2. The effect of the lipid extraction process on $\delta^{13}C$, total percent carbon (C), $\delta^{15}N$, total percent nitrogen (N) and C:N ratio of (a) muscle tissue of prey items fed to the sharks, (b) shark muscle tissue and, (c) shark liver tissue, sampled from aquaria under semi-controlled conditions. Note: LE are values for lipid extracted tissues and BULK are for non-lipid extracted tissue.

For $\Delta^{13}C$, the mean value for shark muscle tissue of 0.90‰ ± 0.33 (all sharks) and 1.01‰ ± 0.29 (sand tigers only) were similar to the widely accepted value of ~1.0‰ but lower than recent estimates for muscle tissue of 2‰ based on a controlled study in European sea bass, *Dicentrarchus labrax*, (Barnes et al., 2007) and 1.8‰ based on a review of 41 estimates (Caut et al., 2009).

Mean $\delta^{15}N$ and $\delta^{13}C$ and calculated $\Delta^{15}N$ and $\Delta^{13}C$ values of LE liver were depleted relative to white muscle tissue in agreement with data for marine and freshwater fish (Pinnegar and Polunin, 1999; MacNeil et al., 2005; Sweeting et al., 2007). Previous work has suggested that lower $\Delta^{15}N$ values in liver tissue reflect the larger proportion of essential amino acids in liver protein (Pinnegar and Polunin, 1999; Kurle and Worthy, 2002). We observed no change in $\delta^{15}N$ of liver tissue following lipid extraction in agreement with the findings of Logan et al. (2008). Whether this reflects low nitrogenous waste and/or urea concentrations (Ballantyne, 1997) requires further investigation. Ingram et al. (2007) reported that the effect of lipid extraction on $\delta^{15}N$ was reduced in muscle tissue with higher lipid content.

Total C values of LE liver tissue were of a similar magnitude to muscle, but there was greater variance in C concentrations of LE liver tissue among sharks. This may indicate variable success of the soxhlet extraction process. Additionally, total N values increased following lipid extraction, in contrast to muscle tissue. Similar to prey muscle tissue, this may be a result of the imbalance of removing large volumes of lipids and hence total C. The variable N and C concentrations in LE shark liver tissue, however, resulted in C:N ratios ranging from 3.33 to 4.74. This may suggest that previously reported C:N ratios for lipid extracted fish liver of 6.36–6.47 (Sweeting et al., 2006) and 3.2–6.4 (Logan et al., 2008), are either influenced by nitrogenous waste content (i.e. N concentration) or that standard lipid extraction methods are not effective for high lipid content liver tissue resulting in both variable C and N values. In previous experimental trials we found that standard chloroform–methanol extraction of shark liver tissue produced highly variable results when compared to soxhlet extraction (Hussey and Fisk unpubl. data). Variable LE liver $\Delta^{13}C$ values among the three sharks sampled, including a negative $\Delta^{13}C$ value for the BP lemon shark, further indicate the complications of; (i) effectively standardising high lipid content tissues and; (ii) the variable metabolic nature of liver tissue in sharks (Hoffmayer et al., 2006; Hussey et al., 2009).

Our estimated $\Delta^{15}N$ values of 1.45‰ ± 0.61 (all sharks) and 1.12 (sand tigers only), for vertebral cartilage, were lower than those for LE muscle tissue in our study and the mean value for collagen (~2‰) reported by Caut et al. (2009). In agreement with our data, MacNeil et al. (2005) found that $\delta^{15}N$ cartilage values drilled from vertebral centra of blue (*Prionace glauca*), shortfin mako (*Isurus oxyrinchus*) and thresher sharks (*Alopias vulpinus*) were depleted relative to muscle tissue. These authors suggested that sampling across the vertebral surface may have resulted in a bias of the $\delta^{15}N$ values by incorporating the larger growth bands of younger animals. Our data would suggest this may not be the case and that the use of a $\Delta^{15}N$ of 3.4‰ for shark vertebral cartilage (Estrada et al., 2006; Kerr et al., 2006) may be inappropriate. Our $\Delta^{13}C$ estimates for vertebral cartilage were highly enriched compared to other tissue types, emphasising the need for future work to isolate the organic collagen portion of the matrix prior to $\delta^{13}C$ analysis (Kerr et al., 2006).

