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Niche metrics suggest euryhaline and coastal elasmobranchs provide trophic connections among marine and freshwater biomes in northern Australia

Sharon L. Every[1,2,3,*], Heidi R. Pethybridge[4], Christopher J. Fulton[3], Peter M. Kyne[1], David A. Crook[1]

[1]Research Institute for the Environment and Livelihoods, Charles Darwin University, Darwin, Northern Territory 0909, Australia

[2]North Australia Marine Research Alliance, Arafura-Timor Sea Research Facility, Brinkin, Northern Territory 0810, Australia

[3]Research School of Biology, The Australian National University, Canberra, Australian Capital Territory 2601, Australia

[4]Oceans and Atmosphere, Commonwealth Scientific and Industrial Research Organisation, Hobart, Tasmania 7000, Australia

ABSTRACT: Tropical elasmobranchs could play significant roles in connecting coastal and river ecosystems, yet few studies have explored the trophic ecology of elasmobranch species that may link these biomes. We investigated the trophic niches of 7 such species in northern Australia during the tropical monsoonal wet and dry seasons, using stable carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes (SI), and fatty acid (FA) biomarkers taken from muscle tissue. Both SI and FA metrics suggested significant niche partitioning between species, with 2 distinct guilds: a marine food web based on epiphytes and seagrass (low $\delta^{13}C$), and an estuarine/freshwater food web with a seston base (higher $\delta^{13}C$). A large overlap in SI niche areas and higher mean trophic positions (4.1–4.8) were evident in species accessing marine diets (*Carcharhinus leucas*, *Rhizoprionodon taylori*) when compared with species predominantly feeding in estuaries (3.2–3.6; *Glyphis garricki*, *G. glyphis*). Across all seasons, *G. garricki* had the greatest FA niche space, and variable overlap with 2 other species (*R. taylori*, *C. leucas*). Although limited seasonal effects were apparent for individual FA biomarkers, SI niche metrics revealed greater niche areas and inter-specific partitioning during the dry season for 3 species. Subtle differences in niche metrics derived from SI and FAs were likely due to disparate turnover times, and the statistical approach of each metric (2-dimensional versus multi-dimensional). Collectively, our analyses suggest that these tropical coastal and euryhaline elasmobranchs consume prey from a range of sources to provide trophic connections across marine, estuarine and freshwater biomes.

KEY WORDS: Niche metrics · Sharks · *Glyphis* · *Carcharhinus* · *Rhizoprionodon* · Biotracers · Stable isotopes · Fatty acids

INTRODUCTION

Resource partitioning, whereby species vary their use of habitat and dietary items over space and time (Ross 1986), is fundamental to our understanding of coastal ecosystem structure and function (Kitchell et al. 2002). As elasmobranchs (sharks and rays) have key roles in aquatic food webs (Stevens et al. 2000) and are demographically vulnerable predators (Dulvy et al. 2014), it is important to understand how they utilize these resources. Elasmobranchs are typically mid to upper level predators, and can provide ecosystem stability by influencing the abundance and health of prey species at multiple trophic levels, and connect otherwise distinct food webs (Rooney et al. 2006, Heithaus et al. 2013). To understand these

*Corresponding author: slevery2001@yahoo.com.au

trophic interactions, it is important to define elasmobranch niches, connections to different biomes, seasonal shifts, and the potential for dietary overlap between species.

Niche theory suggests that species use a set of resources that form their unique space, an '*n*-dimensional niche hypervolume' (Hutchinson 1957), which may be measured and compared among locations, seasons and species. In some cases, species can share resources to produce niche overlap. Whether such overlap leads to competition is dependent on the spatial and temporal extent of shared resource use. In the case of trophic overlap, sympatric species may target different prey items (Yick et al. 2011) and/or have temporal or spatial differences in consumption that negate competitive interactions (Ross 1986). Indeed, the extent of trophic overlap has been found to be highly variable in a number of elasmobranch assemblages (Papastamatiou et al. 2006, Yick et al. 2011, Tilley et al. 2013, Heithaus et al. 2013). Such variation demonstrates the differences between the trophic resource use of elasmobranchs and highlights the importance of determining diet for each species, particularly in assemblages where there are limited data, such as in species that are rare, threatened and/or data deficient, for instance, euryhaline elasmobranchs (Lucifora et al. 2015).

Euryhaline elasmobranchs are capable of tolerating a wide range of salinities and may complete different life history stages in marine, estuarine or freshwater habitats. Tropical regions are particularly important in this regard, given the high diversity of euryhaline elasmobranchs in these biomes (Lucifora et al. 2015). Due to their reliance on rivers and estuaries, euryhaline elasmobranchs must adjust to fluctuations in salinity and turbidity over tidal and seasonal cycles. These fluctuations affect not only their physiology, but also the abundance of their potential prey. Environmental changes are particularly pronounced in tropical rivers due to the variation between high-flow monsoonal 'wet' seasons and low-rainfall 'dry' seasons (Douglas et al. 2005, Warfe et al. 2011). Such changes may affect the utilization of trophic resources and partitioning within tropical elasmobranch assemblages that are poorly understood at present. However, recent studies have indicated the existence of dietary overlap among juvenile pigeye shark *Carcharhinus amboinensis* and bull shark *C. leucas* in northern Australia (Tillett et al. 2014) and among *C. leucas* and largetooth sawfish *Pristis pristis* in Western Australia (Thorburn et al. 2014).

Traditional trophic niche metrics reflecting diet breadth or richness are based on prey composition data from stomach content analysis, which can be invasive or fatal, often requires large sample numbers, and provides only a brief temporal snapshot of diet (Hussey et al. 2012). By analyzing small tissue samples for biochemical tracers, a more time-integrated assessment of trophic resource use can be obtained in a relatively non-invasive manner. Stable isotopes (SI) and fatty acids (FAs) are biotracers found in animal tissues that can be analyzed to estimate time-integrated trophic niches (Hussey et al. 2011, Jackson et al. 2011, Sardenne et al. 2016). An understanding of the fractionation of SI (e.g. $\delta^{13}C$, $\delta^{15}N$) through food webs allows isotopes to be used for a variety of applications, including estimation of niche area, resource partitioning and dietary overlap. Specifically, values of $\delta^{13}C$ may be used to identify species habitat associations through the comparison of $\delta^{13}C$ values with primary producers. The base of a food web may consist of C_3 or C_4 plants, bacteria or detrital matter, each having different biochemical pathways (Peterson & Fry 1987). These differences may result in $\delta^{13}C$ values that can be used to determine which species utilize those food webs. Values of $\delta^{15}N$ can estimate trophic position, because $\delta^{15}N$ is passed up food chains via the consumption of proteins so that higher order predators tend to be more enriched in $\delta^{15}N$ (Hussey et al. 2012).

FAs can also be traced through food webs, and are particularly useful for detecting basal sources and trophic interactions via essential FAs (EFAs) that can only be obtained through dietary sources (Iverson 2009). These EFAs are synthesized by different primary and secondary producers such as dinoflagellates, diatoms and algae or during microbial and bacterial processes (Dalsgaard et al. 2003, Parrish 2013). FAs often have faster turnover rates than isotopes (e.g. 14 wk in shark muscle tissue [Beckmann et al. 2014], cf. 6–12 mo for $\delta^{13}C$ and $\delta^{15}N$ [Malpica-Cruz et al. 2012, Hussey et al. 2012]), and have been used to reveal physiological and environmental changes over relatively fine scales, such as the EFA trophic biomarker ratio omega 3 [ω3]/omega 6 [ω6], which can be used to find seasonal differences within species. For example, albacore tuna *Thunnus alalunga* caught in waters off eastern Australia had an increased ratio of ω3/ω6 with decreasing water temperatures (Pethybridge et al. 2015). The ratio of ω3/ω6 can be influenced by changes in salinity, either increasing (e.g. sturgeon *Acipenser naccarii*; Martínez-Álvarez et al. 2005) or decreasing (e.g. white-edge freshwater whipray *Himantura signifer*; Speers-Roesch et al. 2008) with increasing salinity. Fatty acid 18:2ω6 (LA) is another biomarker that may

be useful in estuarine environments, as it can indicate terrestrial sources when compared with marine phytoplankton (Napolitano et al. 1997, Budge & Parrish 1998).

In this study, we use SI and FA biochemical tracers to evaluate trophic resource partitioning and seasonal variation in trophic resource utilization among 7 species of euryhaline and coastal elasmobranchs in the wet–dry tropical region of northern Australia. Northern Australia provides an excellent setting for studying elasmobranch trophic ecology due to the relatively pristine state of the estuarine and riverine ecosystems (Warfe et al. 2011) and the high diversity of sympatric elasmobranch species (Last 2002). The objectives of our study were to use SI and FA tracers to (1) estimate and compare trophic position estimates among species, (2) identify basal source contributions (marine, estuarine, freshwater), and (3) apply biochemical niche metrics (measurement of the distance between biochemical tracers) (Jackson et al. 2011, Swanson et al. 2015) to determine the extent of dietary overlap within and among species and across seasons.

MATERIALS AND METHODS

Site description, sample collection and preparation

Seven species of euryhaline and coastal elasmobranchs were collected in the South Alligator River, Northern Territory, Australia from March 2013 to July 2014 (Table 1, Fig. 1). This is a relatively pristine, macro-tidal river system with an ~5 km wide mouth (Wolanski & Chappell 1996), and tidal influence to 100 km upstream (Wolanski & Chappell 1996), where it narrows to ~20 m wide. The mean channel depth is ~7.5 m with a largely muddy bed (Wolanski & Chappell 1996), and mangroves dominate the estuary up to the limit of tidal influence (Mitchell et al. 2007). Strong seasonal fluctuations in rainfall, which can be broadly classified as wet season (November to April; 2013–2014 mean [±SE] total rainfall 241.3 ± 36.2 mm per season) and dry season (May to October; 2013–2014 mean total rainfall 18.2 ± 12.7 mm per season; Australian Government Bureau of Meteorology 2016), contribute to large changes in river hydrology, turbidity and salinity. The mouth of the river had mean salinity values (±SD) of 34.5 ± 0.2‰ (dry) and 17.1 ± 4.3‰ (wet), whereas in the mid-lower region of the river it was 21.9 ± 5.3‰ (dry) and 0.4 ± 1.5‰ (wet).

