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Multi-tissue stable isotope analysis reveals resource partitioning and trophic relationships of large reef-associated predators

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ABSTRACT: Defining the role of reef predators is particularly important given the rapid rate at which some species are declining, yet knowledge of trophic relationships is often lacking, particularly for large wide-ranging species that may use coral reefs seasonally or opportunistically. We used a multi-tissue stable isotope approach to investigate the trophic ecology of common reef predators in the central Great Barrier Reef of Australia. Our study revealed significant trophic separation among reef predators, especially when considering isotopic data from muscle, a slower turnover tissue. Based on muscle data, the bull shark *Carcharhinus leucas*, a large wide-ranging coastal species, occupied a higher trophic position and had a larger isotopic niche breadth ($19.1‰^2$) relative to smaller predators, including resident sharks ($4.5 \pm 0.7‰^2$) and large-bodied teleosts ($4.4 \pm 1.8‰^2$). Spanish mackerel *Scomberomorus commerson* and bull sharks had the largest unique total areas of isotopic niche space (expressed as a percentage) that did not overlap with any other species, 95% and 69.4%, respectively, which means they had lower isotopic overlap. In general, faster turnover tissues such as whole blood and plasma showed higher isotopic overlap and smaller niche breadth for all reef predators. These results suggest that bull sharks use similar prey resources to large and small resident predators, at least during short periods. Our findings highlight the importance of investigating dietary changes in faster-turnover tissues of reef predators, particularly large wide-ranging species, which may have key roles in coral reef food webs through direct predation and competition.

KEY WORDS: Trophic ecology · Shark · Food web · Coral reef · Spatial ecology · Great Barrier Reef

1. INTRODUCTION

High trophic level predators fill a variety of ecological roles in terrestrial and aquatic ecosystems, such as regulating prey dynamics, structuring food webs, and ultimately helping maintain ecosystem function and health (Sergio et al. 2008, Terborgh & Estes 2010, Heithaus et al. 2014). Consequently, removal of top predators may propagate down the food chain (i.e. top-down processes), potentially leading to diverse ecological consequences that impact ecosystem processes (Dulvy et al. 2000, Terborgh & Estes 2010, Espinoza et al. 2014, Heithaus et al. 2014). In highly diverse and complex ecosystems such as coral reefs,

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the role of sharks as top predators continues to be debated (Frisch et al. 2016, Roff et al. 2016a,b), because not all sharks act as top predators (Heupel et al. 2014, Casey et al. 2017) or their role may be masked by the high level of functional redundancy with other reef predators (Roff et al. 2016a, Bond et al. 2018). Further still, a recent study found that reef sharks can produce non-consumptive (fear) effects on herbivores, and thus may influence macroalgae distribution and abundance on a coral reef (Rasher et al. 2017). Reef shark functional roles may also vary based on movement behaviour and residency patterns (Speed et al. 2010, Espinoza et al. 2015a), which may create additional layers of complexity. Defining the role of reef predators is important given the rapid rate at which some species are declining (Graham et al. 2010, Ward-Paige et al. 2010, McCauley et al. 2015), and is particularly important as climate-driven changes threaten the health and functioning of coral reefs at a global-scale (Hughes et al. 2018).

Knowledge of the trophic relationships and degree of dietary overlap among co-occurring predators is an important component in understanding their role in a particular ecosystem (Frisch et al. 2016, Bond et al. 2018), and ultimately predicting the ecological and economic consequences of population declines (Heithaus et al. 2008, Ferretti et al. 2010). Given the diverse prey community and dietary links in reef ecosystems, determining how resources are selected and partitioned among predators is crucial to evaluating the extent and characteristics of potential top-down effects.

Stable isotope analysis is a valuable, cost-effective and non-lethal tool for quantifying trophic interactions and niche breadth of large-bodied fish predators (Heithaus et al. 2013, Matley et al. 2016b, Gallagher et al. 2017). Moreover, given that prey items consumed by animals are not immediately incorporated into all tissues, stable isotopes also provide dietary data over multiple scales based on the isotopic turnover rate of the sampled tissue (Cerling et al. 2007, Martínez del Rio & Carleton 2012). For instance, tissues with high turnover rates (e.g. plasma and liver) reflect dietary changes faster than less metabolically active tissues, such as whole blood and muscle (MacNeil et al. 2005). Therefore, a multi-tissue stable isotope approach can reveal temporal habitat and dietary shifts from unique isotopic values of assimilated prey over different temporal scales (MacNeil et al. 2006, Matley et al. 2016a).

Integrating trophic information with shark movement data can further increase our understanding of complex trophic relationships at the top of coral reef food webs. Inter- and intra-specific variation in movement behaviours have been reported in numerous reef-associated sharks which may alter their influence as predators (Chin et al. 2013, Espinoza et al. 2015a). Shark movements are also known to be scale-dependent (Papastamatiou et al. 2009, 2011), biologically and/or environmentally driven (Heupel & Simpfendorfer 2014, Espinoza et al. 2016, Schlaff et al. 2017, Lea et al. 2018), and context-specific (i.e. the behaviour of the same species may vary across reef environments) (Espinoza et al. 2015b), which ultimately may influence foraging strategies and trophic interactions (e.g. degree of dietary overlap). However, few studies have provided detailed information on both the spatial and trophic ecology of reef predators (Papastamatiou et al. 2010, Matley et al. 2016b), particularly larger, wide-ranging non-resident species (Ferreira et al. 2017).

Given that larger predators are more likely to feed at higher trophic levels and forage over larger spatial scales to meet their energetic requirements (Speed et al. 2010), the trophic position of reef-associated predators (within and among species) is expected to increase with size (Hussey et al. 2011, 2014). Moreover, $\delta^{13}C$ values from species that spend a considerable amount of time on a single reef (reef-residents) are expected to reflect an isotopic reef value and are likely to be less variable than $\delta^{13}C$ values from wide-ranging, non-resident reef-associated species (opportunistic or seasonal reef foragers). Given anticipated dietary and movement differences between small and large predators, isotopic niche space is hypothesized to have less overlap between species that occupy intermediate and higher trophic positions. These differences are also expected to be greater in less metabolically active tissues (e.g. muscle) from highly migratory species because such tissues carry with them the baselines and dietary information from previous foraging grounds, which would presumably show greater differences than local foraging behaviour. Therefore, it should be possible to separate functional roles of predators using stable isotopes and isotopic niche analysis.