With the exception of $\delta^{13}C$ in the BP lemon shark LE kidney, LE rectal gland and LE testes, all other LE organs were enriched in $\delta^{15}N$ and $\delta^{13}C$ relative to diet; however the enrichment for other organs of the BP lemon was less pronounced. Generally the pattern of $\delta^{15}N$ and $\delta^{13}C$ LE organ enrichment was similar between individuals but there was a degree of variability in $\Delta^{13}C$ and $\Delta^{15}N$ values of LE organs between individual sharks. It is well understood that isotopes contained in different dietary components are routed differentially to specific tissues (i.e. isotopic routing; Tieszen and Fagre, 1993). It is therefore possible that the varied and mixed diets of the individual experimental sharks may have resulted in variable isotopic routing to organs, producing variable diet–tissue discrimination factors. Of all organs sampled, LE heart tissue was considerably enriched in $\delta^{15}N$. This result was in agreement with Hobson et al. (1996) for captive harp seals (*Pagophilus groenlandicus*) and may reflect the abundance of non-essential amino acids in heart tissue (Wilson and Poe, 1974).

To our knowledge, and accepting the small sample size, our data provide the first estimates of $\Delta^{15}N$ and $\Delta^{13}C$ values determined under semi-controlled conditions for white muscle tissue, liver tissue, vertebral cartilage and internal organs of large sharks. We accept that our approach is simplistic, similar to Hobson et al. (1996) and Kurle (2002), in that the sample size of individual animals was low and they received a varied diet. However, the mass contribution to

Table 4
Stable nitrogen and carbon ($\delta^{15}N$ and $\delta^{13}C$), total percent nitrogen and carbon (N and C) and C:N ratio in muscle and liver tissue, vertebral cartilage and organs of sand tiger sharks (*Carcharias taurus*) and a lemon shark (*Negaprion brevirostris*) sampled from Deep Sea World (DSW), and The Deep (TD) and The Blue Planet (BP) aquaria.

Tissue	*n*	$\delta^{15}N$	N	$\Delta^{15}N$ $DIET_{LE}\|DIET_{BULK}$	$\delta^{13}C$	C	$\Delta^{13}C$ $DIET_{LE}\|DIET_{BULK}$	C:N
DSW sand tiger								
BULK muscle	4[a]	14.99 ± 0.19	16.31 ± 0.16	**1.72|2.28**	−17.12 ± 0.08	43.26 ± 0.28	**0.77|1.13**	2.65 ± 0.03
LE muscle	4[a]	15.54 ± 0.16	14.85 ± 0.05	**2.27|2.83**	−16.63 ± 0.11	44.70 ± 0.24	**1.26|1.62**	3.01 ± 0.01
Vertebrae EDGE		14.18	6.67	**0.91|1.47**	−14.20	18.32	**3.69|4.05**	2.75
TD sand tiger								
BULK muscle	7	15.64 ± 0.19	16.01 ± 0.11	**2.32|2.75**	−16.31 ± 0.08	42.51 ± 0.78	**0.56|0.86**	2.65 ± 0.05
LE muscle	7	15.47 ± 0.30	14.87 ± 0.12	**2.15|2.58**	−15.80 ± 0.19	43.24 ± 0.40	**1.07|1.37**	2.91 ± 0.05