Four main sampling sites (Fig. 1) were chosen along the river to encompass a range of distances from the river mouth and where elasmobranchs were captured (limited by the rarer species). As such, these sites included a spectrum of salinities (see Fig. 1) and habitat characteristics (e.g. distance from mouth, river flow, width of river, abundance and species composition of riparian vegetation). A range of techniques was used at each site to capture species and obtain tissue samples during the live capture and release of individuals in the wild. *Rhizoprionodon taylori* and *Carcharhinus amboinensis* were captured with baited line in the lower estuary and coastal region of the South Alligator River, Kakadu National Park (Fig. 1). Northern river shark *Glyphis garricki*, speartooth shark *Glyphis glyphis*, *Carcharhinus leucas*, *Pristis pristis* and freshwater whipray *Urogymnus dalyensis* were caught in mid-lower estuarine reaches with a combination of 10.2 to 15.2 cm gill nets and baited lines.

Table 1. Number and total length (TL, range) of 7 euryhaline and coastal elasmobranch species from the South Alligator River, Kakadu National Park, Australia, from which muscle tissue samples were taken for stable isotope (SI) and fatty acid (FA) analysis. Included is the division of species between the wet and dry seasons and the sex ratio (male to female)

Taxon	Common name	Number (n)					TL (cm)		Sex ratio M:F		Habitat
		Total	SI		FA		SI	FA			
			Wet	Dry	Wet	Dry			SI	FA	
Carcharhinus amboinensis	Pigeye shark	2	0	2	0	1	68	68	1:1	0:1	Coastal/ marine
Carcharhinus leucas	Bull shark	34	28	6	20	2	72–93	72–139	18:16	13:9	Euryhaline
Glyphis garricki	Northern river shark	42	22	20	12	13	56–141	45–61	23:19	15:10	Euryhaline
Glyphis glyphis	Speartooth shark	8	2	3	2	6	71–120	78–136	1:4	2:6	Euryhaline
Urogymnus dalyensis	Freshwater whipray	2	0	2	0	2	56–110	56–110	0:2	0:2	Euryhaline
Pristis pristis	Largetooth sawfish	2	2	0	0	0	87–96	–	1:1	0:0	Euryhaline
Rhizoprionodon taylori	Australian sharpnose shark	38	8	30	1	28	34–87	34–87	11:27	23:6	Coastal/ marine

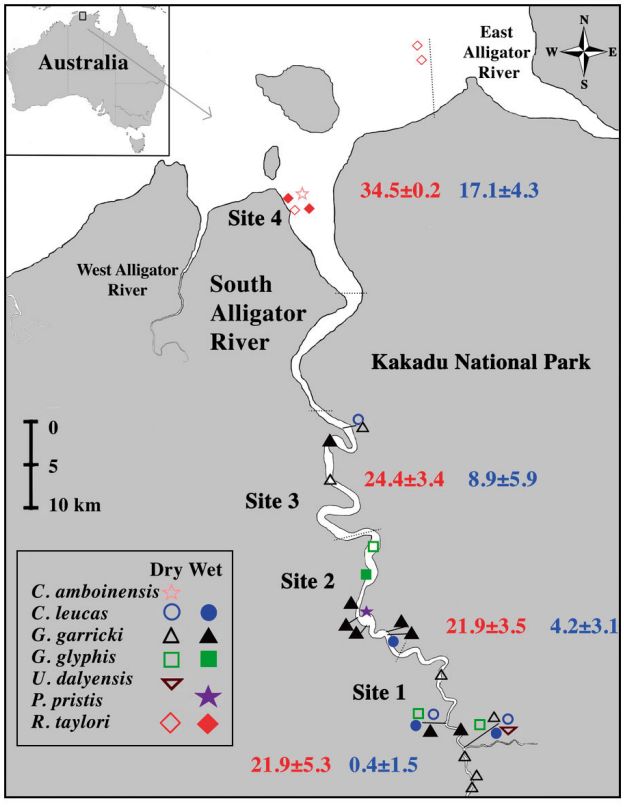

Fig. 1. Map of the South Alligator River, Northern Territory, Australia, showing capture locations of elasmobranchs. Dotted lines represent the approximate boundary of each sampling site. Inset shows where the river is in relation to northern Australia. (Background map from Stamen Toner, and formed using Quantum GIS Development Team 2015). Red (blue) text indicates dry (wet) season salinity (‰) values. For species names, see Table 1

A 5 mm biopsy punch (Stiefel) was used to collect muscle tissue from between the second dorsal and the caudal fin, just anterior and lateral to the caudal peduncle. Total, standard and precaudal length and clasper size were also measured and umbilical scarring in neonates was noted. Within 5 min of capture, the tissue sample was immediately placed on liquid nitrogen (−196°C) for preservation and initial storage in the field. Salinity and other water quality parameters were measured at each site using a hand-held multi-parameter instrument (Sonde 6000, YSI) (Fig. 1) during each sampling event.

Within 1 wk, tissue samples (mean wet mass 30.0 ± 20.0 mg) were transferred to a −20°C freezer, and then later freeze-dried (Alpha 1-4 LSC, Christ) at −20°C for 21 h and then −30°C for 3 h. To avoid degradation of the sample by defrosting and refreezing, frozen muscle samples were dissected in the freezer to remove dermal layers and as much connective tissue as possible. Where sample tissue masses were low, tissue was only used for SI and not FA analysis (Table 1) except for *G. glyphis*, where the opposite was true. Mean (±SD) tissue wet mass for SI was 2.0 ± 1.0 mg and for FAs was 36.0 ± 28.0 mg.

Stable isotopes

Muscle tissue was rinsed in milli-Q water and sonicated to remove excess urea as per Kim & Koch (2012). Tissues were then freeze-dried to a constant mass and pulverized using a combination of micro-scissors and micro-pestle or a coarse pestle and ceramic mortar. A subset of material was weighed to between 400 and 1000 µg in tin cups for combustion in a Sercon Europa EA-GSL elemental analyzer (Sercon Ltd), which were then analyzed with a Sercon Hydra 20-22 duel-inlet gas isotope ratio mass spectrometer at the Australian Rivers Institute, Griffith University, Queensland. The international standards used to determine the relative $\delta^{13}C$ and $\delta^{15}N$ were Peedee Belemnite Carbonate and Atmospheric Nitrogen with a precision of (±1 SD) 0.03 and 0.09‰ for $\delta^{15}N$ and $\delta^{13}C$, respectively. We did not use mathematical models to correct $\delta^{13}C$ values for lipids as lipid content in all samples was deemed to be low as inferred by the percent ratios of carbon to nitrogen (C:N; mass percent composition of tissue sample) being <3.5 (Table 2; Post et al. 2007) and by the low lipid levels inferred from total FAs (Every et al. 2016).

Trophic positions (TPs) for each species were calculated using narrowing discrimination with increasing dietary $\delta^{15}N$ values (Hussey et al. 2014a,b). This works on the concept that as TP increases, the dietary discrimination factors (the increase of $\delta^{15}N$ at each TP) decreases (Hussey et al. 2014a,b). Popeye mullet (*Rhinomugil nasutus*, $\delta^{15}N$ 6.6 ± 1.2‰, TP 2.9; Froese & Pauly 2015) was used as the baseline consumer collected at the same location and over the same time period as the elasmobranchs. Popeye mullet were chosen because they have a wide distribution throughout the river, covering a range of salinities and habitat type (Carpenter & Niem 1999). Also their $\delta^{15}N$ values were similar regardless of where they were caught within the river, negating the need to account for differing baselines. Finally, we suggest that the TP of 2.9 for *R. nasutus* is a reasonable estimate as other Mugilidae grazers—*Mugil bananensis*, *M. curema* and *Liza falcipinnis*—have TPs of 2.8, 2.7 and 3.0, respectively (Faye et al. 2011), and feed in a manner similar to that of *R. nasutus*, consuming algae and insects along the water surface and bank

Table 2. Mean (±SD) values of fatty acids (FA) (% mean of the relative abundance of FA >0.5%), $\delta^{13}C$ (‰)and $\delta^{15}N$ (‰) in muscle tissue from 7 elasmobranch species collected from the South Alligator River, Kakadu National Park, Australia. Included are calculations for trophic position (TP), total area of stable isotope convex hulls (TA, ‰^{2}), ellipse area of stable isotopes (SEA_C, ‰^{2}) and essential fatty acids (EFA) used to calculate niche space (**bold**). SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; wet:dry: wet and dry seasons

SI	*Carcharhinus amboinensis*	*Carcharhinus leucas*	*Glyphis garricki*	*Glyphis glyphis*	*Urogymnus dalyensis*	*Pristis pristis*	*Rhizoprionodon taylori*
$\delta^{13}C$	−13.4	−14.9±2.3	−18.9±1.3	−18.9±1.7	−20.2	−13.4	−15.1±1.3
$\delta^{15}N$	11.7	12.95±2.8	9.3±1.9	7.6±3.6	8.9	12.6	11.1±2.5
C:N	2.6	2.8±0.2	2.8±0.2	2.7±0.1	3.2	2.7	2.8±0.4
TP	4.2	4.8±0.9	3.6±0.4	3.2±0.8	3.4	4.5	4.1±0.6
SEA_C		18.5	6.9				10.3
(wet:dry)		(16.9:17.1)	(9.8:7.0)				(2.1:11.7)
TA		79.1	28.9				39.1
FA							
ΣSFA	42.5	31.9±8.7	30.4±8.1	27.5±7.2	30.3		43.6±12.3
ΣMUFA	24.7	29.7±5.0	22.2±3.6	18.8±2.6	26.1		20.9±3.5
ΣPUFA	26.2	30.3±2.2	38.4±3.4	44.0±4.3	34.7		30.9±3.4
18:1ω9	14.1	16.2±6.3	11.2±4.9	8.2±3.6	13.8±0.5		9.5±2.7
18:2b[a]	0.6	0.6±0.4	0.2±0.2	0.5±0.2	0.4±0.3		0.1±0.1
18:2c[a]	0.1	0.9±0.8	0.1±0.3	0.1±0.1	0.1±0.1		0.13±0.02
18:2ω6	0.6	0.8±0.9	1.8±1.1	1.8±1.1	3.8±3.7		0.9±1.0
20:2[a]	0.2	2.8±2.2	0.5±0.8	0.3±0.2	0.2±0.1		0.1±0.1
20:2ω6	0.4	0.5±0.8	0.9±0.4	0.8±0.2	0.3±0.0		0.6±0.2
20:3ω9[b]	**0.8**	**7.6±6.2**	**1.4±3.4**	**0.3±0.3**	**0.3±0.1**		**0.1±0.1**
20:3ω6	0.2	0.4±0.3	0.8±0.4	0.6±0.3	0.3±0.1		0.4±0.2
22:3[a]	1.7	1.01±0.7	1.7±1.8	2.6±2.4	2.9±3		1.1±1.2
20:4ω6	**9.6**	**5.3±5.5**	**11.3±4.7**	**14.1±4.4**	**14.4±2.4**		**8.1±2.1**
22:4ω6	**3.5**	**2.1±2.1**	**6.4±4.2**	**8.0±6.5**	**0.2±0.2**		**3.0±1.4**
20:5ω3	0.9	0.5±0.2	0.9±0.8	1.1±0.6	5.2±6.8		1.6±0.8
22:5ω3	0.9	1.9±1.2	1.5±2.0	1.6±1.8	1.1±1.6		1.1±1.7
22:5ω6	**1.8**	**1.2±0.7**	**2.3±1.1**	**2.3±1.1**	**1.2±0.8**		**2.2±0.8**
22:6ω3	**4.8**	**4.8±2.3**	**8.3±4.6**	**9.8±8.8**	**4.3±0.2**		**11.4±5.6**
Σω3/Σω6	0.4	0.70±0.4	0.5±0.6	0.4±0.8	0.5±0.0		0.9±1.4
EFA niche space		13413.7	114886.8				455.6
(wet:dry)			40732.8:64824.2				