The Great Barrier Reef (GBR) of Australia is one of the most productive and globally important regions of shark diversity (Espinoza et al. 2014). Previous work in the GBR has provided detailed information on the residency patterns, movements and habitat use of common reef-associated predators (Currey et al. 2014, Espinoza, et al. 2015b,c, 2016, Heupel & Simpfendorfer 2015, Matley et al. 2016c, Heupel et al. 2018). For example, Espinoza et al. (2015b,c) demonstrated that grey reef sharks *Carcharhinus amblyrhynchos* and silvertip sharks *C. albimarginatus* spent most of their time on a single reef (>50% of days detected in more than 2 yr of monitoring), yet behavioural differences between sexes and/or life-stages were also detected. In contrast, Espinoza et al. (2016) revealed high individual variability in reef use patterns of adult bull sharks *C. leucas*, with most individuals (>51%) leaving the study site and undertaking long-range migrations (>500 km) to other coral reefs and/or inshore coastal habitats in Queensland and New South Wales. Similarly, movement data from common coral trout *Plectropomus leopardus* and redthroat emperor *Lethrinus miniatus* tracked in the GBR suggest that these large-bodied teleosts show limited movement and high site fidelity to specific reef habitats (Currey et al. 2014, Matley et al. 2016c), and therefore, may be overlapping with other reef predators that use similar resources. Moreover, stable isotope data from the GBR suggest that reef sharks have functional roles similar to those of large predatory fishes such as snappers, emperors and groupers (Frisch et al. 2016). Therefore, given that movement data from common reef predators is already available in the GBR, integrating trophic information over the same temporal scale, particularly from large wide-ranging sharks, may increase our understanding of the role they play in coral reef ecosystems.

This study used a multi-tissue stable isotope approach to investigate the trophic ecology of common reef predators in the central GBR. Specifically, we determined: (1) the niche breadth and degree of isotopic overlap of predators with different levels of reef-association across multiple tissues; and (2) intra- and inter-specific relationships between biological drivers (sex and size) and isotopic values. This information is needed to adequately define the role of predators in coral reef ecosystems, and ultimately understand how wide-scale population declines may affect top-down processes.

2. MATERIALS AND METHODS

2.1. Study site

Study reefs were part of a large network of mid-shelf reefs located in the central GBR of Australia (Fig. 1). Coral cover in this region is dominated by *Acropora* spp., and reef habitats are characterized by extensive reef flats and a well-developed reef slope (steep on the NE face), with deep (40 to 60 m) soft-bottom channels connecting reef patches (Williams 1982, Graham et al. 2014). The central and northern GBR are periodically subjected to cyclones and prevailing winds from the east to southeast (Done 1982). Reef slope habitats have also experienced extensive coral mortality (>80%) caused by severe coral bleaching events and crown-of-thorns starfish (COTS) outbreaks (Pratchett et al. 2008, Miller et al. 2015, Hughes et al. 2017).

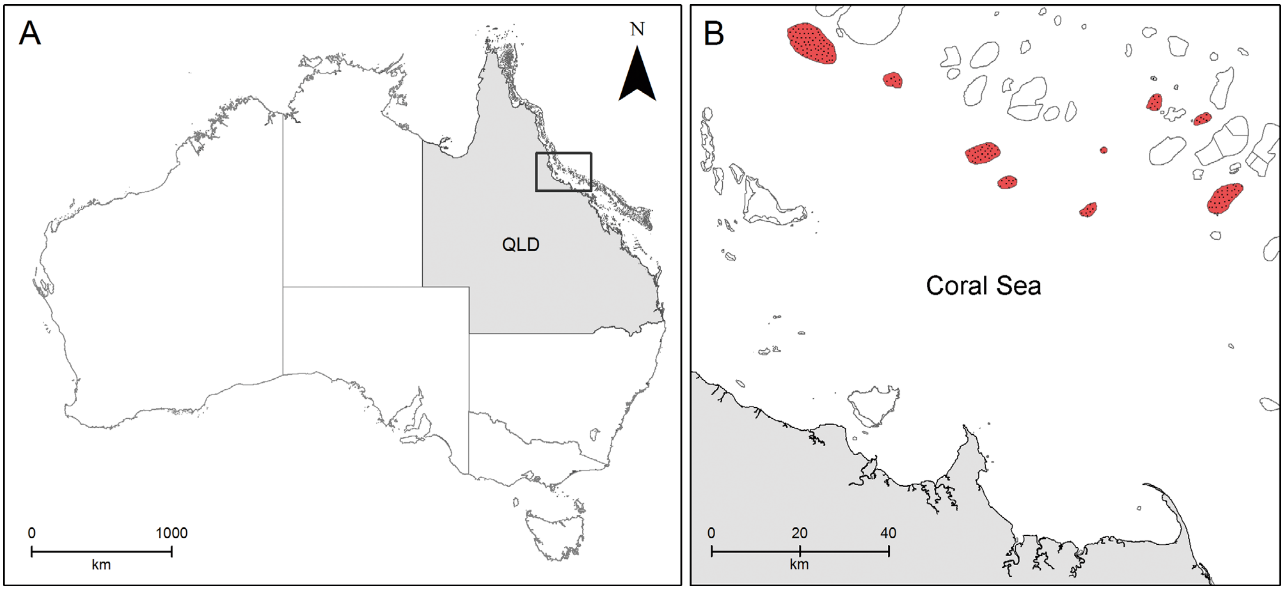

Fig. 1. (A) The study area in the central Great Barrier Reef of Australia. (B) Colored areas indicate reefs where samples were collected

2.2. Study species

To investigate the trophic ecology of reef predators in the central GBR, we selected several sharks (grey reef shark *Carcharhinus amblyrhynchos*, silvertip shark *C. albimarginatus*, whitetip reef shark *Triaenodon obesus*, bull shark *C. leucas*) and large-bodied teleosts (Spanish mackerel *Scomberomorus commerson*, common coral trout *Plectropomus leopardus*, redthroat emperor *Lethrinus miniatus*). These are some of the common predators in the GBR (Espinoza et al. 2014, Rizzari et al. 2014, Emslie et al. 2015), and also economically important reef-associated fishes of Queensland (Mapstone et al. 2004, Buckley et al. 2017). Furthermore, over the past 10 yr we have accumulated extensive knowledge of their spatial ecology, including degree of site fidelity, movement patterns, habitat use, inter-reef connectivity and long-range migratory patterns (Currey et al. 2014, Espinoza et al. 2015a,b,c, 2016, Heupel & Simpfendorfer 2015, Heupel et al. 2015, Matley et al. 2016b,c), making them ideal candidate species for trophic studies.