BULK liver	6	15.27 ± 0.07	3.44 ± 0.70	**1.95|2.38**	−22.30 ± 0.63	64.21 ± 11.94	**−5.42|−5.13**	19.88 ± 7.06
LE liver	6	15.36 ± 0.21	10.48 ± 2.80	**2.04|2.47**	−15.52 ± 0.27	40.74 ± 5.26	**1.35|1.65**	3.33 ± 0.09
LE heart	1	18.14	13.89	**4.82|5.25**	−15.46	43.08	**1.41|1.71**	3.10
LE kidney	1	15.67	13.69	**2.35|2.78**	−15.36	38.95	**1.51|1.81**	2.84
LE spleen	1	15.02	13.85	**2.22|2.65**	−15.21	41.32	**1.86|2.16**	2.98
LE spiral valve	1	15.54	14.35	**1.70|2.13**	−15.01	41.15	**1.66|1.96**	2.87
LE stomach	1	15.24	13.69	**1.92|2.35**	−14.04	40.38	**2.84|3.13**	2.95
BP sand tiger								
BULK muscle	4[b]	16.26 ± 0.18	15.94 ± 0.09	**1.86|2.27**	−16.31 ± 0.09	42.74 ± 0.25	**0.59|0.58**	2.68 ± 0.01
LE muscle	4[b]	16.54 ± 0.22	13.83 ± 0.27	**2.14|2.55**	−16.20 ± 0.08	43.09 ± 0.51	**0.70|0.70**	3.12 ± 0.02
BULK liver	6	15.56 ± 0.22	2.87 ± 0.50	**1.16|1.57**	−22.21 ± 0.17	73.97 ± 5.92	**−5.31|−5.32**	26.39 ± 4.41
LE liver	6	15.36 ± 0.09	11.26 ± 0.99	**0.96|1.37**	−16.58 ± 0.91	45.14 ± 2.45	**0.32|0.31**	4.06 ± 0.61
LE heart	1	17.26	12.89	**2.86|3.27**	−16.00	42.27	**0.90|0.90**	3.28
Vertebrae EDGE		15.72	9.24	**1.32|1.73**	−13.56	24.29	**3.34|3.33**	2.63
LE kidney	1	15.51	13.90	**1.11|1.52**	−14.74	39.84	**2.16|2.16**	2.87
LE spleen	1	15.16	14.06	**0.76|1.17**	−15.46	42.32	**1.44|1.43**	3.01
LE spiral valve	1	15.92	14.10	**1.52|1.93**	−14.86	42.19	**2.04|2.04**	2.99
LE stomach	1	16.53	14.32	**2.12|2.53**	−14.44	40.66	**2.46|2.46**	2.84
LE rectal gland	1	15.75	13.73	**1.35|1.76**	−15.59	41.03	**1.31|1.31**	2.99
LE pancreas	1	15.46	14.06	**1.06|1.47**	−15.23	42.22	**1.67|1.66**	3.00
LE testes	1	15.40	14.05	**1.00|1.41**	−14.51	40.35	**2.39|2.39**	2.87
BP lemon								
BULK muscle	5	12.94 ± 0.23	15.37 ± 0.15	**1.88|2.44**	−17.02 ± 0.15	41.90 ± 0.30	**0.03|0.79**	2.73 ± 0.04
LE muscle	5	13.65 ± 0.14	14.54 ± 0.12	**2.60|3.15**	−16.50 ± 0.24	44.40 ± 0.30	**0.55|1.31**	3.05 ± 0.04
BULK liver	6	12.05 ± 0.19	2.21 ± 0.30	**0.99|1.55**	−24.41 ± 0.18	69.90 ± 1.67	**−7.36|−6.60**	32.22 ± 5.0
LE liver	6	12.55 ± 0.06	9.32 ± 0.22	**1.50|2.05**	−18.05 ± 0.19	44.17 ± 0.63	**−1.00|−0.24**	4.74 ± 0.12
Vertebrae EDGE		13.16	6.35	**2.11|2.66**	−12.83	17.02	**4.22|4.98**	2.68
LE heart	1	15.09	14.03	**4.04|4.59**	−16.73	44.93	**0.32|1.08**	3.20
LE kidney	1	12.60	12.34	**1.54|2.10**	−17.30	42.57	**−0.26|0.50**	3.45
LE spleen	1	11.85	14.13	**0.79|1.35**	−16.96	42.54	**0.09|0.85**	3.01
LE spiral valve	1	13.41	14.14	**2.36|2.91**	−15.90	43.35	**1.15|1.91**	3.07
LE stomach	1	13.62	14.15	**2.57|3.12**	−15.98	43.17	**1.07|1.83**	3.05
LE rectal gland	1	14.59	13.08	**3.54|4.10**	−17.41	44.76	**−0.36|0.40**	3.42
LE pancreas	1	11.89	13.87	**0.83|1.39**	−17.03	42.42	**0.02|0.78**	3.06
LE testes	1	10.87	13.80	**−0.18|0.38**	−17.37	42.11	**−0.32|0.44**	3.05

