Dietary metal and macro-nutrient intakes of juvenile lemon sharks determined from the nutritional composition of prey items

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ABSTRACT: The trace element requirements of sharks are poorly understood and the dietary intake of metals from prey items in wild sharks has not been measured. In this study whole prey of nursery bound juvenile lemon sharks Negaprion brevirostris, from Bimini, Bahamas were analysed for carcass total protein, lipids, carbohydrates, ash content, energy, and elemental composition. Metal analysis included 415 prey items from 18 species (fish: Atherinidae, Belonidae, Gerreidae, Haemulidae, Lutjanidae, Scaridae, Sphyraenidae; decapod crustaceans: Penaeidae, Portunidae). There were some seasonal and location effects (North Sound versus South Bimini shark nurseries), but overall prey metal concentrations were broadly similar to reports for temperate species. Yellow fin mojarra Gerres cinereus dominate the shark diet, in which electrolytes were similar (Na 10, K 10, Ca 65, and Mg 2 mg g⁻¹ dry weight) but trace metals were higher (Cu 2.4, Zn 96, Fe 63, Mn 0.7 µg g⁻¹ dry weight) than in other prey fish species at Bimini. Swimming crabs (Portunidae) were an important source of dietary Ca, Mg, Cu and Mn for lemon sharks. The calculated daily dietary metal intakes (mg d⁻¹) for Cu (0.17), Fe (1.2) and Mn (0.01) of lemon sharks are below previously estimated requirements of other fish. Dietary salt intake (272 mg Na d⁻¹, 1% dietary salt) is similar to other fishes, but the Ca and Zn intakes are higher. Yellow fin mojarra contained 65% protein, 4% lipid, 23% ash. The average juvenile lemon shark (1.85 kg, 24.84 g daily ration) ate a very lean high protein diet (61.9% protein, 4.6% lipid, 24.9% ash, 8.6% carbohydrate), with a calculated energy intake of 397 kJ d⁻¹. Overall, the data suggests the gross macronutrient composition of the shark diet is adequate, but some of the trace metals may be limiting in the diet of wild juvenile lemon sharks at Bimini.

KEY WORDS: Dietary metal · Trace element · Electrolyte · Energy intake · Proximate composition · Nutrition · Negaprion brevirostris · Bimini

INTRODUCTION

The Florida Fish and Wildlife Commission recently implemented a total ban on the harvesting of the lemon shark Negaprion brevirostris, as an urgent measure to conserve the species. Lemon sharks are a threatened species. Classification as ‘Near Threatened’ on the International Union for Conservation of Nature (IUCN) Red List of Threatened Species is based on limited data collected some years ago, leading to an underestimation of the population size and distribution. The species is found in the western Atlantic Ocean from Florida, USA, to Brazil. Pollution, over-fishing, and by-catch are identified as the main threats to the survival of lemon sharks. The species is listed as ‘Near Threatened’ in the IUCN Red List of Threatened Species, and as ‘Vulnerable’ in the United States Endangered Species Act. lemon sharks are an important species for the marine ecosystem, serving as a top predator and playing a key role in the food web. Conservation efforts are necessary to ensure the long-term survival of the species.
mation of their survivability rank on the list (Sundström 2005). Adequate food intake is a critical factor in the survival of any species. For sharks, research has mainly focused on measuring the energy content and the gross nutritional profile (protein, fat, carbohydrate) of teleost prey species so that the energy requirements of the sharks can be calculated (Wetherbee et al. 1990, Lawson et al. 1998, Eder & Lewis 2005). Such information is used to aid understanding of bioenergetics, survival strategies and fish husbandry; as well as to inform conservation measures for the protection of top predators. Yet it is well known in fish nutrition that the energy, protein, fat or carbohydrate content of the food are not the only factors that may limit the growth or health of fishes. Other ingredients in the food such as vitamins and minerals can also have significant effects on fish health (Halver 1989). There has been extensive research on the trace metal requirements of teleost fish, especially those used in aquaculture, and many teleosts need a few milligrams per day of each trace element to remain healthy (Watanabe et al. 1997, Bury et al. 2003, Handy et al. 2005). However the dietary metal requirements of sharks have been given much less attention, although there are some data available on the metal concentrations in the tissues of elasmobranchs (Irwin & Davenport 2002, McMeans et al. 2007, De Boeck et al. 2010). Apart from recent work on gastric handling of salts in sharks (Anderson et al. 2010) and incidental use of oral salt loading in physiological studies (e.g. MacKenzie et al. 2002), the dietary metal intakes and mineral nutritional requirements of most shark species are unknown.

The lemon shark offers a potentially good model of shark nutrition because it has been well studied compared to other species, with information available on diet preference, daily ration, feeding ecology and behaviour (Wetherbee et al. 1990, Morrissey & Gruber 1993, Sundström & Gruber 1998, Newman et al. 2010). Lemon sharks are primarily piscivorous predators that are capable of selective feeding when environmental conditions and prey abundance are favourable (Newman et al. 2010). Juvenile lemon sharks demonstrate habitat selection, and prefer shallow, warm water with rock or sandy substrate (Morrissey & Gruber 1993). The lagoons at Bimini, Bahamas typify this habitat. Juvenile lemon sharks there feed predominantly on teleosts, which make up ~95% of the diet, and the remainder is comprised of crustaceans and the occasional cephalopod or elasmobranch prey (Wetherbee et al. 1990, Newman et al. 2010). The locally abundant mojarra (Gerreidae) are the major prey of lemon sharks at Bimini (Newman et al. 2010), where the sharks have a small home range and benthic feeding patterns (Randall 1967, Newman et al. 2007). Eventually, the sharks mature and move from the shallow lagoons to open sea where the adults are able to feed on larger and more diverse prey (Wetherbee et al. 1990).

Lemon sharks are a protected species that grow to large size, which makes the conventional method of a triplicated fish nutrition trial to determine the feed requirements impractical. Instead, the approach is to collect the stomach contents of sharks captured in the wild to identify the prey species and mass of food eaten (e.g. Newman et al. 2010). Then prey species in the environment are sampled and analysed for their nutritional value, so that nutrient or energy intake may be calculated (e.g. Wetherbee et al. 1990). However, nutritional studies on wild animals are also confounded by other environmental factors, such as temporal and spatial patterns in the availability or nutritional quality of the prey species (Lawson et al. 1998), or abiotic factors such as temperature or salinity. However, lemon sharks and other lamnid sharks are able to partly control body temperature, so this is less of an issue.