2.3. Field sampling

Reef-associated predators were sampled using a variety of fishing methods, including modified drop-lines and long-lines (see Espinoza et al. 2015b for a description of fishing gear). In addition, we used rod and reel to sample yellowtail fusilier *Caesio cuning*, a small planktivorous fish. as a reference baseline species from coral reefs. All captured individuals were measured (pre-caudal length, PCL; fork length, FL; stretch total length, STL), and sexed if possible. Blood and muscle samples were collected from captured individuals for subsequent analyses. Blood samples (2 ml) were collected from the caudal vein of sharks and teleosts. During collection, 1 ml of blood was placed into sterile collection vials with no additives or interior coating. Plasma was separated immediately by centrifugation for 1 min at 906 × *g* using a portable centrifuge. The remaining 1 ml of blood was retained in its original composition (whole blood, hereafter). Based on previous laboratory studies, plasma and whole blood serve as short and medium-term dietary indicators, respectively, for upper level consumers such as sharks (MacNeil et al. 2006, Kim et al. 2012). Muscle samples (~0.5 cm^3) were collected via a biopsy punch in the dorsal musculature at the base of the dorsal fin (i.e. first dorsal fin for sharks). Muscle samples provide a longer-term diet indicator relative to plasma and whole blood (MacNeil et al. 2006). All tissues were frozen (−80°C), freeze dried and ground to a fine powder for subsequent analysis. All sampling procedures were conducted following protocols approved by James Cook University Animal Ethics (A1933).

2.4. Stable isotope analysis

Lipids from all predator samples were extracted using a solution of chloroform and methanol (1:1) as they are depleted in ^{13}C compared with proteins and carbohydrates (Hussey et al. 2012a,b). The ratio of C:N for all samples ranged from 2.7 to 4.1 (3.2 ± 0.2, mean ± SD), suggesting that lipid extraction was sufficient. We used a Thermo Finnigan DeltaPlus mass spectrometer to quantify stable isotope ratios of ^{13}C:^{12}C and ^{15}N:^{14}N. Isotope ratios were expressed as δ^{13}C and δ^{15}N, according to the following equation: $X = (R_{\text{sample}} / R_{\text{standard}} - 1) \times 1000$ (‰), where X is the heavy isotope, R_{sample} is the ratio of heavy to light isotope in the sample, and R_{standard} is the ratio of heavy to light isotope in the reference standard. We used PeeDee Belemnite and atmospheric N_2 as standard reference materials for carbon and nitrogen, respectively. Analytical precision of the equipment was determined every 12 samples based on NIST standards. The analytical precision (standard deviation) for NIST standard 1577c (bovine liver, n = 93) and an internal laboratory standard (tilapia muscle, n = 93) were 0.07 and 0.11‰ for δ^{13}C, respectively, and 0.11 and 0.11‰ for δ^{15}N, respectively. Although C:N ratios for some samples were greater than 4.0, relationships between C:N and δ^{13}C for specific tissue-taxa subsets were weak ($R^2 < 0.15$) based on linear regressions; therefore, the influence of lipid was not significant for δ^{13}C.

2.5. Niche breadth and degree of isotopic overlap

Niche breadth and isotopic overlap among species were calculated using the SIAR library (Jackson et al. 2011) in R version 3.4.3 (R Development Core Team 2018). Bayesian standard ellipse areas corrected for sample size (SEA$_c$) were calculated to represent the 'average' isotopic niche breadth of the population (Jackson et al. 2011), which was expressed as an area (‰^{2}). Differences in SEA$_c$ size were considered significant if the 95% credibility intervals of posterior draws did not overlap between species. Measures of central tendency such as mean isotopic values and

SEA_c can often mask ecologically meaningful variation within species and potential individual level overlap in resource use (Layman et al. 2012). Therefore, we also used the total area (TA; expressed as ‰^{2}), which was calculated as the smallest convex hull that encompassed all $\delta^{13}C$ and $\delta^{15}N$ values, as a measure of isotopic trophic diversity (or niche breadth) within a species and tissue type (Layman et al. 2007, Jackson et al. 2011). To assess the degree of isotopic overlap, we quantified the unique area of a species' isotopic niche space that did not overlap with any other species using the convex hull approach. The degree of overlap was expressed as a percent (%) of the shared isotopic space and measured using ArcMAP 10.4.1 (ESRI). Where applicable, data were checked for normality and homogeneity of variance using diagnostic plots in R (R Development Core Team 2018).

2.6. Relationships between biological drivers and isotopic values

Analysis of variance (ANOVA) was used to explore variation in mean isotopic values ($\delta^{13}C$ and $\delta^{15}N$) among species/ecological groups and tissue type. Differences in isotopic values were investigated using post-hoc tests (Tukey's HSD; $p < 0.05$). Multiple linear regression models were used to test relationships between sex and size (fork length) on $\delta^{13}C$ and $\delta^{15}N$ values for each species and tissue type. Initially, trophic position (TP) was calculated to account for inter-reef variability in baseline isotope values, which consequently may influence $\delta^{15}N$ values further up the food web (Munroe et al. 2015). However, TP estimations were biased due to the limited sample size of lower trophic-level organisms across all of the study reefs. As opposed to using a common baseline value across all reefs for TP estimates, we chose to use $\delta^{15}N$ instead due to these sampling limitations and because conversion to TP would result in minor differences (see Fig. A1 in the Appendix). All analyses were conducting using R (R Development Core Team 2018).