[a]Unable to identify bonds as standard was not available at the time of analyses
[b]20:3ω9 identified based on comparison with other *C. leucas* fatty acid literature; a standard was not available at the time of analysesinaerg

edges (Faye et al. 2011). The following equation was used, with β_0 and β_1 values from the meta-analysis of Hussey et al. (2014a,b):

$$TP = \frac{\log\left(\delta^{15}N_{lim} - \delta^{15}N_{base}\right) - \log\left(\delta^{15}N_{lim} - \delta^{15}N_{TP}\right)}{k} + TP_{base} \quad (1)$$

where TP_{base} is the trophic position of 'baseline' consumer (*R. nasutus*) and $\delta^{15}N_{TP}$ is the consumer $\delta^{15}N$ value. k (rate at which $\delta^{15}N_{TP}$ approaches $\delta^{15}N_{lim}$) is calculated via the formula:

$$k = -\log\frac{\left(\beta_0 - \delta^{15}N_{lim}\right)}{-\delta^{15}N_{lim}} \quad (2)$$

where:

$$\delta^{15}N_{lim} = \frac{-\beta_0}{\beta_1} \quad (3)$$

Here, β_0 is the slope, β_1 is the intercept and k is calculated from β_0 = 5.9 [4.5, 7.3] and β_1 = −0.3 [−0.4, −0.1] (highest posterior density median [95% uncertainty intervals]) from Hussey et al. (2014a,b).

Fatty acids

Lipid was extracted quantitatively using the modified Bligh & Dyer (1959) method (detailed in Every et al. 2016), which utilizes an overnight one-phase extraction process of methanol:dichloromethane (DCM): milli-Q water (2:1:0.8 by volume). The following day, DCM and saline milli-Q water were added so that the final volume was 1:1:0.9. The lower phase was removed into a round bottom flask and solvents

were evaporated using a rotary evaporator in a bath of 40°C. Remaining lipid was transported with DCM into a pre-weighed vial, blown down with nitrogen gas and dried to a constant mass. All vials were made up to final concentration of 10 mg lipid to 1.5 ml DCM and were stored in a −20°C freezer until further analysis within a couple of days.

To liberate the FAs from the lipid backbone, the total lipid extract was transmethylated based on the methods used in Parrish et al. (2015) and Miller et al. (2006). Briefly, 50 µl of the extract was transferred to a test tube and blown down with nitrogen gas before adding 3 ml of a methylating solution (methanol, hydrochloric acid and chloroform at a ratio of 10:1:1 volume). After heating for 2 h at 100°C and cooled, 1 ml of milli-Q water was added to the test tube, and then extracted with 1.8 ml of hexane and chloroform (4:1), vortexed and centrifuged for 5 min. The upper, organic layer containing the fatty acid methyl esters (FAMEs) was placed into a clean vial. This extraction process was repeated 3 times. After adding a known amount of internal standard (C23 or C19 FAME), 0.2 µl was injected into an Agilent Technologies 7890B gas chromatograph (GC) equipped with an Equity-1 fused silica capillary column (15 × 60.1 mm internal diameter and 0.1 µm film thickness), a flame ionization detector, a splitless injector and an autosampler. Peaks were quantified using Agilent Technologies ChemStation software. Confirmation of peak identifications was by GC-mass spectrometry (GC-MS), using a column of similar polarity to the Equity-1 fused silica capillary column and a Finnigan Thermoquest DSQ GC-MS system.

Statistical analysis

ANOVA was used to determine differences in stable isotopes among the fixed factors of species and seasons. Due to a significant relationship between $\delta^{13}C$ and total length (TL) in *C. leucas* (which was not evident in the other taxa), an analysis of covariance (ANCOVA) was run with TL as a covariate to account for this species. Assumptions of normality, variance, homogeneity of slopes and colinearity were tested through visual inspection of boxplots, calculating leverage, residuals and Cook's *D*, and graphed in a residual versus fitted, normal Q-Q and residual versus fitted values plots prior to accepting the models. No transformations were necessary.

FAs were calculated as a percentage of total FAs, and those with group means of <0.5% were excluded from further analyses. To evaluate interspecific differences in FA profiles among species, a permutational multivariate ANOVA (PERMANOVA) was applied to a Euclidean distance matrix comprising the FAs (variables) for all elasmobranch samples, using Type III sum of squares (partial) and a minimum of 9900 unique permutations. To determine which FAs contributed most to the observed interspecific differences, an analysis of similarity (SIMPER) was conducted. To explore FA profiles between the species and season, a principal coordinates analysis (PCA) was constructed and arranged into a difference matrix based on Euclidean distances. PERMANOVA analysis was used to explore the significance of seasonal effects in *G. garricki* (the only species with sufficient seasonal replication; Table 1). ANOVA was then used to explore interspecific differences in ω3/ω6 between *G. garricki, G. glyphis, C. leucas* and *R. taylori*. As significant effects were found, we then used an ANCOVA with TL as a covariate between each shark's ω3/ω6 ratio to consider possible allometric effects. 18:2ω6 (LA) was also explored for interspecific differences to determine which species of shark may have the most terrestrial input, which is suggestive of floodplain resources (Napolitano et al. 1997).

Univariate analyses were performed in R using the R core packages (R Development Core Team 2014), SIBER (Jackson et al. 2011) and one FA multivariate analysis used the package NicheROVER to determine overlap between sharks (Swanson et al. 2015). All other multivariate analyses were performed in PRIMER (v.6) and PERMANOVA$^+$ (v.1) (Clarke & Gorley 2006). All p-values 0.05 and below were considered significant.

Niche area calculations

Using the R package Stable Isotopes Analysis in R (SIAR), a SI Bayesian ellipse model was calculated and the SI ellipse area (‰^{2}) was corrected for sample size (SEA_C) (Jackson et al. 2011). SEA_C was calculated from the covariance matrix determined via Bayesian inference. Another metric of isotopic niche areas, the convex hull total area (‰^{2}) (TA) was also calculated (Layman et al. 2007a, Jackson et al. 2011); however, this was not compared across seasons due to low sample numbers. Although TA in a $\delta^{15}N$–$\delta^{13}C$ biplot is strongly affected by sample size (Jackson et al. 2011, Syväranta et al. 2013), it was calculated to explore total niche width as a proxy for the extent of trophic diversity within a food web, presence of outliers, and a conservative estimate of maximum poten-

tial overlap among species (Layman et al. 2012, Heithaus et al. 2013). Drawing SEA_C in isotopic space ($\delta^{13}C$ and $\delta^{15}N$) that incorporates ~40% of the sampled individuals is thought to provide a more accurate measure of niche width than TA, and is less biased by sample size (Jackson et al. 2011, Syväranta et al. 2013). Ellipse confidence intervals for the area of the ellipses were calculated from Bayesian likelihoods, whereby the probability of one ellipse being bigger than the other can be determined by the uncertainty in the probable values. Overlaps of SEA_C were calculated by finding the area of species 'a' ellipse and the area of the overlap of species 'b' with species 'a' and then converting to a percentage. This could only be performed for the 3 species *C. leucas*, *G. garricki* and *R. taylori*, for which total n-values for samples over both seasons were >30, which is considered optimum for these analyses (Syväranta et al. 2013). It should be noted that samples were not even across seasons, with wet:dry sampling for *C. leucas* and *R. taylori* being 28:6 and 8:30, respectively. As such, the *C. leucas* dry season ellipse may be slightly biased due to their small n-value (8 is considered the minimum; Jackson et al. 2011), yet it was included to provide a descriptive comparison. *Glyphis garricki* seasonal samples were relatively even, at 22:20 wet:dry. Ellipses were also calculated for the combined sampling period to ensure that these results were not overly biased.

FA niche space and overlap was explored by calculating hyper-ellipses, using a multivariate extension on the bivariate approach of Jackson et al. (2011) described above. Here, Bayesian priors are used to determine the hyper-volume and the probability of finding one species in another species' niche space (Swanson et al. 2015). To avoid degenerate covariance matrices in the absence of prior information, the number of niche dimensions fitted from the dependent variables should be lower than the number of sample observations (Swanson et al. 2015). Therefore, only the 5 most abundant EFAs were selected (20:4ω6, 20:3ω9, 22:5ω6, 22:6ω3 and 22:4ω6), given the sample numbers (~20) for each of the 3 shark species analyzed (*G. garricki*, *C. leucas*, *R. taylori*). Each EFA was then represented as an axis, calculated in FA hyperspace and projected onto a 2-dimensional plot as a probabilistic projection to display FA niche space. Niche space was calculated based on the volume of ellipses, under the assumption that they were multivariate normal. This differs from niche area calculations for SI, as they are not multi-dimensional and area is calculated from 2 dimensions. Percentage overlap was calculated based on the probability that one species would be found in the niche of another species.

Throughout this article, we refer to niche metric as the measurement of the 2-dimensional bivariate aspect of niche, niche area (as for SI) and the multivariate niche space (as for FAs).