In this study, the field sites were selected at Bimini, Bahamas to enable some consideration of the environmental factors. The lagoon that comprises the North Sound of Bimini is no more than 2 to 3 m deep, except within channels. It thus has restricted tidal flow and is subject to more pronounced seasonal salinity (dilution in the wet season) and temperature variations compared with the adjacent South Bimini, which is a more exposed site open to the sea. In addition, the North Sound has suffered anthropogenic impact with removal of over 30% of the mangrove fringe and mobilisation of sediment deposits (see Newman et al. 2007 for a detailed habitat description). Jennings et al. (2008) suggest this may contribute to less diverse bottom level species in the North Sound. We recently detailed the stomach contents and prey preference of juvenile lemon sharks at these locations (Newman et al. 2010). This included collecting specimens of the prey species from the environment at the same time that the sharks were sampled. The primary aim of the current study was to conduct a detailed nutritional trace element analysis of these prey items to provide detailed estimates of dietary essential metal intake in wild sharks. In addition, the energy content and proximate composition (protein, fat, ash, carbohydrate) of each prey species was also measured so that any changes in whole body metals could be interpreted against the general nutritional profile. The data on energy and protein content also add to the sparse literature on estimates of energy intake for sharks. Finally, sorting of the data by season (wet versus dry) and location (North versus South Sound) enabled an indication of the importance of environment or location in the overall nutritional intake.
MATERIALS AND METHODS

Study site and sampling protocol. Known prey species of juvenile lemon sharks were collected from Bimini, Bahamas, located 40 nautical miles due east of Miami, Florida (25°44’N, 79°16’W). A total of 415 samples were recovered for nutritional analyses based on their relative importance to the diet of lemon sharks (Newman et al. 2010). The Index of Relative Importance (% IRI) values used were those calculated in Newman et al. (2010) from original ingested weight using a precise bone regression technique (% IRI = [% number + % weight] × % occurrence). Collections from 2 ecologically contrasting locations, North Sound and South Bimini, took place during wet and dry seasons between March 2000 to March 2003 (Newman et al. 2010, see Newman et al. 2007 for detailed information on the collection sites, general ecological conditions and prey communities at Bimini). Table 1 lists prey items taken from juvenile lemon sharks, including biometric information, condition factors and the number of samples analysed.

Prey of lemon sharks were collected from mangroves using block nets; and from seagrass using seine nets, trawl, and gillnets as described in Newman et al. (2010). Briefly, seine and block nets were 75 m long (2 m deep with a 5 mm mesh), and the trawl opening was 1 × 0.5 m with a 5 mm mesh. Following collection, all samples were identified to species and weighed (±0.1 g, Ohaus Scout scales; ±1 g for large organisms). Measurements (mm) were taken of fork length (FL) and/or total length (TL) or carapace width (CW) according to species (Newman et al. 2010). Samples were oven dried to a constant weight, roughly ground in a clean pestle and mortar, then stored in air-tight 20 ml vials until analysis. Samples were then analysed for trace element, energy, ash, lipid, protein and carbohydrate content (see below).

Trace element analyses. Trace element concentration of whole prey items of lemon sharks was determined using inductively coupled plasma–optical emission spectrometry (ICP-OES; VARIAN 725–ES) following the method of Handy et al. (1999) with minor modifications. Analar grade reagents or above were used for all solutions, and spectrophotometric grade reagents were used for analytical standards. Analysis focused on the nutritionally required elements most important to the survival of most complex organisms (NRC 1993) including Cu, Fe, Zn, Mn and the major electrolytes Na, K, Ca, Mg. One percent Ytrium (Y) was used as an internal standard, and samples were matrix matched with blanks and calibration standards. Samples (~1 g dry weight) were digested in 5 ml of concentrated Aristar grade nitric acid in a water bath at 40°C using 20 ml polythene screw-top digestion vials (VWR International). Samples were allowed to cool overnight, and then diluted to 18 ml with deionised water. In some cases where dry weights were <1 g, then 1 ml of acid was used in the digestion, and samples were diluted to 5 ml instead. Instrument calibrations were performed using acidified, serial dilutions of the relevant certified standard. Top standards were 4 mg l–1 for Cu, Fe and Zn; 200 mg l–1 for Mg, Ca and Na; 0.4 mg l–1 for Mn; and 400 mg l–1 for K. Standards were analysed at the beginning of each run, and checked every 10 samples. Certified reference material DORM-2 (Dogfish Squalus acanthias muscle tissue, National Research Council Canada) was analysed using identical methods, and gave good recoveries (all within 100 ± 10%). In addition, spiked sample recoveries from analysis of Ca, Cu, K and Zn at 50 µg ml–1 in Sphyraena barracuda tissues were all within 102 ± 4% (mean ± SE; n = 5 per element), indicating good recovery of metals from our digestion procedure and subsequent analysis.