3. RESULTS

Tissue samples of large-bodied reef-associated predators from the central GBR were collected between 2013 and 2014, and included 4 shark (n = 119) and 3 teleost (n =116) species (Table 1). Shark size ranged from 58 to 235 cm FL (125 ± 40 cm FL, mean ± SD); the bull shark *Carcharhinus leucas* was the largest sampled (197 ± 22 cm FL) and the whitetip reef shark *Triaenodon obesus* was the smallest (102 ± 16 cm FL). Significant size differences were detected between bull sharks and the rest of the studied species ($F_{3,112} = 63.7$, $p < 0.001$); however, size ranges of the grey reef shark *C. amblyrhynchos* (110 ± 25 cm FL), silvertip shark *C. albimarginatus* (120 ± 29 cm FL) and whitetip reef shark (102 ± 16 cm FL) sharks were not statistically different. Teleost size range varied from 31.5 to 125.0 cm FL (63 ± 28 cm FL); Spanish mackerel *Scomberomorus commerson* were significantly larger (104 ± 14 cm TL) than common coral trout *Plectropomus leopardus* (45 ± 9 cm FL) and redthroat emperor *Lethrinus miniatus* (46 ± 5 cm FL), which had similar sizes ($F_{2,75} = 333$, $p < 0.001$). Tissue samples were also taken from the yellowtail fusilier *Caesio cuning*, a small (24.7 ± 1.1 cm FL) planktivorous fish.

3.1. Isotopic values from reef-associated species

Stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) of sharks and teleosts are included in Table 1. The common coral trout (range of $\delta^{13}C$: 6.74‰) and bull shark (range of $\delta^{13}C$: 6.29‰) had the largest range of $\delta^{13}C$ values, while the yellowtail fusilier had the smallest (range of $\delta^{13}C$: 0.70‰). The bull shark and yellowtail fusilier also had the largest and smallest range of $\delta^{15}N$ values, with 4.32‰ and 0.91‰, respectively. A significant interaction effect between species and tissue type was detected for both $\delta^{13}C$ ($F_{12,452} = 2.741$, $p = 0.0013$) and $\delta^{15}N$ ($F_{12,452} = 9.554$, $p < 0.0001$). Muscle samples from bull sharks had statistically similar $\delta^{13}C$ to small (common coral trout and whitetip reef sharks) and large (grey reef and silvertip shark) predators (Figs. 2A & 3A). Values of $\delta^{13}C$ from redthroat emperor, however, were significantly higher than those from bull sharks (Fig. 3A). In contrast, Spanish mackerel had significantly lower $\delta^{13}C$ relative to all species sampled. Similar $\delta^{13}C$ values were also observed between common coral trout and grey reef and whitetip reef sharks (Figs. 2A & 3A). Silvertip sharks, however, had significantly lower $\delta^{13}C$ than common coral trout (Fig. 3A).

Whole blood and plasma from redthroat emperor and whitetip reef sharks had similar $\delta^{13}C$ (Fig. 2B,C), but these were significantly higher than values from Spanish mackerel, common coral trout and grey reef and silvertip sharks (Fig. 3A). Bull sharks showed statistically similar $\delta^{13}C$ compared to small (redthroat emperor, common coral trout and whitetip reef

Table 1. Stable isotopes (mean ± SD) of reef predators in the central Great Barrier Reef of Australia. Tissue samples: M, muscle; WB, whole blood; P, plasma. N: number of individuals sampled; FL: fork length; Sex ratio: expressed as male to female ratio; TA: total area or isotopic niche breadth; Unique area: area of a species' TA that does not overlap with any other species. SEA$_c$: corrected standard ellipse area, which is an estimate of the trophic niche breadth. –: sex not determined

Species	Tissue	N	FL range (cm)	FL mean ± SD (cm)	Sex ratio (%♀)	δ^{13}C (‰)	δ^{15}N (‰)	TA (‰^{2})	Unique area (%)	SEA$_c$ (‰^{2})
Sharks										
Bull shark	M	22	150–235	199 ± 20	0.77	−14.60 ± 1.58	13.53 ± 1.08	19.1	69.4	5.4
(*Carcharhinus leucas*)	WB	19	150–235	197 ± 22	0.74	−14.63 ± 0.60	11.82 ± 0.68	4.1	15.4	1.3
	P	18	150–235	200 ± 22	0.83	−14.37 ± 0.80	11.28 ± 0.83	6.3	1.7	2.2
Silvertip shark	M	22	72–163	116 ± 29	0.55	−15.59 ± 0.69	12.43 ± 0.60	4.2	1.4	1.4
(*Carcharhinus*	WB	24	72–163	120 ± 29	0.58	−15.53 ± 0.61	11.84 ± 0.49	3.1	7.2	0.9
albimarginatus)	P	23	72–163	122 ± 32	0.57	−15.21 ± 0.73	11.51 ± 0.55	5.0	7.9	1.3
Grey reef shark	M	10	58–135	100 ± 33	0.20	−15.06 ± 0.72	11.53 ± 1.23	5.3	7.1	3.1
(*Carcharhinus*	WB	64	58–140	110 ± 25	0.45	−14.85 ± 1.04	11.20 ± 0.68	8.5	32.4	2.2
amblyrhynchos)	P	57	58–140	111 ± 25	0.51	−14.75 ± 1.09	10.77 ± 0.72	12.1	16.8	2.4
Whitetip reef shark	M	9	70–120	102 ± 16	0.50	−14.71 ± 0.88	10.43 ± 0.83	3.9	13.2	2.4
(*Triaenodon obesus*)	WB	9	70–120	102 ± 16	0.50	−14.09 ± 0.88	9.92 ± 0.83	3.8	5.8	2.4
	P	8	70–120	103 ± 17	0.43	−13.34 ± 1.06	9.48 ± 0.93	3.9	1.1	2.8
Teleosts										
Spanish mackerel	M	29	80–125	103 ± 12	0.59	−16.73 ± 0.44	12.32 ± 0.47	2.4	95	0.6
(*Scomberomorus*	WB	13	80–125	104 ± 14	0.67	−17.58 ± 0.25	11.78 ± 0.22	0.3	100	0.2
commerson)	P	12	94–124	104 ± 10	0.73	−17.97 ± 0.38	12.01 ± 0.31	0.9	100	0.4
Common coral trout	M	28	31–63	45 ± 9	–	−14.42 ± 1.62	10.73 ± 0.46	5.6	14.6	1.8
(*Plectropomus*	WB	22	33–63	45 ± 9	–	−15.47 ± 1.36	9.96 ± 0.27	3.1	36.3	1.2
leopardus)	P	8	33–58	45 ± 8	–	−15.31 ± 1.39	11.00 ± 0.66	3.9	14.3	2.9
Redthroat emperor	M	26	35–59	47 ± 6	–	−13.37 ± 1.65	10.65 ± 0.67	5.3	26.4	1.8
(*Lethrinus miniatus*)	WB	31	35–59	45 ± 5	–	−13.90 ± 1.35	8.79 ± 0.59	6.3	80.2	2.0
	P	19	38–52	52 ± 5	–	−14.10 ± 1.21	9.33 ± 0.56	6.0	30.4	2.2
Yellowtail fusilier	M	28	21–27	24.7 ± 1.1	–	−17.83 ± 0.20	9.89 ± 0.23	0.4	100	0.1
(*Caesio cuning*)										

sharks) and large (grey reef and silvertip sharks) predators. Whole blood and plasma δ^{13}C from Spanish mackerel, however, were lower than the rest of the species sampled.