RESULTS

Stable isotope values, trophic position estimates, and niche metrics

Muscle $\delta^{13}C$ and $\delta^{15}N$ values in the 7 elasmobranch species revealed 2 distinct trophic guilds (Fig. 2). The highest values of both elements were in *Carcharhinus leucas*, *Rhizoprionodon taylori*, *Carcharhinus amboinensis* and *Pristis pristis* (Table 2, Fig. 2). The elasmobranch guild with the lowest mean $\delta^{13}C$ and $\delta^{15}N$ values included *Glyphis glyphis*, *Urogymnus dalyensis* and *Glyphis garricki* (Table 2, Fig. 2). Taxa

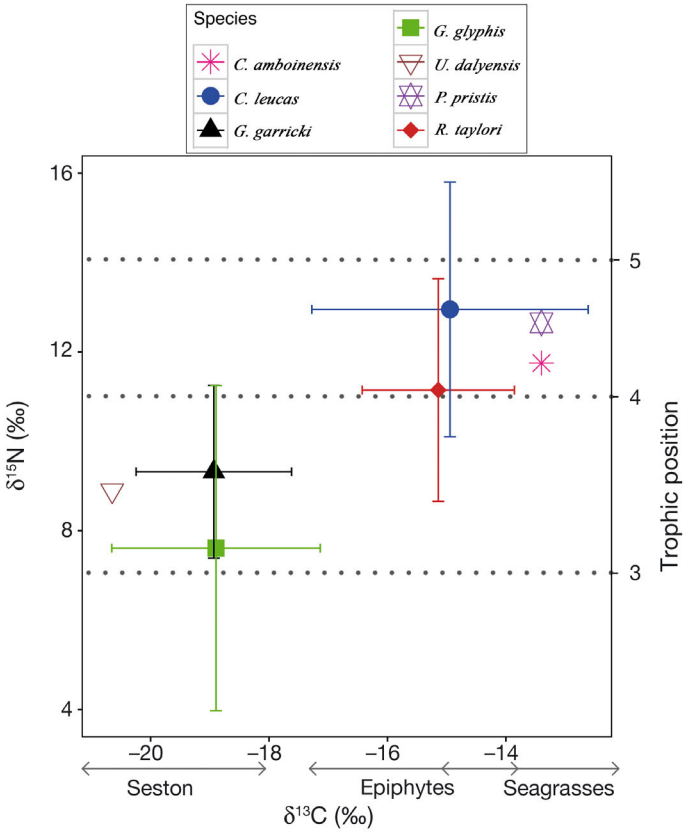

Fig. 2. Biplot of mean (±SD) $\delta^{13}C$ and $\delta^{15}N$ values for 7 species of euryhaline and coastal elasmobranchs from South Alligator River, Kakadu National Park, Australia. Primary producer values along the *x*-axis are $\delta^{13}C$ ranges for seston, epiphytes and seagrasses from the combined wet and dry season, Embley River Estuary, Gulf of Carpentaria (Loneragan et al. 1997)

had a significant effect on $\delta^{15}N$ values ($p < 0.01$, $F_{1,115} = 16.6$, $R^2 = 0.3$) and $\delta^{13}C$ values ($p < 0.01$, $F_{1,115} = 50.7$, $R^2 = 0.6$). The post hoc test for $\delta^{15}N$ showed no significant differences between *R. taylori* and *C. leucas*, or between *G. garricki* and *G. glyphis*, whereas all others species differed significantly. For $\delta^{13}C$ values, only *G. garricki* and *G. glyphis* were not significantly different from each other (Table 3).

The relationship between isotope values and TL of sharks were compared and were not significant except for $\delta^{13}C$ values in *C. leucas*, which had a negative relationship with TL ($p < 0.01$, $F_{1,30} = 10.3$, $R^2 = 0.3$). There was a significant relationship between the C:N ratio and $\delta^{13}C$ ($p < 0.01$, $F_{1,117} = 14.8.7$, $R^2 = 0.1$). Seasonal effects for $\delta^{13}C$ values were not found in *C. leucas*, as the inclusion of TL as a covariate negated the seasonal effect and revealed TL to be the only significant variable (Table 3). Based on $\delta^{15}N$ values, the calculated TP ranged from 3.2 ± 0.8 to 4.8 ± 0.9 and was lowest in *G. glyphis* and highest in *C. leucas* (Table 2).

Of the 3 species for which convex hull TA was calculated, *C. leucas* was the largest followed by *R. taylori* and *G. garricki*. *Carcharhinus leucas* moderately overlapped with *R. taylori*, although some individuals almost fell into the isotopic niche area of *G. garricki*. *Glyphis garricki* had the least overlap with other species: only the outliers of *R. taylori* and *C. leucas* shared their TA.

SEA_C of stable isotopes ranged from 6.86 to 18.46‰^{2}, with confidence intervals ranging from 97 to 99%. *Carcharhinus leucas* had the largest SEA_C, followed by *R. taylori* and *G. garricki* (Table 4, Fig. S1 in the Supplement at www-int-res.com/articles/suppl/m565p181_supp.pdf). When both seasons were combined, the SEA_C of *C. leucas* and *R. taylori* overlapped and were clearly separated from *G. garricki* (Table 4, Fig. S1). The wet season SEA_C of *R. taylori* was almost completely within the wet season ellipse of *C. leucas* and over the dry season niche space increased in both species, particularly in *R. taylori* (Table 4, Fig. 3). Seasonal differences in niche metrics (Table 2, Fig. 3) and overlap (Fig. 2, Table 4) of SEA_C were found in each of the 3 species, with the least change occurring in *G. garricki*. The SEA_C of *C. leucas* in the dry season overlapped with that of

Table 3. ANOVA and ANCOVA (shaded) of stable isotope values, the ratio of ω3/ω6 and the fatty acid 18:2ω6 from the muscle of *Carcharhinus leucas*, *Glyphis garricki*, *G. glyphis* and *Rhizoprionodon taylori* from the South Alligator River, Kakadu National Park, Australia, compared with the 'wet' and 'dry' season, with total length (TL) as the covariate. Significant values are in **bold**

Species	Test	Variable(s)	Tracer	df	*F*	p	Residual standard error	R^2	*t*-value	Significant Tukey post hoc comparisons ($p < 0.05$)
All	ANOVA	Species	$\delta^{15}N$	115	16.6	**<0.01**	2.5	0.3		*G. garricki – C. leucas* *G. glyphis – C. leucas* *R. taylori – G. garricki* *R. taylori – G. glyphis*
			$\delta^{13}C$	115	50.7	**<0.01**	1.7	0.6		*G. garricki – C. leucas* *G. glyphis – C. leucas* *R. taylori – G. garricki* *R. taylori – C. leucas* *R. taylori – G. glyphis*
			ω3/ω6	3, 76	7.2	**<0.01**	0.6	0.2		*G. garricki – C. leucas* *G. glyphis – C. leucas*
			18:2ω6	3, 76	6.4	**<0.01**	1	0.2		*G. garricki – C. leucas* *R. taylori – G. garricki*
C. leucas	ANOVA	Season	$\delta^{15}N$ $\delta^{13}C$	30 30	0.8 5.4	0.4 **0.03**	2.6 1.9	0 0.2		
	ANCOVA	Season + TL Season TL	$\delta^{13}C$ $\delta^{13}C$ $\delta^{13}C$	29	5.1	**0.01** 0.7 **0.05**	1.8	0.3	 0.4 −2.1	
G. garricki	ANOVA	Season	$\delta^{15}N$ $\delta^{13}C$	40 40	0 1.3	0.9 0.2	1.9 1.3	0 0		
R. taylori		Season	$\delta^{15}N$ $\delta^{13}C$	36 36	1.4 1.7	0.2 0.2	2.5 1.3	0 0		

Table 4. Isotopic overlap (%) of stable isotopes (SI) ellipses (SEA_C) and convex hull total area (TA) of *Carcharhinus leucas*, *Glyphis garricki* and *Rhizoprionodon taylori* from the South Alligator River, Kakadu National Park, Australia. Also included are the probabilities of sharks being in the major essential fatty acids (EFA) niche space of each other with a set confidence interval (CI) of 95%. –: no test completed

Biochemical method:	SI			SI	EFA
Niche metric:	% of species *a* among species *b* ellipse (SEA_C)			% Overlap TA	% Overlap probability[a]
Season:	Both	Dry	Wet	Both	Both
Species comparisons (*a* × *b*)					
C. leucas × *G. garricki*	0	27.4	0	22.5	61.7
C. leucas × *R. taylori*	36.9	27.6	12.2	37.0	0.4
G. garricki × *R. taylori*	0	0	0	8.4	0.6
G. garricki × *C. leucas*	0	66.5	0	–	13.2
R. taylori × *C. leucas*	66.1	40.2	99.8	–	11.2
R. taylori × *G. garricki*	0	0	0	–	79.9
Season comparisons					
G. garricki Dry × Wet	94.5	–	50.3		
G. garricki Wet × Dry	67.8	–	35.4		
C. leucas Dry × Wet	36.3	–	–		
C. leucas Wet × Dry	36.6	–	–		
R. taylori Dry × Wet	79.0	–	–		
R. taylori Wet × Dry	14.1	–	–		

[a]of one species being in another's niche space

G. garricki. However, there was no overlap between *C. leucas* and *G. garricki* when all samples were combined (Fig. 3, Table 4).

Fatty acid biomarkers and niche metrics

Sixty-five FAs were identified in the elasmobranch muscle tissue, of which 31 were detected with mean values above 0.5% for any one species (Table 2, Table S1 in the Supplement). Relative abundance of saturated FAs (SFAs) were similar in *C. leucas*, *G. garricki*, *G. glyphis* and *U. dalyensis*, ranging from 27.5 ± 7.2 to 32.0 ± 8.7%, but were higher in *R. taylori* and *C. amboinensis* (43.6 ± 12.3% and 42.5, respectively). The monounsaturated FAs (MUFAs) were relatively low in *G. glyphis*, *G. garricki* and *R. taylori* (means ranging from 18.8 to 22.2%) compared with the other elasmobranchs, whose mean relative abundance ranged from 24.8 to 29.7%. Relative amounts of polyunsaturated FAs (PUFAs) were highly variable between species (means ranging from 26.2 to 44.0%), with the lowest in *C. amboinensis* and highest in the *Glyphis* species. PUFAs were similar between *C. leucas* and *R. taylori* (30.3 ± 2.2% and 30.9 ± 3.4%, respectively) and were dominated by 3 EFAs: 20:4ω6 (ARA), 22:6ω3 (DHA) and 22:4ω6 (adrenic acid).