Proximate composition analyses. Key species in the diet of lemon sharks (see % IRI in Newman et al. 2010) were selected for proximate composition analyses using the standard method for whole fish (AOAC 1995). A total of 60 samples were analysed; from 9 species of fish and 3 species of crab (5 to 6 replicates of each species). Samples were initially cut into small pieces with clean scissors, then ground to a fine powder using an electric grinder, or pestle and mortar as appropriate. Carcass protein was determined by the Kjeldal digestion method (AOAC 1995). Briefly, 85 to 265 mg of dry sample (in triplicate) was digested with a Kjeldal tablet (KJELTABS TCT, Thompson & Capper) diluted up to 25 ml with 0.1 M H2SO4. Samples were heated (Kjeldatherm digestion block) using the following manifold temperatures: 105°C for 15 min, 225°C for 60 min, 380°C for 45 min, then lowered to 105°C. Samples were then distilled (Gerhardt Vapodest 40), and converted to % protein using a conversion factor of 6.25 in accordance with AOAC (1995). Recovery of protein from casein reference material (Standard materials, International Feed Number: 5-01-162) analysed on each sample run was 101.41 ± 0.25% (n = 6). To prevent foaming, silicone anti-foaming agent (1 g, Fisher Scientific) was added to crustacean samples prior to analyses. Lipid was determined by anhydrous ether extraction (Soxtec System HT Extraction Unit) according to AOAC (1995), using 325 to 1060 mg samples (in triplicate). Ash was determined by the direct method (AOAC 1995) using 2 to 3 repeated measures on each sample and an initial dry weight of around 500 mg. Samples were heated to 550°C in a muffle furnace for a minimum of 8 h, and the resulting ash was weighed. Energy content of ground tissue was determined according to AOAC (1995) using a bomb
Table 1. *Negaprion brevirostris*. Nursery bound juvenile lemon shark prey from Bimini, Bahamas. Sample sizes (n) are: **Total**: North Sound/South Bimini, wet season/dry season. Teleosts and crustaceans are listed in order of Index of Relative Importance (% IRI) to the diet (Newman et al. 2010). Mean ± SE (range in parentheses, n > 5) fork length (FL), total length (TL) or crustacean carapace width (CW) and wet weight (W). Different superscript letter means significantly different by ANOVA from within column species, progressing alphabetically from most important prey species *Gerres cinereus*. Correlation coefficients (Corr.) and $r^2$ were obtained using logarithmic (log10) linear regression analysis. $K_n$ is the relative condition factor obtained from the equation $K_n = W/aTL^n$ where W is wet weight (kg), TL is total length or carapace width (mm), and a and n are the exponential form of the intercept and slope, respectively, of the logarithmic length-weight equation (Craig et al. 2005).

<table>
<thead>
<tr>
<th>Sample size (n)</th>
<th>FL (mm)</th>
<th>TL or CW (mm)</th>
<th>W (g)</th>
<th>$K_n$ Equation</th>
<th>$r^2$</th>
<th>Corr.</th>
<th>$K_n$</th>
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<td><strong>TELEOSTS</strong></td>
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<td><em>Gerreidae</em> (mojarras)</td>
<td>93: 49/44, 46/47</td>
<td>109 ± 5 (50 – 183)$^b$</td>
<td>137 ± 6 (62 – 233)$^b$</td>
<td>41.38 ± 4.99 (2.76 – 152.60)$^b$</td>
<td>W/<a href="TL$%5E%7B2.895%7D$">2.155 × 10$^{-8}$</a>]</td>
<td>94.03</td>
<td>0.970</td>
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<td><em>Gerres cinereus</em></td>
<td>46: 23/23, 24/22</td>
<td>82 ± 2 (51 – 123)$^c$</td>
<td>97 ± 3 (60 – 147)$^b$</td>
<td>11.16 ± 0.99 (2.42 – 34.64)$^c$</td>
<td>W/<a href="TL$%5E%7B2.868%7D$">2.043 × 10$^{-8}$</a>]</td>
<td>91.40</td>
<td>0.956</td>
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<td><em>Eucinostomus jonesii</em></td>
<td>47: 26/21, 22/25</td>
<td>–</td>
<td>97 ± 4 (54 – 153)$^b$</td>
<td>16.15 ± 2.38 (2.55 – 56.16)$^c$</td>
<td>W/<a href="TL$%5E%7B2.878%7D$">2.043 × 10$^{-8}$</a>]</td>
<td>91.40</td>
<td>0.956</td>
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<td><strong>SCARIDAE</strong> (parrotfishes)</td>
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<td><em>Sparisoma chrysopterum</em></td>
<td>30: 11/19, 19/11</td>
<td>–</td>
<td>82 ± 2 (51 – 123)$^c$</td>
<td>97 ± 3 (60 – 147)$^b$</td>
<td>11.16 ± 0.99 (2.42 – 34.64)$^c$</td>
<td>W/<a href="TL$%5E%7B2.868%7D$">2.043 × 10$^{-8}$</a>]</td>
<td>91.40</td>
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<td><em>Lutjanidae</em> (snappers)</td>
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<td><em>Lutjanus apodus</em></td>
<td>18: 2/16, 7/11</td>
<td>116 ± 8 (71 – 210)$^b$</td>
<td>122 ± 9 (75 – 222)$^b$</td>
<td>39.27 ± 9.32 (2.42 – 34.64)$^c$</td>
<td>W/<a href="TL$%5E%7B3.087%7D$">1.175 × 10$^{-8}$</a>]</td>
<td>98.92</td>
<td>0.995</td>
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<td><em>Sphyraenidae</em> (barracudas)</td>
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<td><em>Sphyraena barracuda</em></td>
<td>38: 17/21, 17/19</td>
<td>196 ± 16 (71 – 396)$^b$</td>
<td>69.68 ± 10.62 (2.03 – 213.79)$^b$</td>
<td>W/<a href="TL$%5E%7B3.074%7D$">4.433 × 10$^{-8}$</a>]</td>
<td>99.04</td>
<td>0.995</td>
<td>1.45</td>
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<td><strong>CRUSTACEANS</strong></td>
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<td><em>Portunidae</em> (crabs)</td>
<td>29: 21/8, 12/17</td>
<td>72 ± 7 (38 – 119)$^b$</td>
<td>30.34 ± 10.40 (2.97 – 79.26)$^b$</td>
<td>W/<a href="TL$%5E%7B2.958%7D$">6.975 × 10$^{-8}$</a>]</td>
<td>97.34</td>
<td>0.987</td>
<td>1.24</td>
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<td><em>Callinectes ornatus</em></td>
<td>13: 10/3, 6/7</td>
<td>–</td>
<td>92 ± 10 (47 – 129)$^b$</td>
<td>105.07 ± 28.63 (45.09 – 169.30)$^b$</td>
<td>W/<a href="TL$%5E%7B2.971%7D$">1.441 × 10$^{-8}$</a>]</td>
<td>97.67</td>
<td>0.988</td>
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<td><em>Penaeidae</em> (shrimps)</td>
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<td><em>Penaeus duorarum</em></td>
<td>49: 25/24, 27/22</td>
<td>–</td>
<td>76 ± 2 (49 – 111)$^b$</td>
<td>3.09 ± 0.28 (0.75 – 8.32)$^b$</td>
<td>W/<a href="TL$%5E%7B2.966%7D$">5.312 × 10$^{-9}$</a>]</td>
<td>97.13</td>
<td>0.986</td>
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calorimeter (PARR 1356) pressurised with 30 bar O₂. Briefly, 879 to 1170 mg of powdered sample was pressed into a pellet and measured in the calorimeter (in triplicate, or duplicate for small animals). The water jacket on the instrument was filled to 2000 ± 0.500 g with distilled water, 2 to 3°C lower than room temperature. Bucket water weight was checked after every run, and changed every 4 to 5 tests. The remaining sample following bomb calorimetry was calculated as 99.746 ± 0.157% ash (mean ± SE, n = 16).