Corresponding diet–tissue discrimination factors are detailed ($\Delta^{15}N$ and $\Delta^{13}C$ in bold) for both lipid extracted diet ($DIET_{LE}$) and non-lipid extracted diet ($DIET_{BULK}$). Note, for muscle tissue, *n* is the number of samples analysed along the length of each shark and for liver is upper/mid and lower samples of each lobe. Data are ± 1 SD. Note: BULK are values for non-lipid extracted tissue and LE for lipid extracted tissue.

[a] Note muscle section 2 (at ~20 cm posterior to gill slits) and muscle section 4 (at ~60 cm posterior to gill slits) were lost due to freezer failure.

[b] Four muscle samples were taken: posterior to gills/anterior to dorsal fin/posterior to second caudal fin and anterior to caudal fin.

diet, $\delta^{15}N$ and $\delta^{13}C$ signatures of prey items and N and C concentrations in individual prey were quantified in the diet calculation of all four sharks prior to euthanasia and considered in the estimates for diet–tissue discrimination factors. The inclusion of multiple dietary components, of mixed proximate composition, also provides the first realistic comparison to the diet of wild sharks. Given the threatened status of global stocks of sand tiger sharks (Musick et al., 2000; Otway et al., 2004), these data are of particular importance.

Considering the known effects of growth rate on $\Delta^{15}N$ and $\Delta^{13}C$ values (Gaye-Siessegger et al., 2003; Trueman et al., 2005), future work should aim to investigate stable isotopes in both slow and fast growing juvenile sharks; dusky, *Carcharhinus obscurus* and bonnethead sharks, *Sphyrna tiburo*, may be suitable candidates. The relatively small size at birth of these sharks will enable a detailed examination of diet–tissue discrimination factors under controlled laboratory conditions. Accepting that sharks typically consume a diverse prey base (teleost, cephalopod, crustacean, marine mammal, elasmobranch and bird; Wetherbee and Cortés, 2004) and that the largest species are planktivorous, further aquaria/laboratory investigation in to the effects of diets with variable $\delta^{15}N$, N and $\delta^{13}C$, C values on derived $\Delta^{15}N$ and $\Delta^{13}C$ estimates is of critical importance to advancing our knowledge of stable isotopes in this unique group of marine vertebrates.

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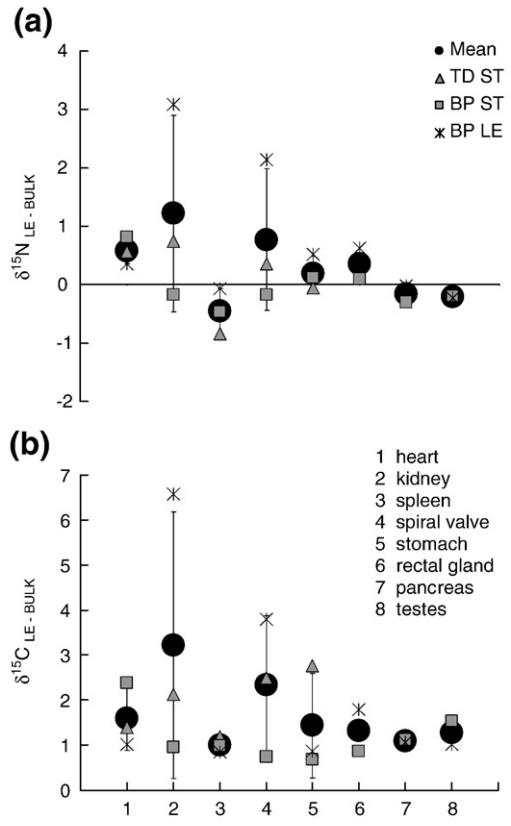

Fig. 3. The effect of the lipid extraction process on (a) δ^{15}N and (b) δ^{13}C of the organs of sand tiger (*Carcharias taurus*) and lemon (*Negaprion brevirostris*) sharks sampled from aquaria under semi-controlled conditions. Note: LE are values for lipid extracted tissues and BULK are for non-lipid extracted tissue.

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Table 5
Summary diet–tissue discrimination factors for non-lipid extracted (BULK) and lipid extracted (LE) muscle and liver tissue and vertebral cartilage for; (i) all sharks (fish and cephalopod diet) and (ii) sand tiger sharks only (fish diet), calculated from both LE and BULK total diet ($DIET_{LE}$ and $DIET_{BULK}$).

Summary					
Tissue	*n*	Δ^{15}N		Δ^{13}C	
		$DIET_{LE}$	$DIET_{BULK}$	$DIET_{LE}$	$DIET_{BULK}$
All sharks					
BULK muscle	4	1.95 ± 0.26	2.44 ± 0.22	0.49 ± 0.32	0.84 ± 0.23
LE muscle	4	2.29 ± 0.22	2.78 ± 0.28	0.90 ± 0.33	1.25 ± 0.39
BULK liver	3	1.37 ± 0.51	1.83 ± 0.47	−6.03 ± 1.15	−5.68 ± 0.80
LE liver	3	1.50 ± 0.54	1.96 ± 0.56	0.22 ± 1.18	0.57 ± 0.97
Vertebrae EDGE	3	1.45 ± 0.61	1.95 ± 0.63	3.75 ± 0.44	4.12 ± 0.83
Sand tiger sharks only					
BULK muscle		1.97 ± 0.31	2.43 ± 0.27	0.85 ± 0.20	0.86 ± 0.28
LE muscle	3	2.19 ± 0.07	2.65 ± 0.15	1.01 ± 0.29	1.23 ± 0.48
BULK liver	2	1.56	1.98	−5.37	−5.23
LE liver	2	1.50	1.92	0.84	0.98
Vertebrae EDGE	2	1.12	1.60	3.52	3.69
LE heart	3	3.91 ± 0.99	4.37 ± 1.01	1.16[a]	1.31[a]
LE kidney	3	1.67 ± 0.63	2.13 ± 0.63	1.84[a]	1.99[a]
LE spleen	3	1.26 ± 0.83	1.72 ± 0.81	1.65[a]	1.80[a]
LE spiral valve	3	1.86 ± 0.44	2.32 ± 0.52	1.85[a]	2.00[a]
LE stomach	3	2.20 ± 0.33	2.67 ± 0.40	2.65[a]	2.80[a]

Summary diet–tissue discrimination factors for lipid extracted (LE) organs are also presented. Data ± 1SD.
[a] Calculated excluding BP lemon shark Δ^{13}C values.

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