There were significant differences in FA profiles among species (PERMANOVA: $F_3 = 9.2$, $p < 0.01$) and there was no effect of season on the FA profile of *G. garricki* ($F_1 = 0.7$, $p = 0.60$). Elasmobranch FAs showed some grouping between and within species, such as in *R. taylori* and *G. garricki* (Fig. 4). The greatest overlap was between *G. glyphis* and *G. garricki* and a few individuals of *C. leucas*. The least overlap was in *R. taylori*, separated by the SFAs 17:0, 18:0 and 16:0. However, there were also a few dry season *R. taylori* outliers near *G. glyphis* and *U. dalyensis*. A large subgroup of *C. leucas* (and some individuals of *G. garricki*) were also separated from the other species by the FAs 18:2c, 20:3ω9, 18:2b, 18:1ω9 and 20:2, and notably these sharks did not appear to have any unique differences such as those related to size or sex. In contrast, the *Glyphis* spp. were largely separated by the EFAs 20:3ω6, 22:6ω3, 22:4ω6, 22:5ω6 (ω6 DPA) and 20:4ω6 (ARA). There was a shift in posterior means from the dry to the wet season from ~35 to 50%, which may suggest an increase in niche space during the wet season (Fig. 5, Fig. S2B in the Supplement).

The highest mean ratio of ω3/ω6 was in *R. taylori* and the lowest was in *G. glyphis*, ranging from 0.9 ± 1.4 to 0.4 ± 0.8 (Table 2). An ANOVA using shark species (consisting of *C. leucas*, *G. garricki*, *G. glyphis* and *R. taylori*) and ω3/ω6 as factors revealed significant interspecific differences ($p = 0.01$, $F_{3,76} = 7.2$, $R^2 = 0.2$), whilst post hoc tests found significant differences between *G. garricki* and *C. leucas*, and *G. glyphis* and *C. leucas*, but not between *R. taylori* and the other sharks (i.e. *G. garricki*, *G. glyphis* and *C. leucas*). TL had a positive significant effect on ω3/ω6 ratios in *C. leucas* ($p = 0.05$, $F_{1,21} = 4.2$, $R^2 = 0.18$) and *G. garricki* ($p = 0.02$, $F_{1,23} = 6.2$, $R^2 = 0.9$) but not in *R. taylori* or *G. glyphis*. A significant difference was also found between the elasmobranches using the terrestrial marker 18:2ω6 ($p < 0.01$, $F_{3,76} = 6.4$, $R^2 = 0.2$), and post hoc tests found that *G. garricki* and *C. leucas*, and *G. garricki* and *R. taylori* (Table 3), were the only pairs that had significantly different means.

The largest FA niche space occurred in *G. garricki*, followed by *C. leucas* and *R. taylori* (Table 2, Figs. 5 & 6) across all sites and seasons. The

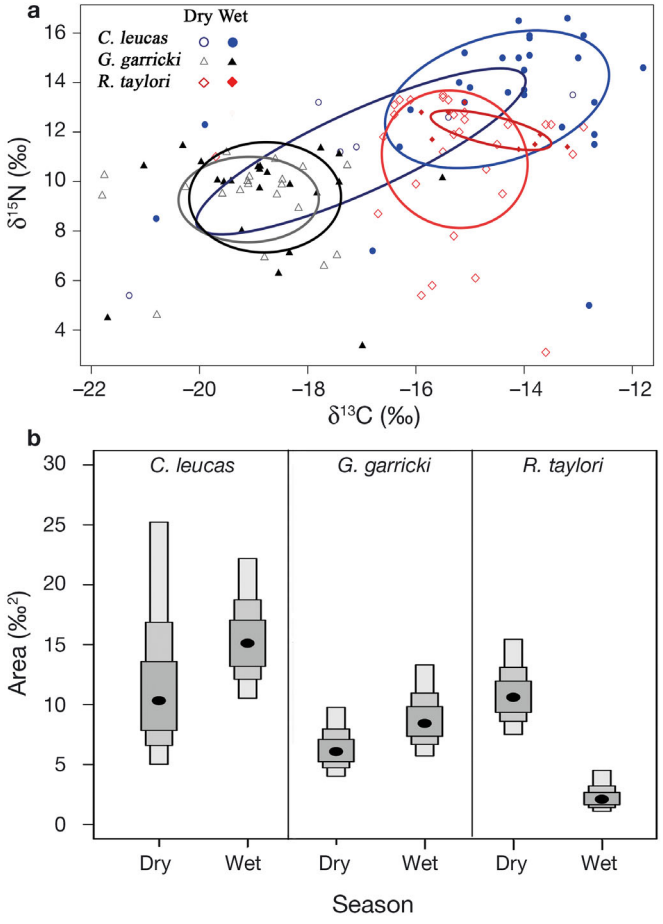

Fig. 3. (a) Bivariate plot of isotopic space depicting wet versus dry season niche areas within standard ellipse (SEA_C) of $\delta^{13}C$ and $\delta^{15}N$ values of *Carcharhinus leucas* (blue), *Glyphis garricki* (black) and *Rhizoprionodon taylori* (red) from Kakadu National Park, Australia. (b) Bayesian confidence intervals of isotopic niche area; black dots represent their mode, shaded boxes represent the 50, 75 and 95% credible intervals from dark to light grey

chance of *G. garricki* being in another species' FA niche space was low (0.6% for *R. taylori* and 13.2% for *C. leucas*) yet *C. leucas* and *R. taylori* had larger probabilities for overlapping with *G. garricki* FA niche space (79.9% for *R. taylori* and 61.7% for *C. leucas*). Slight seasonal differences were evident in *G. garricki* FA niche space, with a 15% probability of overlap between the wet and dry seasons (Fig. 5).

Trophic niche metric comparisons

Differences in niche metrics, including inter-specific overlap, were apparent between the 2 SI niche metrics (SEA_C and TA) and between both these and EFA niche spaces. Between the 2 SI niche metrics, TA consistently gave larger area values and showed greater percentage overlap between species compared with SEA_C. Despite this, the ordering of species niche areas was similar, being highest in *C. leucas* followed by *R. taylori*, and *G. garricki* (Table 2). In contrast, FA niche spaces were highest in *G. garricki*, followed by *C. leucas* and *R. taylori* (Table 2). When comparing percent overlap in SEA_C and EFA niche metrics, differences in model output were observed (Table 4). The greatest differences between methods were observed between *R. taylori* and *G. garricki*, where there was a large chance of overlap in EFA niche space but not in SEA_C. *Carcharhinus leucas* and *R. taylori* overlap was also different, with less chance of overlap for SI than EFA niche metrics (Table 4). Seasonal differences between *G. garricki* SI and FA niche metrics were also apparent, with greater overlap in SI SEA_C (67.8 to 94.5%) and FA niche space (35.4 to 50.3%).

DISCUSSION

The complementary analyses of SI and FA niche metrics in the present study revealed significant differences in the trophic resource use of tropical elasmobranchs, with indications of resource partitioning among 3 species (and limited evidence in another 4 species) occupying the South Alligator River and adjacent coastal waters. Although SI niche metrics indicated clear separation between coastal/marine and euryhaline elasmobranchs, the difference was less pronounced based on FA niche metrics alone. This is likely due to the faster turnover of FAs (Kirsch et al. 1998, Beckmann et al. 2014) compared with SI (Logan & Lutcavage 2010), differences in the statistical methods employed, and the fact that both tracers provide different representations of an organism's niche. Among a community of organisms, FA niche space reflects the trophic transfer of marker FAs from basal sources to predators, whilst SI niche areas represent both an organism's trophic position and its basal carbon source. Not surprisingly, differences between these biochemical niche metrics and more traditional niche metrics based on taxonomic descriptions of diet composition have also been observed through recent efforts to compare SI, TA and the omnivory index derived from the Ecopath ecosystem modeling framework (Layman et al. 2007a, Navarro et al. 2011). Despite these differences, each method gives complementary understanding of ecological interactions and habitat use.

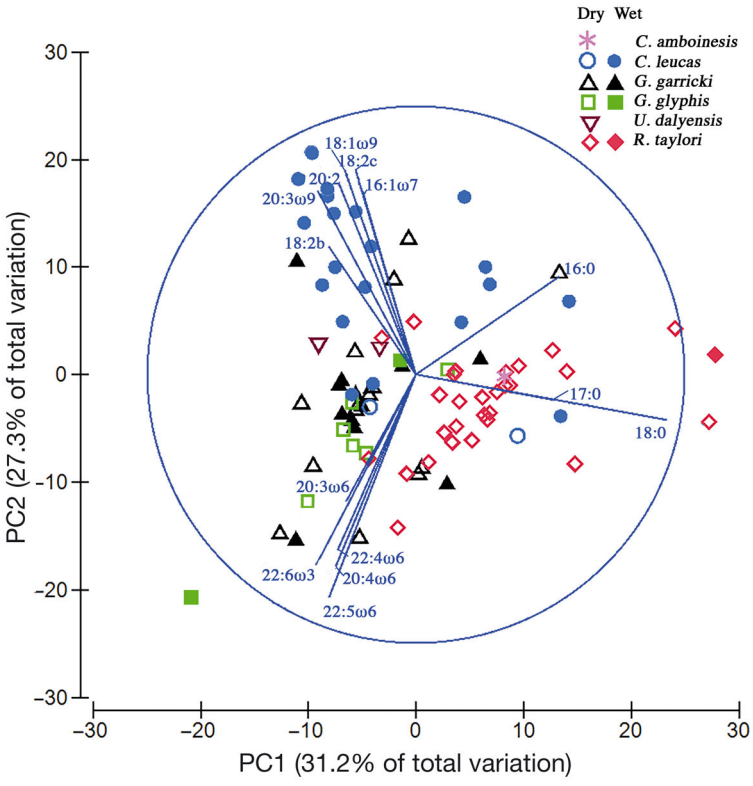

Fig. 4. Principal coordinates analysis of fatty acids in muscle tissues of *Carcharhinus amboinensis*, *Carcharhinus leucas*, *Glyphis garricki*, *Glyphis glyphis*, *Urogymnus dalyensis* and *Rhizoprionodon taylori* in both wet and dry seasons from the South Alligator River, Kakadu National Park, Australia

In this study, interspecific differences in the SI niche area of elasmobranchs appear to be largely driven by $\delta^{13}C$, indicating that the feeding locations of each species are variable or that mobile prey species are being consumed by specific elasmobranchs. Several species (*Rhizoprionodon taylori*, *Carcharhinus amboinensis*, *C. leucas* and *Pristis pristis*) were characterized by values that are typically reported in marine environments (−14 to −16‰; Hussey et al. 2011, Munroe et al. 2015). $\delta^{13}C$ values for *P. pristis* and *C. amboinensis* were similar to those from previous studies (Thorburn et al. 2014, Tillett et al. 2014), which may have been sourced from seagrass, whilst *C. leucas* values were between both marine epiphytes and seagrass, and *R. taylori* had values closer to epiphytes (Loneragan et al. 1997). In contrast, *Glyphis garricki*, *G. glyphis* and *Urogymnus dalyensis* had lower $\delta^{13}C$ values, suggestive of estuarine/freshwater seston signatures (−18.8 to −23.2‰; Loneragan et al. 1997).