Significant differences in δ^{15}N between large and small predators were detected across tissue types (Figs. 2 & 3B). In muscle, bull sharks exhibited significantly higher δ^{15}N than the rest of the species (Figs. 2A & 3B). Similar δ^{15}N were found in Spanish mackerel and grey reef and silvertip sharks, which were significantly higher than values found in other smaller reef predators (e.g. whitetip reef shark, common coral trout and redthroat emperor). These smaller reef predators all exhibited similar δ^{15}N values (Figs. 2A & 3B). In whole blood, larger predators such as Spanish mackerel and bull and silvertip sharks had similar δ^{15}N, and these were significantly higher than those of the rest of the species sampled (Figs. 2B & 3B). Significant differences in δ^{15}N were also detected between grey reef sharks and smaller reef predators (e.g. whitetip reef shark and common coral trout). Redthroat emperor had the lowest δ^{15}N values relative to other species (Figs. 2B & 3B). In plasma, δ^{15}N from bull sharks, Spanish mackerel, common coral trout and silvertip and grey reef sharks were not significantly different (Figs. 2C & 3B). Bull sharks, however, had significantly higher δ^{15}N than whitetip reef sharks and redthroat emperor (Fig. 3B). Significant differences in δ^{15}N among silvertip, grey reef and whitetip reef sharks were also detected (Figs. 2C & 3B).

3.2. Niche breadth and degree of isotopic overlap among predator species

For muscle, bull sharks had the largest isotopic niche breadth (TA = 19.1‰^{2}; SEA$_c$ = 5.4‰^{2}) and Spanish mackerel the smallest (TA = 2.4‰^{2}; SEA$_c$ = 0.6‰^{2}) (Table 1, Figs. 2D & 4). The remaining species had TA values ranging from 3.9 to 5.6‰^{2} (4.9 ± 0.8‰^{2}) and SEA$_c$ values ranging from 1.4 to 3.1‰^{2}

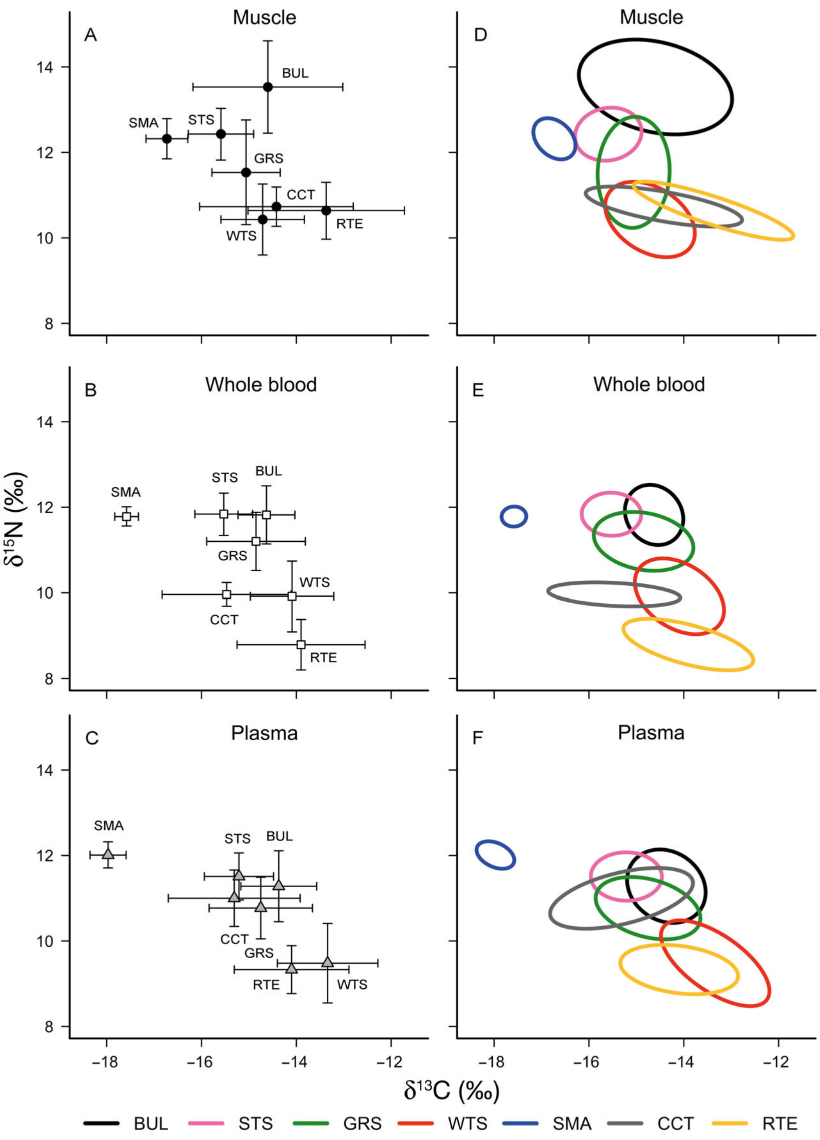

Fig. 2. Isotopic values ($\delta^{13}C$ and $\delta^{15}N$), niche breadth and degree of trophic niche overlap of common reef-associated predators. Left panels show $\delta^{13}C$ and $\delta^{15}N$ values for (A) muscle, (B) whole blood and (C) plasma. Error bars are 95% CI. Right panels (D–F) show standard ellipse areas corrected for sample size (SEA_c). Coloured lines indicate the SEAc from sharks (BUL: bull shark *Carcharhinus leucas*, STS: silvertip shark *C. albimarginatus*, GRS: grey reef shark *C. amblyrhynchos*, WTS: whitetip reef shark *Triaenodon obesus*) and teleosts (SMA: Spanish mackerel *Scomberomorus commerson*, CCT: common coral trout *Plectropomus leopardus*, RTE: redthroat emperor *Lethrinus miniatus*)