**Statistics and calculations.** Statistics were conducted using Statgraphics 5.1. After descriptive statistics and a variance check (Cochran’s C-test), data for each parameter (macronutrient, energy or metal) were analysed using 1-way Analysis of Variance (ANOVA) for season (wet versus dry), and location (North Sound versus South Bimini), after checking that body size did not affect the data for individual species by season or location; and in 3 rare dietary items where this did occur, body size or other morphometrics of the prey was used as a covariate in the ANOVA. Differences in metal concentration by species (regardless of season or location) were similarly analysed by ANOVA. In all cases, the default Fisher’s Least Squares Differences (LSD) post-hoc test was used to identify the individual differences in the ANOVA. Where the variance check failed, data were transformed; where data were non-parametric and could not be transformed the Kruskal-Wallis test was used. All statistical analysis used the default rejection level of p = 0.05. In the text, all p-values that were transformed are for ANOVAs, unless otherwise stated.

Relative condition factors in Table 1 reported are for ANOVAs, unless otherwise stated. Where these are expected (e.g. Fe and Cu interactions that were similar to yellow fin mojarra, although both Fe and Mn were significantly higher in both mojarras (Atherinidae), yellow fin and slender mojarra Euclinostomus jonesii, than in all other fishes except silversides (Atherinidae; Atherinomorus stipes) and needlefishes (Belonidae; Strongylura notata). Slender mojarra had Zn, Ca, and Na concentrations that were similar to yellow fin mojarra, although both Fe and Mn were significantly higher in the yellowfin within the mojarra family (Table 2). Similar findings applied to Na and Ca between slender and yellow fin mojarra. Among the fishes, silversides and needlefish had the highest Na, Ca and Mg, and barracuda (Sphyraenaenid; Sphyraena barracuda) had the highest K. Not surprisingly, concentrations of the minerals Ca, Cu, Na, Mg and Mn were generally higher in crustaceans than teleosts (Table 2). Pink shrimp (Penaeidae; Penaeus duorarum) had the highest Na concentration of all animals, and a K concentration comparable to many of the teleost fish. Swimming crabs (Portunidae; Callinectes spp.) contained the highest Ca and Mg of all the prey species analysed, but K was generally half that of fishes (Table 2). The crabs,

Metal concentrations varied between all 15 species of lemon shark prey (Table 2). The most common prey of the lemon shark, yellow fin mojarra, contained some of the highest trace element concentrations of all the teleost species analysed (Table 2). Yellow fin mojarra showed significantly higher Fe and Mn concentrations than all other fishes, and the highest Fe of all the animals examined. Zn was also significantly higher in both mojarras (Gerreidae), yellow fin and slender mojarra Euclinostomus jonesii, than in all other fishes except silversides and needlefishes. Sphyraenaenid; Sphyraena barracuda) had the highest K. Not surprisingly, concentrations of the minerals Ca, Cu, Na, Mg and Mn were generally higher in crustaceans than teleosts (Table 2). Pink shrimp (Penaeidae; Penaeus duorarum) had the highest Na concentration of all animals, and a K concentration comparable to many of the teleost fish. Swimming crabs (Portunidae; Callinectes spp.) contained the highest Ca and Mg of all the prey species analysed, but K was generally half that of fishes (Table 2). The crabs,
as expected from their respiratory pigments, contained higher Cu and significantly lower Fe compared to the teleosts (Table 2). The crabs were a particular good source of Mn, yet parrotfish (Scaridae; Sparisoma chrysopterum) and yellow fin mojarra contained more than the most common monly predated crustacean, Callinectes ornatus.

Comparison of concentrations of different metals within each species did produce correlations that were not random (i.e., > 5%), but most gave r² values of 20% or much less. Nonetheless, there were some notable exceptions. For example, Zn negatively correlated with Cu (e.g., barracuda: Zn = 83.3696 – 39.4415/Cu, r² = 37.57, correlation coefficient = –0.61), and Fe positively with Cu (e.g., parrotfish: Fe = 83.0521 – 62.426/Cu, r² = 40.18, correlation coefficient = –0.63) except in yellow fin mojarra, and crustaceans. Several elements correlated positively with Mn (e.g., parrotfish: Ca = 15.6095 + 51.4775 × Mn, r² = 77.64, correlation coefficient = 0.88; Fe = 32.7835 + 36.4913 × Mn, r² = 40.77, correlation coefficient = 0.64). There were no body size effects between locations or seasons at the level of species.