In terms of TP, the 7 elasmobranch species were broadly similar, based on the spread of $\delta^{15}N$ values, with *C. leucas* having the highest values and mean TP (4.8 ± 0.9). This result conforms with *C. leucas* stomach contents analyzed in northern Australia, which mainly consisted of teleost fish and occasional larger predators such as *P. pristis* and the freshwater crocodile *Crocodylus porosus* (Thorburn & Rowland 2008). Although our analysis used juveniles, our TP estimates were higher than adult TP (4.6 ± 0.2) and sub-adult TP (4.4 ± 0.3) values for *C. leucas* reported in Mozambique (Daly et al. 2013) and Western Australia TP (~4.4) (Thorburn et al. 2014), and in the same range (~3.8 to 5.4) as those from South Africa (Hussey et al. 2014a). This variation is likely due to the different statistical methods of determining TP as well as dietary and environmental variation (Peterson & Fry 1987). There is currently no literature reporting TP estimates for *Glyphis* spp. and *U. dalyensis*, and this is only the second report of TP for *P. pristis*, which was in the same range (3.6 to 4.7) as Thorburn et al. (2014). The low TP calculated in this study for *G. glyphis* (3.2 ± 0.8) suggests that they consume lower order prey and that their TP was more similar to rays than sharks of this body size (Hussey et al. 2014a). The TPs in *R. taylori* were in the same range as those of specimens caught in Cleveland Bay, Queensland, but were higher than those caught in nearby bays (Munroe et al. 2014a). This could be attributed to differences in prey TP, environmental conditions or baseline $\delta^{15}N$ values, which are all closely coupled.

Based on the analysis of elasmobranch FA profiles and biomarkers, clear interspecific differences were apparent which mostly supported the 2 guilds found from SIs. All species had marked variation in their individual FAs, which was similar to other northern Australian tropical sharks (Nichols et al. 2001) and *C. leucas* (Belicka et al. 2012). The *Glyphis* spp. grouped together and had high levels of ω6 FA biomarkers and 18:2ω6 (LA), which are all characteristic of terrestrial and freshwater sources (Napolitano et al. 1997, Budge & Parrish 1998). In contrast, *R. taylori* had higher relative levels of the EFAs 20:5ω3 (EPA) and 22:6ω3 (DHA), which are characteristic of marine food webs based on diatoms and flagellates, respectively (Parrish 2013). Higher relative levels of the FA biomarker 18:1ω9, linked to piscivory (Dalsgaard et al. 2003, Kelly & Scheibling 2012), was reported for *C. leucas*, which supports the high TP estimates based on $\delta^{15}N$ values. However, the complete FA

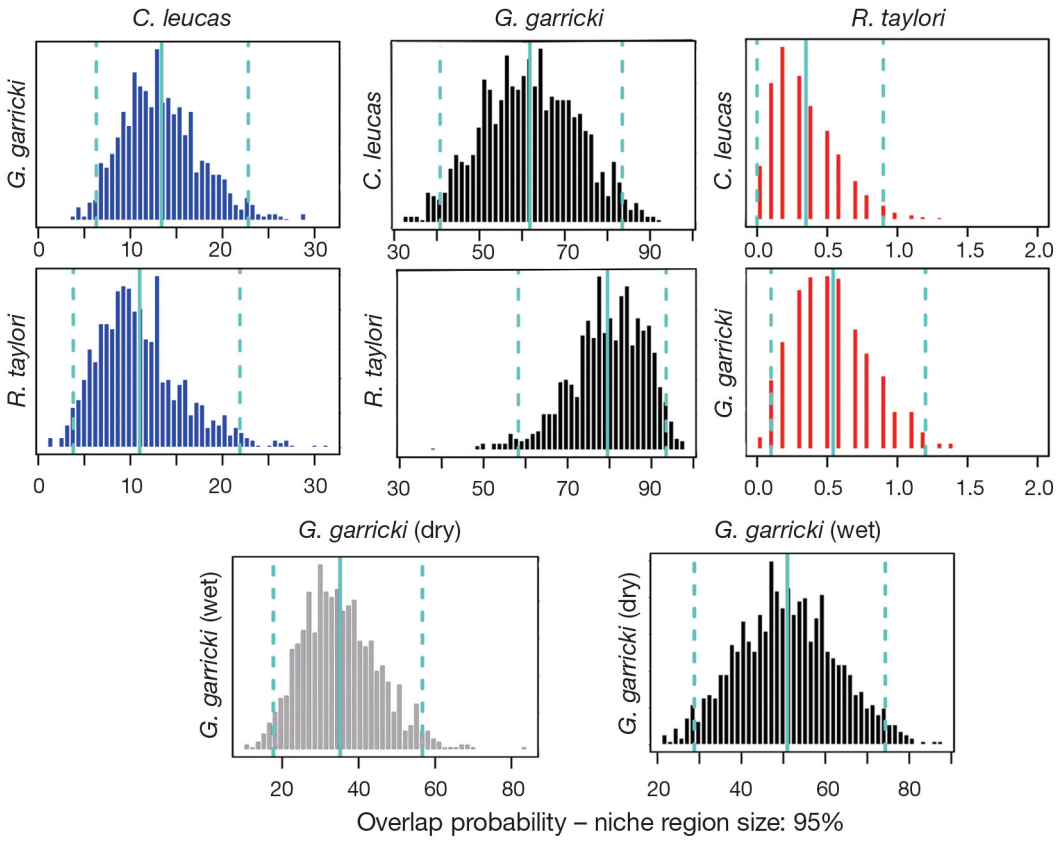

Fig. 5. Comparisons of the posterior distributions of the probabilistic niche overlap metrics of 5 major essential fatty acids (EFAs) between 3 shark species: *Carcharhinus leucas* (blue), *Glyphis garricki* (black) and *Rhizoprionodon taylori* (red) from the South Alligator River, Kakadu National Park, Australia. Also shown on the bottom row is seasonal overlap for *G. garricki* (wet season = black, dry season = grey). Niche overlap is characterized by the probability that an individual from one shark species (or season) is found within the niche region of another shark species (or season). Posterior means and 95% credible intervals are displayed in light blue

profile of *C. leucas*, which consisted of high relative levels of 18 PUFA, 16:1ω7 and low 22:6ω3 (DHA), suggests a greater intake of estuarine than marine prey, contrary to the predominantly marine-based SI signatures. This suggests that *C. leucas* are utilizing food webs across the marine to freshwater spectrum, which was previously found in this species (Matich & Heithaus 2014). *Glyphis* spp. also had links to marine sources, indicated by the moderate proportion of 22:6ω3 (DHA) and a higher amount of 20:4ω6 (ARA), which implies links between benthic marine and estuarine/coastal prey (Hall et al. 2006, McMeans et al. 2013).

The FA biomarker ω3/ω6 can help further define a species' niche and their basal sources, as higher ratios (>~1.5) indicate a preference for marine resources whilst low ratios (<~1.5) suggest a dependence on freshwater resources (Martínez-Álvarez et al. 2005, Özogul et al. 2007). The elasmobranchs in the present study appeared to all have a freshwater

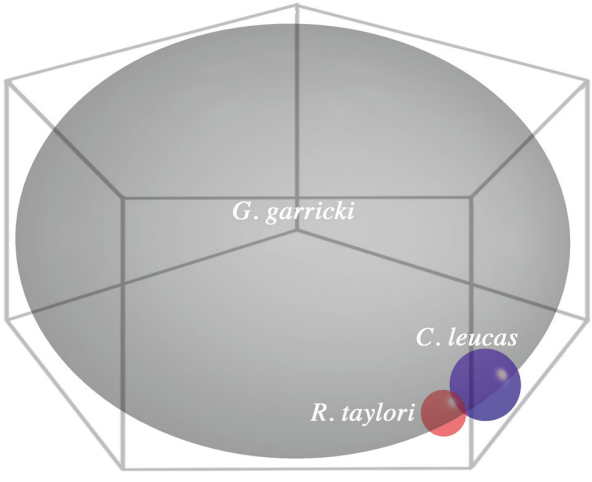

Fig. 6. Schematic of the essential fatty niche space and extent of overlap among 3 shark species, *Carcharhinus leucas* (blue), *Glyphis garricki* (grey) and *Rhizoprionodon taylori* (red), in the South Alligator River, Kakadu National Park, Australia. Niche spaces are scaled relative to each other; central axis omitted

influence based on the above range; however, there was a difference between species, in that *R. taylori* (a marine species) had the highest ratio (0.9), whilst *G. garricki* (0.5) and *G. glyphis* (0.4) collected upstream tended to be lower. The ω3/ω6 ratio in *C. leucas* and *G. garricki* were significantly different, which may reflect a preference for feeding in differing salinities, especially since *C. leucas* had $\delta^{13}C$ values that were more indicative of marine sources (although caught in similar areas). The ω3/ω6 ratio may also be related to condition, growth and maturation in elasmobranchs, as a significant (positive) relationship was found between TL and the ratio in *C. leucas* and *G. garricki*, which were mostly juveniles. This relationship may reflect physiological changes that occur during sexual maturation, such as that found in juvenile teleost fish, where the ω3/ω6 ratios decreased during sexual maturation (Uysal & Aksoylar 2005), or even changes in the way they use the river during ontogeny.