(1.8 ± 0.8‰^{2}). A considerable reduction in the size of the isotopic niche breadth of bull sharks was observed in whole blood (TA = 4.1‰^{2}; SEA_c = 1.3‰^{2}) and plasma (TA = 6.3‰^{2}; SEA_c = 2.2‰^{2}) relative to muscle (Figs. 2E,F & 4). The rest of the species showed TA values ranging from 0.3 to 8.5‰^{2} (4.2 ± 2.9‰^{2}) and SEA_c values ranging from 0.2 to 2.4‰^{2} (1.5 ± 0.8‰^{2}) in whole blood, and TA values from 0.9 to 12.1‰^{2} (5.3 ± 3.7‰^{2}) and SEA_c values ranging from 0.4 to 2.9‰^{2} (2 ± 1.0‰^{2}) in plasma (Table 1, Fig. 4). Spanish mackerel consistently showed the lowest isotopic niche breadth among reef predators across tissue types, whereas whitetip reef shark had the largest isotopic niche breadth in both whole blood and plasma (Table 1).

In muscle, Spanish mackerel and bull sharks had the largest unique areas, with 95 and 69.4%, respectively, indicating these species exhibit a relatively low degree of isotopic overlap with other reef predators (Table 1, Fig. 2D). In contrast, unique areas of common coral trout and whitetip reef, grey reef and silvertip sharks were less than 15%, indicating they consumed isotopically similar prey items. Analysis of whole blood and plasma samples revealed that bull sharks had a lower unique isotopic niche space area (whole blood (WB): 15.4%; plasma (P): 1.7%) than smaller reef predators such as redthroat emperor (WB: 80.2%; P: 30.4%) and common coral trout (WB: 36.3%; P: 14.3%) (Fig. 2E,F).

3.3. Relationships between biological drivers and isotopic values

There was a significant positive relationship between the mean reef predator size and mean $\delta^{15}N$ ($p < 0.05$), in which larger species such as silvertip and bull sharks had higher $\delta^{15}N$ values than smaller ones (Fig. 5). This relationship was significant across all tissue types, with the slope of muscle (β_1 = 0.0199 ± 0.004) being slightly higher than, but not significantly different from the slopes from whole blood (β_1 = 0.0177 ± 0.007) and plasma (β_1 = 0.0082 ± 0.007) ($F_{3,17}$ = 0.84, p = 0.488; Fig. 5). When examining the effects of sex and body size on $\delta^{13}C$ and $\delta^{15}N$ values from each of the reef predators, we found that sex did not have a significant effect on $\delta^{13}C$ or $\delta^{15}N$ (Table 2). However, size-related differ-

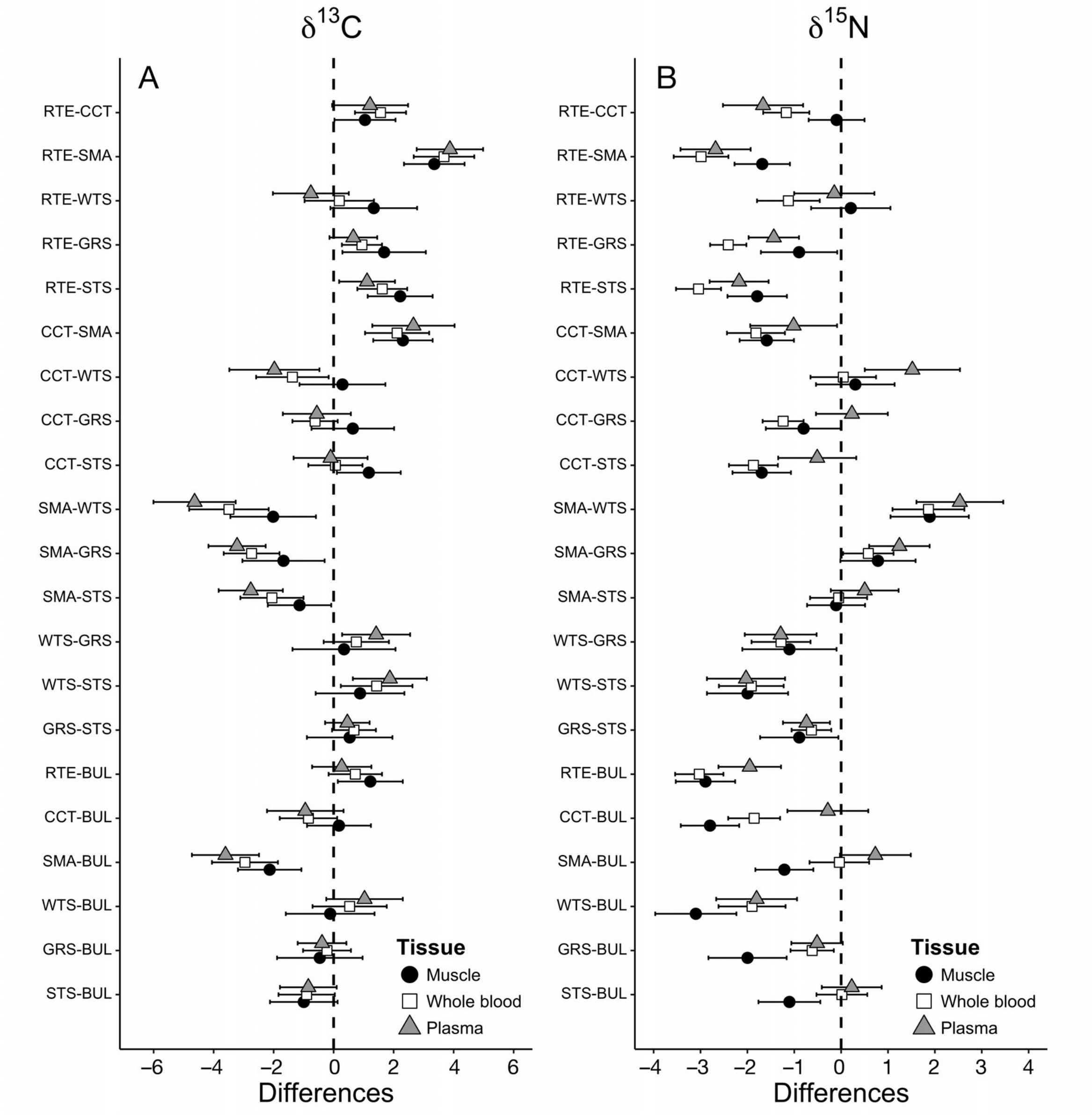

Fig. 3. Results from Tukey's post-hoc tests showing pairwise comparisons (mean ± 95 % CI) in isotopic values (δ^{13}C and δ^{15}N) of muscle, whole blood and plasma samples from sharks and teleosts. See Fig. 2 legend for species abbreviations