Spatial variation in metal concentrations of prey items

There were a number of statistically significant differences between the metal concentrations in the animals from the North Sound compared to South Bimini (38 out of 80 comparisons were different, Fig. 1). When location differences occurred in the major electrolytes, these tended to be higher in animals from South Bimini. There were only a few location differences in whole body Na. Grunts, parrotfish and shrimps showed statistically significant higher concentrations of Na in South Bimini compared to the North Sound. Barracuda and silversides contained higher K concentrations in South Bimini but, notably, silversides.
Fig. 1. *Negaprion brevirostris*. Concentrations of (a–d) electrolytes Na, K, Ca, Mg (mg g$^{-1}$ dry weight) and (e–h) trace elements Cu, Zn, Fe, Mn (µg g$^{-1}$ dry weight) in major prey of nursery bound juvenile lemon sharks from North Sound Bimini and South Bimini, Bahamas (mean ± SE, n > 5, see Table 1 for sample sizes). Dashed line separates crustaceans (right) and fishes (left); species are otherwise listed left to right in order of Index of Relative Importance (% IRI) to the diet (Newman et al. 2010). Analyses were conducted using ICP-OES standard techniques (AOAC 1995) adapted for whole fish and crustaceans. Asterisks indicate significant differences between spatial and seasonal samples by ANOVA: *p < 0.05, **p < 0.01, ***p < 0.001
had lower Ca in South Bimini. In the few species where location differences in Mg occurred, all showed higher concentrations in South Bimini (Fig. 1d). The trace elements showed more location effects than the bulk electrolytes. In particular, all fish species showed higher Cu concentrations in South Bimini compared to the North Sound (Fig. 1e), as did many of the fishes for Zn (Fig. 1f). There were also some strong differences in Fe concentration between locations, with Fe concentration being higher in all animals from South Bimini except slender mojarra, snappers, and silversides, for which no location effects could be detected (Fig. 1g). For Mn, only parrotfish, some grunts, needlefish, and pink shrimp showed a location effect, with higher concentration in specimens from South Bimini.

### Seasonal variation in the metal concentrations of prey items

There were some seasonal effects on metal concentrations and, when these occurred, metals were usually higher in animals collected during the dry season (Fig. 2). Yellow fin mojarra showed an increase of Na in the dry season, but needlefish, silversides, and pink shrimp showed the reverse (Fig. 2a). Yellow fin mojarra showed a decrease of K in the dry season, but some grunts, needlefish and silversides showed increases (Fig. 2b). Grunts showed the biggest seasonal increase of Ca in the dry season (Fig. 2c). Conversely, where seasonal effects in Mg occurred, concentrations were generally lower in animals from the dry season (Fig. 2d). There were no seasonal effects on Cu, except a small increase in Cu concentrations in grunts in the dry season (Fig. 2e). Only snappers and shrimps showed a seasonal effect for Fe (Fig. 2g). Among the trace metals, the most pronounced seasonal effects were found for Zn (Fig. 2f), with yellow fin mojarra showing higher concentrations in South Bimini, compared to the North Sound. There were also strong differences in Fe concentration between all animals from Fe concentration being higher in all animals from South Bimini than in those from the North Sound. In the few species showed higher concentrations in South Bimini (Fig. 1f). The trace elements showed more location effects (Fig. 2h).

### Proximate carcass composition and energy content

The teleost fish gave higher energy values than the crustaceans, and among the fishes, highest values were found in the slender mojarra, followed by the barracuda, grunts also showed some large increases in Mn in the dry season (Fig. 2h).

#### Table 3. Negaprion brevirostris. Proximate nutrient and energy composition of nursery bound juvenile lemon shark whole dry prey from Bimini, Bahamas (mean ± SE, n > 5). Teleosts and crustaceans are listed in order of Index of Relative Importance (% IRI) to the diet (Newman et al. 2010), of which 97 % IRI was analysed for nutritional composition. Analyses were conducted using standard methods (adapted from AOAC 1995). Carb. = carbohydrate. Different superscript letter means significantly different by ANOVA from within column species, progressing alphabetically from the dominant prey Gerres cinereus.

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<th>Family</th>
<th>IRI</th>
<th>% IRI</th>
<th>Energy (kJ g⁻¹)</th>
<th>Energy (kcal g⁻¹)</th>
<th>Moisture (%)</th>
<th>Lipid (%)</th>
<th>Protein (%)</th>
<th>Ash (%)</th>
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<td>74.92 ± 0.38A</td>
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Fig. 2. *Negaprion brevirostris*. Concentrations of (a–d) electrolytes Na, K, Ca, Mg (mg g⁻¹ dry weight) and (e–h) trace elements Cu, Zn, Fe, Mn (µg g⁻¹ dry weight) in major prey of nursery-bound juvenile lemon sharks from Bimini, Bahamas during wet and dry seasons (mean ± SE, n > 5 see Table 1 for sample sizes). Dashed line separates crustaceans (right) and fishes (left); species are otherwise listed left to right in order of Index of Relative Importance (% IRI) to the diet (Newman et al. 2010). Analyses were conducted using ICP-OES standard techniques (AOAC 1995) adapted for whole fish and crustaceans. Asterisks indicate significant differences between spatial and seasonal samples by ANOVA: *p < 0.05, **p < 0.01, ***p < 0.001.
had the highest lipid contents of all the animals examined, although the grunts and snappers were also a good source of lipid (Table 3). The crabs provided the richest source of carbohydrates and ash, but were low in protein compared to teleost prey items. Of the fishes, the parrotfish provided the most protein on a percent of carcass basis (Table 3). The mojarras and parrotfishes generally had slightly higher moisture contents than the other fishes (Table 3). There were not enough individuals from each species to do a comparison of proximate composition and energy content by location (North Sound versus South Bimini) or season (wet versus dry). An analysis of the data pooled by species showed no location effect on protein, lipid or carbohydrate content of the prey (ANOVA: p > 0.05, data not shown). Similarly, there were no seasonal effects on proximate composition, except an overall 3% increase in carbohydrate in the wet season (Kruskal-Wallis: p = 0.01, data not shown).

Calculated daily nutritional intake

Table 4 displays the calculated daily nutritional intake of the macronutrients (protein, fat and carbohydrate), energy, and the intake of metals obtained from the different prey items. The data are illustrative for an average 1.85 kg juvenile lemon shark from Bimini with an average ration size of 24.84 g d⁻¹. Then, using data on prey preference of lemon sharks, an estimate of the gross proportions of the diet was calculated (Fig. 3). Notably, the diet was mostly protein (61.9%), and much of the remainder is ash (24.9%) which includes the mineral component of the diet. Lipid is only 4.6% of the diet, and carbohydrates are also a low proportion (8.6%, Fig. 3). Calculated absolute values of dietary intake for a 1.85 kg lemon shark were: 397.06 kJ energy, 15.34 g protein, 6.17 g ash, 2.14 g carbohydrate per day (Table 4). The average lemon shark ingested ~1 mg or less of the trace metals, but much more of the bulk electrolytes, and >1 g of Ca each day (Table 4). For the metals, most of the elemental content of the daily ration is Ca (6.6%) and, of the metals measured, Ca accounted for 73% of the entire metal intake by mass. Na and K contributed 1% of the daily ration, respectively. Magnesium was 0.3% of the daily ration, and the trace elements together contributed only 0.01% of the daily ration (Fig. 3).