The niche metrics of elasmobranch species in this study suggest that there is a broad prey base in the South Alligator River. The largest SI niche area was observed for *C. leucas*, a species reported to consume a broad range of taxa including catfish (ariids), rays (batoids) and other carcharhinid sharks (Snelson et al. 1984, Tillett et al. 2014). However, the FA niche space for *C. leucas* was intermediate between that of *G. garricki* (being very large) and *R. taylori* (very small), which suggests individual dietary specializations in *C. leucas* (Matich et al. 2011), prey switching according to the local abundance of prey (Matich & Heithaus 2014) and/or that there is a maternal influence (Olin et al. 2011, and see below). In contrast, *R. taylori* exhibited an intermediate SI niche area, and a much smaller FA niche space, which is somewhat at odds with the relatively broad diet (e.g. prawns and teleost fishes) identified in stomach contents (Simpfendorfer 1998). Interestingly, some *R. taylori* in Queensland occupied areas near the river outflows during the wet season rather than moving to seagrass beds with the majority of the population (Munroe et al. 2014b). In light of these findings, it may be that the narrow niche of *R. taylori* in the South Alligator estuary results from some individuals of this species targeting prey around riverine outflows. Collectively, these trophic niches suggest some important considerations for conservation and management of these species. For example, the strong overlap between *R. taylori* and *C. leucas* and the relatively narrower trophic niche of *R. taylori* suggests that this species may be in competition with the more abundant *C. leucas* and vulnerable to disturbances (Layman et al. 2007b). Similarly, the greater dependence of *G. garricki* to riverine resources compared with the other elasmobranchs highlights the importance of this habitat to *G. garricki* in order for them to forage, grow and maintain healthy populations.

Temporal changes in trophic niche size or overlap among populations are likely in environments that are highly dynamic. Seasonal differences in biota are relatively common in tropical river systems due to the large outflow of freshwater during the wet season, changing the sources of energy and nutrients, and increasing primary and secondary production (Winemiller & Jepsen 1998). Our results may indicate seasonal changes in the niche metrics of all species tested, but not in the individual SI or FA biomarkers. This may be because the ellipses are highlighting subtle population differences as they compare the posterior mean values of the 2 isotopes and the covariance matrix of each species, while PERMANOVA and ANOVA compare individual treatment means. The largest seasonal differences in niche metrics were for SI areas for *R. taylori*, which were much smaller in the wet than the dry season. This is likely to reflect a preference for a particular food type that is more prevalent in the wet season whereas in the dry season they may need to source a variety of food to meet their nutritional requirements. For both *C. leucas* and *G. garricki*, larger SI areas were observed in the wet than the dry season, reflecting a greater breadth of available food and habitat, although the large overlap between niche areas suggests that they are consuming similar prey type over both seasons. This was further supported by *G. garricki* having the larger wet season EFA niche space.

Observed differences between SI and FA niche metrics, particularly within *C. leucas*, may also be a result of maternal influences, driven by temporal differences in muscle tissue turnover rates between the 2 biochemical tracers. The significant correlation between TL and *C. leucas* $\delta^{13}C$ values suggests that these sharks rely on different food sources as they grow. Maternal signatures have been found in *C. leucas* in both $\delta^{13}C$ and the FA 20:3ω9, which was used as a biomarker for EFA deficiency (Matich et al. 2010, Olin et al. 2011, Belicka et al. 2012), and very weakly in $\delta^{15}N$ (Matich et al. 2010). The lack of maternal signature in isotopes within our sharks may be attributed to *C. leucas* feeding at a similar TP to their mothers, to their size range being too small to find a size-related difference or to differences caused from the baseline consumer. Tissues in *C. leucas* have shown a decline in isotopic values until they reach 110 cm TL (Matich et al. 2010), whereas our TL

range was from 72 to 93 cm and open umbilical scars were found on ~50% of individuals. The 2 juvenile *P. pristis* may also have had some maternal influence as they shared higher SI values.

Differences detected between the biochemical niche metrics are also associated with the statistical methods used. FA niche space is a probability projection rather than geometric (Swanson et al. 2015), and thus calculates the likelihood of one species being in the other's niche space. In contrast, SEA_C is reliant on the confidence interval set for the ellipse and only one measurement of overlap is calculated. For example, *R. taylori* had a high probability of being in *G. garricki*'s FA niche space, but there was no SEA_C overlap between these species. In determining FA niche space, only 5 EFAs could be used, which meant there was an underlying assumption that these FAs were the only FAs within the species that were important for differentiating niches. A more complete understanding of trophic niche based on FA profiles could be achieved with larger sample sizes. Better understanding of FA trophic markers in tropical watersheds would increase our ability to distinguish food sources within and among rivers, estuaries and coastal ecosystems. Likewise, understanding how biomarkers respond to environmental cues, and profiling potential prey items, would greatly increase our ecological knowledge of species resource use and trophic interactions in coastal and estuarine ecosystems.

This study identified 2 separate feeding guilds and was the first to use SI and FA analysis in combination to measure trophic niche metrics and explore resource partitioning among an assemblage of elasmobranchs. Differences in FA and SI niche metrics highlighted the advantages of combining such analyses. Our approach has demonstrated that elasmobranchs within the South Alligator River display partitioning in trophic resource use, particularly across marine and freshwater food webs. In particular, some species (*C. leucas* and *G. garricki*) are utilizing food webs across the marine to freshwater spectrum, which suggests that these fish provide broad and important cross-biome trophic linkages in tropical coastal ecosystems. In contrast, other species (*R. taylori*) have a predominantly marine-based niche that is relatively narrow but still provides limited connections through to the estuary. Given the potential for complexity in resource use, also highlighted by seasonal shifts in SI and FA niche metrics, it appears likely these elasmobranchs play important roles in food web connectivity in tropical aquatic ecosystems.

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LITERATURE CITED

Australian Government Bureau of Meteorology (BOM) (2016) Daily rainfall (Table). www.bom.gov.au/climate/data/

Beckmann CL, Mitchell JG, Stone DAJ, Huveneers C (2014) Inter-tissue differences in fatty acid incorporation as a result of dietary oil manipulation in Port Jackson sharks (*Heterodontus portusjacksoni*). Lipids 49:577–590

Belicka LL, Matich P, Jaffé R, Heithaus MR (2012) Fatty acids and stable isotopes as indicators of early-life feeding and potential maternal resource dependency in the bull shark *Carcharhinus leucas*. Mar Ecol Prog Ser 455: 245–256

Bligh EG, Dyer WJ (1959) A rapid method of total lipid extraction and purification. Can J Biochem Physiol 37: 911–917

Budge SM, Parrish C (1998) Lipid biogeochemistry of plankton, settling matter and sediments in Trinity Bay, Newfoundland. II. Fatty acids. Org Geochem 29:1547–1559

Carpenter KE, Niem VH (eds) (1999) FAO species identification guide for fishery purposes. The living marine resources of the Western Central Pacific, Vol 4. Bony fishes part 2 (Mugilidae to Carangidae). FAO, Rome

Clarke K, Gorley R (2006) PRIMER v6. PRIMER-E Ltd, Plymouth

Dalsgaard J, St. John MA, Kattner G, Müller-Navarra D, Hagen W (2003) Fatty acid trophic markers in the pelagic marine environment. Adv Mar Biol 46:225–340

Daly R, Froneman PW, Smale MJ (2013) Comparative feeding ecology of bull sharks (*Carcharhinus leucas*) in the coastal waters of the southwest Indian Ocean inferred from stable isotope analysis. PLOS ONE 8:e78229

Douglas MM, Bunn SE, Davies PM (2005) River and wetland food webs in Australia's wet–dry tropics: general principles and implications for management. Mar Freshw Res 56:329–342

Dulvy NK, Fowler SL, Musick JA, Cavanagh RD and others (2014) Extinction risk and conservation of the world's sharks and rays. eLife 3:e00590

Every SL, Pethybridge HR, Crook DA, Kyne PM, Fulton CJ

(2016) Comparison of fin and muscle tissues for analysis of signature fatty acids in tropical euryhaline sharks. J Exp Mar Biol Ecol 479:46–53

Faye D, Tito de Morais L, Raffray J, Sadio O, Thiaw OT, Le Loc'h F (2011) Structure and seasonal variability of fish food webs in an estuarine tropical marine protected area (Senegal): evidence from stable isotope analysis. Estuar Coast Shelf Sci 92:607–617

Froese R, Pauly D (2015) FishBase. www.fishbase.org (accessed 15 Oct 2015)

Hall D, Lee SY, Meziane T (2006) Fatty acids as trophic tracers in an experimental estuarine food chain: tracer transfer. J Exp Mar Biol Ecol 336:42–53

Heithaus MR, Vaudo JJ, Kreicker S, Layman CA and others (2013) Apparent resource partitioning and trophic structure of large-bodied marine predators in a relatively pristine seagrass ecosystem. Mar Ecol Prog Ser 481:225–237

Hussey NE, Dudley SFJ, Mccarthy ID, Cliff G, Fisk AT (2011) Stable isotope profiles of large marine predators: viable indicators of trophic position, diet, and movement in sharks? Can J Fish Aquat Sci 68:2029–2045

Hussey NE, MacNeil MA, Olin JA, McMeans BC, Kinney MJ, Chapman DD, Fisk AT (2012) Stable isotopes and elasmobranchs: tissue types, methods, applications and assumptions. J Fish Biol 80:1449–1484

Hussey NE, Macneil MA, McMeans BC, Olin JA and others (2014a) Rescaling the trophic structure of marine food webs. Ecol Lett 17:239–250

Hussey NE, Macneil MA, McMeans BC, Olin JA and others (2014b) Corrigendum to Hussey et al. (2014). Ecol Lett 17:768

Hutchinson GE (1957) Concluding remarks. Cold Spring Harb Symp Quant Biol 22:415–427

Iverson SJ (2009) Tracing aquatic food webs using fatty acids: from qualitative indicators to quantitative determination lipids in aquatic ecosystems. In: Kainz M, Brett MT, Arts MT (eds) Lipids in aquatic ecosystems. Springer, New York, NY, p 281–307

Jackson AL, Inger R, Parnell AC, Bearhop S (2011) Comparing isotopic niche widths among and within communities: SIBER—Stable Isotope Bayesian Ellipses in R. J Anim Ecol 80:595–602

Kelly JR, Scheibling RE (2012) Fatty acids as dietary tracers in benthic food webs. Mar Ecol Prog Ser 446:1–22

Kim SL, Koch PL (2012) Methods to collect, preserve, and prepare elasmobranch tissues for stable isotope analysis. Environ Biol Fishes 95:53–63

Kirsch PE, Iverson SJ, Bowen WD (1998) Dietary effects on the fatty acid signature of whole Atlantic cod (*Gadus morhua*). Can J Fish Aquat Sci 55:1378–1386