ences in δ^{13}C were detected in whole blood and plasma samples from grey reef sharks (Fig. 6E), and in muscle and whole blood samples from common coral trout (Table 2, Fig. 7C). A significant relationship between size and δ^{15}N was also found in silvertip (Fig. 6D) and grey reef sharks (Table 2, Fig. 6F); however, this relationship was only detected in whole blood and plasma samples from silvertip sharks. In teleosts, a positive relationship between δ^{15}N and size was detected in Spanish mackerel (Fig. 7B), whereas the redthroat emperor had a negative relationship (Fig. 7F).

4. DISCUSSION

Defining the role of sharks on coral reefs has sparked increasing interest among scientists (Roff et al. 2016a, Ruppert et al. 2016), especially since some species have already experienced significant population declines (Graham et al. 2010, Ward-Paige et al. 2010, Nadon et al. 2012). In the GBR, there is a high diversity of sharks and other large-bodied predators that use coral reefs, including non-resident species (Ceccarelli et al. 2014, Espinoza et al. 2014), which could cause strong intra- and inter-specific competi-

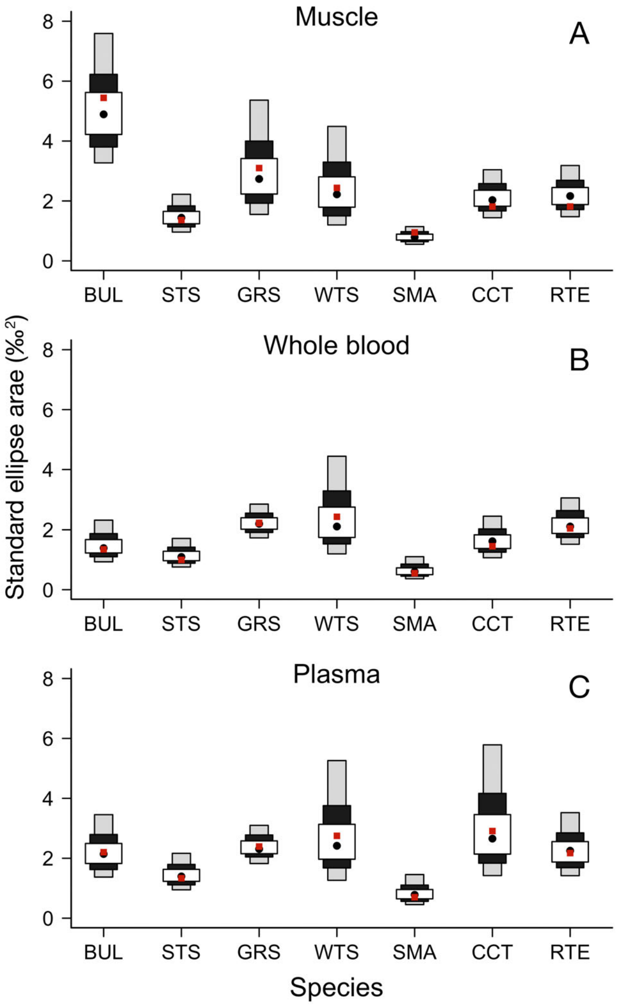

Fig. 4. Credibility intervals of Bayesian standard ellipse areas (SEA). Black circles are mode SEA and red squares small sample size-corrected SEA (SEA_C). Shaded boxes show the 50, 75 and 95% credibility intervals for each species. See Fig. 2 legend for species abbreviations

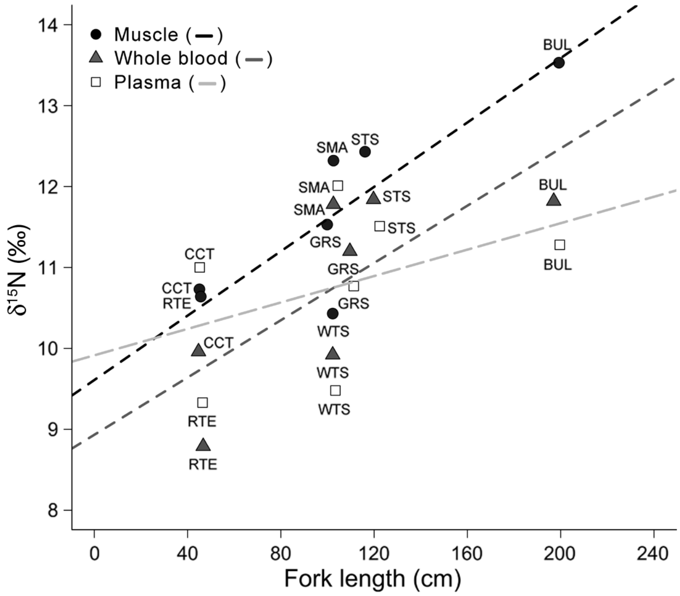

Fig. 5. Relationship between mean $\delta^{15}N$ and mean fork length in multiple tissues of sharks and teleosts, central Great Barrier Reef of Australia. See Fig. 2 legend for species abbreviations

tion for common resources such as food and space. However, previous studies have focused on reef shark trophic interactions (Frisch et al. 2016, Bond et al. 2018), but not on other larger reef-associated predators that may only use coral reefs opportunistically and/or seasonally. This complexity makes it difficult to define direct coral reef food web links and species functional roles. Therefore, a more detailed assessment of the trophic ecology of reef-associated predators, particularly non-resident species such as bull sharks, may provide key evidence to fill these gaps, improving our understanding of the role they play in coral reef food webs.