The stomach contents of sharks can often only be usefully analysed to family level (see Newman et al. 2010). When the daily nutritional intake is considered according to the importance of each family of prey in the diet, it is clear that the mojarras (50.85% of the daily ration) contribute most of the major nutrients and

<table>
<thead>
<tr>
<th>Family</th>
<th>IRI</th>
<th>DR (g)</th>
<th>R (kJ)</th>
<th>Ash (g)</th>
<th>Carb (g)</th>
</tr>
</thead>
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<td>Gerreidae (Mojarras)</td>
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<tr>
<td>Belonidae (Needlefishs)</td>
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<td>0.35</td>
<td>6.21</td>
<td>1.48</td>
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</tr>
<tr>
<td>Atherinidae (Silversides)</td>
<td>0.35</td>
<td>0.08</td>
<td>nd</td>
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</table>

Table 4. Nutritional composition data (Tables 2 & 3) and dietary intake of prey formed the basis of calculations for daily nutritional intake. Totals are based on the estimate of absolute dietary intake (100%) from the known content of 97% of prey. nd: no data. Consult Fig. 3 for a graphical representation of relative proportion of nutrients in the diet.
minerals to the daily diet of lemon sharks at Bimini (Table 4). Even animals with high nutritional value on a carcass composition basis, had little effect on the overall daily nutritional profile when the % IRI was low. For example, snappers contained a high level of energy compared to other species (16.14 ± 0.41 kJ g⁻¹) yet, at little more than 5% IRI, contributed only 21.43 kJ d⁻¹ or 5% of the daily energy intake. However, notably, needlefish provided 0.24 g of the 1.15 g of daily lipid intake equal to 20% of the daily lipid intake, despite this family making up only 1.5% of the diet (Table 4). The mojarras dominated the daily mineral intake, but 20% of the daily Ca (332 g out of 1641 g) and nearly 28% of the daily Mg (20.7 g out of 74.5 g) was obtained from swimming crabs (Table 4). Notably, 63 and 13% of the daily Cu and Mn intake, respectively, was also obtained from swimming crabs (Table 4). Despite being only 10% of the daily ration by family, the crabs therefore provided an important contribution to the overall trace metal intake of sharks.

**DISCUSSION**

To our knowledge this study is one of the first to provide detailed trace element nutritional information on the prey items of juvenile lemon sharks, and for prey items collected in the field at the same time/locations as the stomach contents of the sharks (Newman et al. 2010). Data sets on the macronutrient and metal composition of carcasses of tropical fish are relatively limited compared to temperate species. The data shows that the metal content of the tropical species of fish and crustaceans eaten by lemon sharks are broadly similar to the temperate species used in aquaculture and those well known in fish nutrition. Calculation of a typical daily nutritional intake showed that the lemon shark eats a very lean, high protein diet and that the mineral intake is dominated by Ca. Juvenile lemon sharks get most of their daily nutritional intake from eating yellow fin mojarras, but parrotfish provide the main intake of lipid. Notably although invertebrates are less frequent in the diet of lemon sharks, they do provide an important contribution to the mineral intake; especially Cu, Ca, and Mg.

**Metal concentrations in prey items and metal intake by lemon sharks**

There are relatively few reports of the tissue concentrations of the major electrolytes (i.e. Na, K, Ca, Mg) in tropical species of fish, compared to the wealth of data on temperate species (for reviews see: Holmes & Donaldson 1969, electrolytes in the body; Eddy 2009, Na; Salman 2009, Na; Flik et al. 2009, Ca; Bijvelds et al. 1998, Mg). For the fish species in Table 2 there appear to be no previous reports detailing the major electrolyte composition of the whole carcass. However, the whole body Na concentration in this study (8–16 mg g⁻¹, Table 2) were, as expected, slightly higher than that of rainbow trout in freshwater (e.g. 0.7–1 mg g⁻¹, Salman 2009); but close to trout in seawater (~2.6 mg g⁻¹, Eddy & Bath 1979) and similar to marine teleosts (e.g. sea bream: 9.2 mg g⁻¹, Van Anholt et al. 2004). Whole body K appears to be rarely reported in fish, but the concentrations here (7–12 mg g⁻¹, Table 2) were similar to freshwater trout (~6 mg g⁻¹, Eddy & Bath 1979). Calcium concentrations in fish in this study (50–84 mg g⁻¹, Table 2) are similar to those reported for marine scorpion fish (Sebastiscus marmoratus: ~55 mg g⁻¹, Hossain & Furuichi 2000). Carcass Mg concentration in fish depends especially on the amount of hard tissue (scales, bones) which can
contribute 70% of the total Mg pool (Bijvelds et al. 1998). In freshwater-adapted tilapia Oreochromis mossambicus the skeletal muscle contains ~1.4 mg g⁻¹, and the bone 3.1 mg g⁻¹ of Mg, which are broadly similar to the values for whole fish in this study (~2 mg g⁻¹, Table 2). For crustaceans, the mineral contents are often reported just for the edible parts of the flesh and exclude the exoskeleton (Gökoðlu & Yerlikaya 2003, Naczk et al. 2004). However, the analysis here also included the exoskeleton, and the values for Mg and Ca (Table 2) are at least broadly similar to reports for the whole body of Callinectes spp. when tissue wet weight is taken into account (Neufeld & Cameron 1992).