Kitchell JF, Essington TE, Boggs CH, Schindler DE, Walters CJ (2002) The role of sharks and longline fisheries in a pelagic ecosystem of the Central Pacific. Ecosystems 5: 202–216

Last PR (2002) Freshwater and estuarine elasmobranchs of Australia. In: Fowler SL, Reed TM, Dipper FA (eds) Elasmobranch biodiversity, conservation and management. Proceedings of the International Seminar and Workshop, Sabah, Malaysia, July 1977. IUCN SSC Shark Specialist Group. IUCN, Gland, p 185–192

Layman CA, Arrington DA, Montaña CG, Post DM (2007a) Can stable isotope ratios provide for community-wide measures of trophic structure? Ecology 88:42–48

Layman CA, Quattrochi JP, Peyer CM, Allgeier JE (2007b) Niche width collapse in a resilient top predator following ecosystem fragmentation. Ecol Lett 10:937–944

Layman CA, Araujo MS, Boucek R, Hammerschlag-Peyer CM and others (2012) Applying stable isotopes to examine food-web structure: an overview of analytical tools. Biol Rev Camb Philos Soc 87:545–562

Logan JM, Lutcavage ME (2010) Reply to Hussey et al.: The requirement for accurate diet-tissue discrimination factors for interpreting stable isotopes in sharks. Hydrobiologia 654:7–12

Loneragan NR, Bunn SE, Kellaway DM (1997) Are mangrove and seagrasses sources of organic carbon for penaeid prawns in a tropical estuary? A multiple isotope study. Mar Biol 130:289–300

Lucifora LO, de Carvalho MR, Kyne PM, White WT (2015) Freshwater sharks and rays. Curr Biol 25:R971–R973

Malpica-Cruz L, Herzka SZ, Sosa-Nishizaki O, Lazo JP (2012) Tissue-specific isotope trophic discrimination factors and turnover rates in a marine elasmobranch: empirical and modeling results. Can J Fish Aquat Sci 69: 551–564

Martínez-Álvarez RM, Sanza A, García-Gallegoa M, Domezain A and others (2005) Adaptive branchial mechanisms in the sturgeon *Acipenser naccarii* during acclimation to saltwater. Comp Biochem Physiol A 141: 183–190

Matich P, Heithaus MR (2014) Multi-tissue stable isotope analysis and acoustic telemetry reveal seasonal variability in the trophic interactions of juvenile bull sharks in a coastal estuary. J Anim Ecol 83:199–213

Matich P, Heithaus MR, Layman CA (2010) Size-based variation in intertissue comparisons of stable carbon and nitrogen isotopic signatures of bull sharks (*Carcharhinus leucas*) and tiger sharks (*Galeocerdo cuvier*). Can J Fish Aquat Sci 67:877–885

Matich P, Heithaus MR, Layman CA (2011) Contrasting patterns of individual specialization and trophic coupling in two marine apex predators. J Anim Ecol 80:294–305

McMeans BC, Arts MT, Lydersen C, Kovacs KM, Hop H, Falk-Petersen S, Fisk AT (2013) The role of Greenland sharks (*Somniosus microcephalus*) in an Arctic ecosystem: assessed via stable isotopes and fatty acids. Mar Biol 160:1223–1238

Miller MR, Nichols PD, Barnes J, Davies NW, Peacock EJ, Carter CG (2006) Regiospecificity profiles of storage and membrane lipids from the gill and muscle tissue of Atlantic salmon (*Salmo salar* L.) grown at elevated temperature. Lipids 41:865–876

Mitchell AL, Lucas RM, Donnelly BE, Pfitzner K, Milne AK, Finlayson M (2007) A new map of mangroves for Kakadu National Park, Northern Australia, based on stereo aerial photography. Aquat Conserv 17:446–467

Munroe SEM, Heupel MR, Fisk AT, Simpfendorfer CA (2014a) Geographic and temporal variation in the trophic ecology of a small-bodied shark: evidence of resilience to environmental change. Can J Fish Aquat Sci 72:343–351

Munroe SEM, Simpfendorfer CA, Heupel MR (2014b) Habitat and space use of an abundant nearshore shark, *Rhizoprionodon taylori.* Mar Freshw Res 65:959–968

Munroe SEM, Heupel MR, Fisk AT, Logan JM, Simpfendorfer CA (2015) Regional movement patterns of a small-bodied shark revealed by stable-isotope analysis. J Fish Biol 86:1567–1586

Napolitano GE, Pollero RJ, Gayoso AM, MacDonald BA, Thompson RJ (1997) Fatty acids as trophic markers of

phytoplankton blooms in the Bahia Blanca estuary (Buenos Aires, Argentina) and in Trinity Bay (Newfoundland, Canada). Biochem Syst Ecol 25:739–755

Navarro J, Coll M, Louzao M, Palomera I, Delgado A, Forero MG (2011) Comparison of ecosystem modelling and isotopic approach as ecological tools to investigate food webs in the NW Mediterranean Sea. J Exp Mar Biol Ecol 401:97–104

Nichols PD, Rayner M, Stevens J (2001) A pilot investigation of Northern Australian shark liver oils: characterization and value-adding. Final Report FRDC Project 99/369

Olin JA, Hussey NE, Fritts M, Heupel MR, Simpfendorfer CA, Poulakis GR, Fisk AT (2011) Maternal meddling in neonatal sharks: implications for interpreting stable isotopes in young animals. Rapid Commun Mass Spectrom 25:1008–1016

Özogul Y, Özogul F, Alagoz S (2007) Fatty acid profiles and fat contents of commercially important seawater and freshwater fish species of Turkey: a comparative study. Food Chem 103:217–223

Papastamatiou YP, Wetherbee BM, Lowe CG, Crow LG (2006) Distribution and diet of four species of carcharhinid shark in the Hawaiian Islands: evidence for resource partitioning and competitive exclusion. Mar Ecol Prog Ser 320:239–251

Parrish CC (2013) Lipids in marine ecosystems. ISRN Oceanogr 2013:1–16

Parrish CC, Nichols PD, Pethybridge HR, Young JW (2015) Direct determination of fatty acids in fish tissues: quantifying top predator trophic connections. Oecologia 177: 85–95

Peterson BJ, Fry B (1987) Stable isotopes in ecosystem studies. Annu Rev Ecol Syst 18:293–320

Pethybridge HR, Parrish CC, Morrongiello J, Young JW, Farley JH, Gunasekera RM, Nichols PD (2015) Spatial patterns and temperature prediction of tuna fatty acids: tracing essential nutrients and changes in primary producers. PLOS ONE 10:e0131598

Post DM, Layman CA, Arrington DA, Takimoto G, Quattrochi J, Montaña CG (2007) Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. Oecologia 152: 179–189

Quantum GIS Development Team (2016) Quantum GIS Geographic Information System. Open Source Geospatial Foundation Project. http://qgis.osgeo.org

R Core Team (2016) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna. www.R-project.org

Rooney N, McCann K, Gellner G, Moore JC (2006) Structural asymmetry and the stability of diverse food webs. Nature 442:265–269

Ross ST (1986) Resource partitioning in fish assemblages: a review of field studies. Copeia 1986:352–388

Sardenne F, Bodin N, Chassot E, Amiel A, Fouché E, Degroote M, Pethybridge HR (2016) Trophic niches of sympatric tropical tuna in the Western Indian Ocean inferred by stable isotopes and neutral fatty acids. Prog Oceanogr 146:75–88

Simpfendorfer CA (1998) Diet of the Australian sharpnose shark, *Rhizoprionodon taylori*, from northern Queensland. Mar Freshw Res 49:757–761

Snelson FFJ, Mulligan TJ, Williams SE (1984) Food habits, occurrence, and population structure of the bull shark, *Carcharhinus leucas*, in Florida coastal lagoons. Bull Mar Sci 34:71–80

Speers-Roesch B, Ip YK, Ballantyne JS (2008) Plasma nonesterified fatty acids of elasmobranchs: comparisons of temperate and tropical species and effects of environmental salinity. Comp Biochem Physiol A 149:209–216

Stevens JD, Bonfil R, Dulvy NK, Walker PA (2000) The effects of fishing on sharks, rays, and chimaeras (chondrichthyans), and the implications for marine ecosystems. ICES J Mar Sci 57:476–494

Swanson HK, Lysy M, Power M, Stasko AD, Johnson JD, Reist JD (2015) A new probabilistic method for quantifying n-dimensional ecological niches and niche overlap. Ecology 96:318–324

Syväranta J, Lensu A, Marjomäki TJ, Oksanen S, Jones RI (2013) An empirical evaluation of the utility of convex hull and standard ellipse areas for assessing population niche widths from stable isotope data. PLOS ONE 8: e56094

Thorburn DC, Rowland AJ (2008) Juvenile bull sharks *Carcharhinus leucas* (Valenciennes, 1839) in northern Australian rivers. Beagle 24:79–86

Thorburn DC, Gill HS, Morgan DL (2014) Predator and prey interactions of fishes of a tropical Western Australia river revealed by dietary and stable isotope analyses. J R Soc West Aust 97:363–387

Tillett BJ, Meekan MG, Field IC (2014) Dietary overlap and partitioning among three sympatric carcharhinid sharks. Endang Species Res 25:283–293

Tilley A, López-Angarita J, Turner JR (2013) Diet reconstruction and resource partitioning of a Caribbean marine mesopredator using stable isotope Bayesian modelling. PLOS ONE 8:e79560

Uysal K, Aksoylar MY (2005) Seasonal variations in fatty acid composition and the n-6/n-3 fatty acid ratio of pikeperch (*Sander lucioperca*) muscle lipids. Ecol Food Nutr 44:23–35

Warfe DM, Pettit NE, Davies PM, Pusey BJ and others (2011) The 'wet-dry' in the wet-dry tropics drives river ecosystem structure and processes in northern Australia. Freshw Biol 56:2169–2195

Winemiller KO, Jepsen DB (1998) Effects of seasonality and fish movement on tropical river food webs. J Fish Biol 53: 267–296

Wolanski E, Chappell J (1996) The response of tropical Australian estuaries to a sea level rise. J Mar Syst 7:267–279

Yick JL, Tracey SR, White RWG (2011) Niche overlap and trophic resource partitioning of two sympatric batoids co-inhabiting an estuarine system in southeast Australia. J Appl Ichthyol 27:1272–1277

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