Similar considerations apply to the trace metals (Fe, Cu, Zn, Mn). In fish, the Fe concentrations (36–52 µg g⁻¹, Table 2) in our study are similar to previous reports for trout carcass (11–41 µg g⁻¹, Carriquiriborde et al. 2004). Copper and Zn concentrations (Cu 1–3 µg g⁻¹, Zn 39–156 µg g⁻¹; Table 2) are also similar to previous reports for marine fish (grey mullet Chelon labrosus: Cu ~7 µg g⁻¹, Zn ~43 µg g⁻¹; Baker et al. 1998). The carcass Mn concentrations for wild fish (0.1–0.7 µg g⁻¹, Table 2) are lower than those for marine fish fed a normal Mn supplement in aquaculture (juvenile groupers Epinephelus coioides: 4–10 µg g⁻¹, Ye et al. 2009), but are comparable to reports for Atlantic salmon Salmo salar (~0.2 µg g⁻¹, Maage et al. 2000). Trace metal concentrations in the crustaceans are also broadly comparable with previous reports (Scott-Fordsmand & Depledge 1997, Gökoðlu & Yerlikaya 2003).

The calculated electrolyte intakes for an average lemon shark (Table 4) also show some similarity with other fishes. Most teleosts require a dietary salt intake of ~1% (Salman 2009) and this is at least matched by similar Na and K intakes in the lemon shark (~1%, Table 4). In seawater, Flik et al. (1995) argue that dietary Ca has a negligible role in Ca metabolism in fishes, with Ca influx at the gills being more important. Seawater typically contains 10 mmol l⁻¹ Ca and, with elasmobranchs having a plasma Ca concentration of ~4 mmol l⁻¹ (e.g. Trivett et al. 2001), it would also seem that sharks could achieve their Ca requirements through Ca uptake at the gills. It is therefore curious that the lemon shark ingests a diet of ~6% Ca as dry matter. Calcium plays a role in biomineralisation of secreted bicarbonate to help maintain acid-base balance in flounder (Whittamore et al. 2010), which obtain their Ca through drinking seawater as part of their osmoregulatory strategy. Sharks are osmoconformers and do not normally drink much seawater, but may still need ingested Ca for biomineralisation processes. Alternatively, it is more likely that the Ca is just an incidental component of the diet not required for Ca homeostasis. Similar osmoregulatory arguments apply to Mg, where Mg concentration in seawater is typically at ~50 mmol l⁻¹, but tissue normally contains only a few mmols, and therefore any dietary requirement would be negligible (Bijvelds et al. 1998, Flik et al. 2009). Nonetheless, ~0.3% of the daily ration is Mg (74.49 mg of Mg for 24.84 g of ingested food, Table 4).

The dietary trace element requirements of sharks are mostly unknown. In this study the amount of ingested Zn was ~0.008% of the daily ration (Table 4 & Fig. 3). Typical fish meals used in aquaculture contain 80 to 100 mg Zn per kg food (i.e. 0.001% or less of the diet, Watanabe et al. 1997). It would therefore seem that the 1.97 mg daily Zn intake of the lemon sharks exceeds the minimum requirements of fish. This does not seem to be the case for the other dietary metals. Fish have a minimum dietary Fe requirement of at least 30 mg kg⁻¹ of food or more (~0.03% of the ration in trout, see Carriquiriborde et al. 2004). In comparison to teleosts, the lemon shark has a low iron intake at ~0.005% Fe in the daily ration (Table 4 & Fig. 3). Assuming that iron metabolism in sharks is similar to other fish, this could tentatively be interpreted as evidence that the lemon shark may be Fe deficient. Fish normally require a few mg kg⁻¹ of dietary Cu each day (Handy et al. 1999) and, with the lemon sharks eating only ~0.1 mg Cu per day (Table 4), this is also at least an order of magnitude below the requirements of other fish. Little is known about Cu metabolism in elasmobranchs but, for spotted dogfish Scyliorhinus canicula at least, Cu exposure results in the expected metallothionein induction and tissue Cu accumulation (De Boeck et al. 2010). Thus it seems unlikely that Cu metabolism in sharks is vastly different from other fish, and would therefore suggest that lemon sharks are probably Cu deficient. Similarly, most fish need a few milligrams of Mn per day, and the Mn intake of 0.01 mg (Table 4) is at least 2 orders of magnitude below the requirements of most fishes (Watanabe et al. 1997). There also appears to be no information on Mn metabolism in sharks, but it would be extremely unlikely that a 100-fold lower Mn intake in the lemon shark could be compensated by comparably more efficient Mn uptake or retention mechanisms, given that metal transporters are well conserved across species (Bury et al. 2003).

There were some seasonal and location effects on the metal contents of prey items (Figs. 1 & 2). The reasons for the small differences in prey metal concentrations by location could relate to anthropogenic activity (e.g. building construction on the shore line) mobilising minerals into the aquatic food chain, or simply changes in geochemical cycling (e.g. by removal of vegetation, Jennings et al. 2008). Tolerable dilution during wet season could also explain the slightly lower electrolyte concentration in the tissues in the winter.
However, these effects on overall elemental intake were mostly small and not likely to have a big nutritional impact. For example, the preferred prey, yellow fin mojarra, showed one of the biggest changes in dietary Fe intake by location (from ~55 µg g⁻¹ in the North Sound to ~70 µg g⁻¹ in South Bimini, Fig. 1g). An additional 15 µg Fe per g⁻¹ of mojarra represents ~198 µg of extra Fe in the diet (sharks eat 12.63 g of mojarra per day, Table 4), or a 16% increase in the daily Fe intake. Nutritionally, this would bring Fe slightly closer to the requirements of other fish. For Cu, the mojarra in South Bimini had about 2 µg g⁻¹ more Cu than those in the North (Fig. 1e). A similar calculation yields an additional 25 µg Cu in the daily ration from mojarra. This would still leave the nutritional Cu intake for lemon sharks far below the milligram concentrations reported for other fish in the nutrition literature (Watanabe et al. 1997). Similar arguments apply to the seasonal effects. For example, mojarra showed one of the biggest increases in Mn in the dry season (an additional 0.3 µg g⁻¹ of mojarra, Fig. 2h). This represents an additional 3.7 µg of Mn in the diet (37% rise), but this microgram increase remains far below the milligram Mn requirements of most fish (Watanabe et al. 1997).

Macronutrients in prey items and estimated protein, lipid and carbohydrate intake by lemon sharks

Practical fish diets for carnivorous or omnivorous fish in aquaculture can contain around 400 to 600 g kg⁻¹ dry matter of crude protein, but the digestible protein may only be 30 to 40% of the dry matter (Halver 1989). These levels are set to maximise growth, and it is clear that the average lemon shark has a very high protein diet (61.9% of dry matter, Fig. 3). The lipid intake of the lemon shark is ~5% of the diet (Fig. 3) and is consistent with the minimum requirement for other tropical carnivorous fish (e.g. cobia: ~5%, Fraser & Davies 2009). Many carnivorous fish do not have a specific minimum requirement for total carbohydrate in the diet because they are able to use protein and/or lipids as an energy source (Halver 1989). Sharks also use carbohydrate as an energy source, but the overall contribution of carbohydrates to energy metabolism is unclear (Speers-Roesch & Treberg 2010).

However, there is a notable difference in obtaining energy from protein in elasmobranchs compared to other fish. Sharks rely more on the oxidation of amino acids for fuel (Speers-Roesch & Treberg 2010), and this may explain the high protein intake of the lemon shark (Fig. 3). The lemon sharks at Bimini also seem to be selecting prey fish species with high carcass protein contents (around 60–70%, Table 3). Even a well fed rainbow trout in the laboratory might have a proximate composition of 55% protein (e.g. Carriquiriborde et al. 2004), so these warm water fish have higher protein contents. However, even for wild prey, the lemon shark seems to be feeding at the upper end of the protein content range of teleosts. For example, Payne et al. (1999) analysed 13 fish species from the northeast Pacific and found a carcass protein range of approximately 40 to 60% dry mass protein, depending on species. Eder & Lewis (2005) produced a similar range of carcass protein levels from an analysis of 27 prey fish species from the southwest Atlantic, which ranged from about 40 to 69% dry mass.

The ash and lipid contents of an adult rainbow trout in aquaculture are around 10 and 20% respectively (Carriquiriborde et al. 2004) and it is clear that the wild tropical fish eaten by the lemon shark are leaner (1–5% lipid, Table 3); and have a higher ash content (17–25% Table 3), probably because the fish have relatively more hard tissues (bone, scales etc.) than a trout. The yellow fin mojarra is the preferred prey of juvenile lemon sharks at Bimini (Reeve et al. 2009, Newman et al. 2010). This preference appears not be on the basis of gross nutritional value per se because several other families of fish also provide similar protein, lipid, carbohydrate and ash contents at Bimini. It would seem that the abundance in the environment, colour and shape, behaviour, or swimming speed of the mojarra (see Reeve et al. 2009, Newman et al. 2010 for discussion) are the more likely explanations for this prey choice by the lemon shark. Parrotfishes make a notable contribution to lipid intake, and the portunid crabs provided most of the Cu intake (Table 4), but it remains unclear if sharks will feed on particular prey items to obtain specific nutrients.

Energy intake of lemon sharks

The overall energy content of the food is also an important facet of survival for any animal. For a fish to survive in the long term, the assimilation of daily energy (after the cost of digestion and absorption), must slightly exceed daily energy expenditure (Priede 1985). In the present study the total energy intake as food for an average juvenile lemon shark (body weight 1.85 kg, 24 g daily ration) was calculated as 397 kJ kg⁻¹d⁻¹ (Table 4). Wetherbee & Gruber (1993) estimate the energy absorption efficiency of the lemon shark to be around 62 to 83%. Even if the lower end of this suggested absorption efficiency is used in calculations, this would give an available daily energy (after digestion and absorption costs) of around 246 kJ. The estimated routine metabolic rate of juvenile lemon sharks of comparable weight to this study is about 106 kJ d⁻¹.
(Sundström & Gruber 1998). This suggests the energy intake from food is more than enough to meet the needs of routine metabolism, and the juvenile shark has around 140 kJ of energy to spend on metabolic scope each day (i.e. activity in excess of routine metabolism).

DiBattista et al. (2007) argue for a bioenergetic strategy that favours slow growth of juvenile lemon sharks at Bimini. The lemon sharks are therefore not spending their metabolic scope on extra growth. Bioenergetic theory suggests that fish which keep a greater reserve of metabolic scope have a better probability of survival in the long term (Priede 1977). For example, they can use this metabolic scope to evade a predator or take a risk by chasing more nutritionally valuable prey. Lemon sharks do not seem to do the latter at Bimini. The preferred prey of yellowfin mojarra have a small home range, are slow moving, relatively inactive feeders; and are widely abundant at Bimini (Randall 1967, Newman et al. 2010). This suggests the sharks at Bimini have some ‘spare’ metabolic scope, but despite this, the apparent survival rates of juvenile sharks is typically around 50 to 60% (DiBattista et al. 2007). This implies that bioenergetics is not limiting the survival of sharks at Bimini, but some other factor. This, of course, could be one of the minor components of the diet such as a trace element deficiency (see above), but anthropogenic activity and its environmental implication will also be important in survival (e.g. Jennings et al. 2008). Active predators also spend the majority of their daily energy on locomotion (Priede 1985), and so the precise amount of metabolic scope in reserve each day will depend especially on daily activity patterns. Telemetry measurements of locomotion/daily activity in lemon sharks at Bimini (Chapman et al. 2009), may help elucidate the short term daily changes in metabolic scope.

In conclusion, analysis of prey species caught in the wild shows that the calculated dietary metal intake of the lemon shark has some similarities with other fish, with Na and K being close to the 1% dietary salt intake of other fishes. Ca dominates the mineral intake of other fishes. The Zn intake of lemon sharks exceeds those of other fishes. The Cu, Fe and Mn intakes are much lower than the minimal nutritional requirements of other fishes. The latter are more easily explained by deficiency, as it seems unlikely that sharks are orders of magnitude better at retaining metals compared to other fish. Overall, although some seasonal and location effects on the metal content of prey items were observed, this had a negligible impact on trace element nutrition. The lemon shark also eats a very high protein diet, and an energy intake that exceeds that estimated to be required for routine metabolism by more than 100 kJ d⁻¹. Overall, the data suggest that lemon sharks have an adequate gross intake of macronutrients and energy that is consistent with their predatory tactics, but possible trace element deficiencies require clarification by detailed studies of trace metal metabolism and turnover in sharks.

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