Here we have revealed significant trophic separation among some of the most common upper level reef predators from the central GBR, especially when considering isotopic data from muscle, a slower turnover tissue that provides an integration of diet over a longer time period. Based on our data, the bull shark, a large wide-ranging coastal species (Heupel et al. 2015), occupies a higher trophic position and larger niche breadth relative to smaller predators, including resident sharks and large-bodied teleosts. In contrast, faster turnover tissues such as whole blood and plasma showed a higher degree of isotopic overlap between bull sharks and other reef predators. These results suggest that bull sharks have similar prey sources to, and are likely interacting with, large and small resident predators, at least over shorter timescales. Consequently, bull sharks may play an important role in coral reef food webs through direct predation, competition and/or through fear effects. Although reef predators may be feeding on similar prey types, their movement patterns are known to differ over different spatial and temporal scales (Papastamatiou et al. 2009, Speed et al. 2011, Espinoza et al. 2015b), which may reduce competition for resources by accessing these prey types in different areas. For example, reef sharks have been shown to partition habitat by depth (Heupel et al. 2018) and exhibit different degrees of reef connectivity (Chin et al. 2013, Espinoza et al. 2015a), whereas other common reef predators from the GBR, such as redthroat emperor and common coral trout, show different space use and movement patterns (Currey et al. 2014, Matley et al. 2016b,c).

Table 2. Linear regression model results testing the effects of sex and fork length (cm) on δ^{13}C and δ^{15}N values (muscle tissue) of the reef predators bull shark *Carcharhinus leucas*, silvertip shark *C. albimarginatus*, grey reef shark *C. amblyrhynchos*, whitetip reef shark *Triaenodon obesus*, Spanish mackerel *Scomberomorus commersoni*, common coral trout *Plectropomus leopardus* and redthroat emperor *Lethrinus miniatus*

Species	δ^{13}C					δ^{15}N				
	Estimate	SE	*t*-value	p	R^2	Estimate	SE	*t*-value	p	R^2
Bull shark										
Intercept	−21.321	3.428	−6.220	<0.001	0.17	19.475	2.119	9.187	<0.001	0.33
Sex (female)	0.216	0.769	0.280	0.782		0.222	0.476	0.467	0.645	
Fork length	0.033	0.017	1.973	0.063		−0.031	0.010	−2.975	0.007	
Silvertip shark										
Intercept	−15.951	0.652	−24.452	<0.001	0.02	11.412	0.507	22.518	<0.001	0.23
Sex (female)	−0.007	0.309	−0.024	0.981		0.372	0.240	1.552	0.137	
Fork length	0.003	0.005	0.571	0.574		0.007	0.004	1.645	0.116	
Grey reef shark										
Intercept	−15.631	0.833	−18.753	<0.001	0.22	8.131	0.872	9.324	<0.001	0.71
Sex (female)	0.536	0.672	0.798	0.451		−0.723	0.703	−1.029	0.338	
Fork length	0.005	0.009	0.539	0.607		0.035	0.009	3.915	0.006	
Whitetip reef shark										
Intercept	−16.958	2.559	−6.627	<0.001	0.19	7.724	2.233	3.458	0.018	0.23
Sex (female)	0.255	0.784	0.326	0.758		−0.122	0.684	0.865	0.865	
Fork length	0.021	0.027	0.763	0.479		0.026	0.023	1.135	0.308	
Spanish mackerel										
Intercept	−17.339	0.816	−21.240	<0.001	0.03	10.498	0.801	13.098	<0.001	0.18
Sex (female)	0.039	0.184	0.214	0.823		−0.055	0.181	−0.304	0.763	
Fork length	0.006	0.008	0.705	0.488		0.018	0.008	2.298	0.031	
Common coral trout										
Intercept	−22.739	1.584	−14.358	<0.001	0.77	12.060	0.716	16.832	<0.001	0.28
Sex (female)	0.576	0.607	0.949	0.36		−0.233	0.275	−0.849	0.411	
Fork length	0.188	0.030	6.336	<0.001		−0.030	0.013	−2.211	0.456	
Redthroat emperor										
Intercept	−16.100	2.494	−6.455	0.003	0.52	11.288	0.932	12.115	<0.001	0.26
Sex (female)	−1.218	0.739	−1.647	0.175		0.256	0.276	0.928	0.406	
Fork length	0.068	0.053	1.259	0.276		−0.015	0.020	−0.744	0.498	

Isotopic values (δ^{13}C and δ^{15}N) from reef-associated predators sampled in the central GBR varied considerably across tissues. While stable isotopes from muscle revealed clear trophic separation among bull sharks and large (Spanish mackerel, grey reef and silvertip shark) and small reef-associated predators (whitetip reef shark, common coral trout, redthroat emperor), the degree of trophic overlap increased significantly in faster turnover tissues such as whole blood and plasma. Based on muscle samples, for example, adult bull sharks had higher δ^{15}N and significantly broader δ^{13}C relative to other predators, indicating that: (1) they feed at higher trophic positions over a longer period of months to years, assuming consistent δ^{15}N baselines (Malpica-Cruz et al. 2012, Hussey et al. 2012a); and (2) they forage from a more diverse range of carbon sources, likely over a wider geographical range (Matich & Heithaus 2014). Moreover, given that they are highly migratory and show a wide isotopic niche based on muscle, it would seem likely that baseline effects are at least partly explaining the elevated δ^{15}N. Similar findings have been reported by Daly et al. (2013) in the southwest Indian Ocean. These authors suggested that adult bull sharks may have the ability to exploit a more diverse range of coastal habitats and prey on a wider resource pool than other large predators found in the same region. Bull sharks are opportunistic feeders, and their diet consists mainly of teleosts and small sharks, but other groups such as sea turtles, birds, dolphins, crustaceans, echinoderms and stingrays have also been reported (Snelson et al. 1984, Last & Stevens 2009). Therefore, over the course of a longer time period and given the extent of their movement patterns (Heupel et al. 2015, Espinoza et al. 2016), adult bull sharks are feeding from multiple ecosystems, which provides a rational explanation for their broader isotopic niche relative to other reef predators.

Examination of faster turnover tissues such as whole blood and plasma, however, revealed that bull

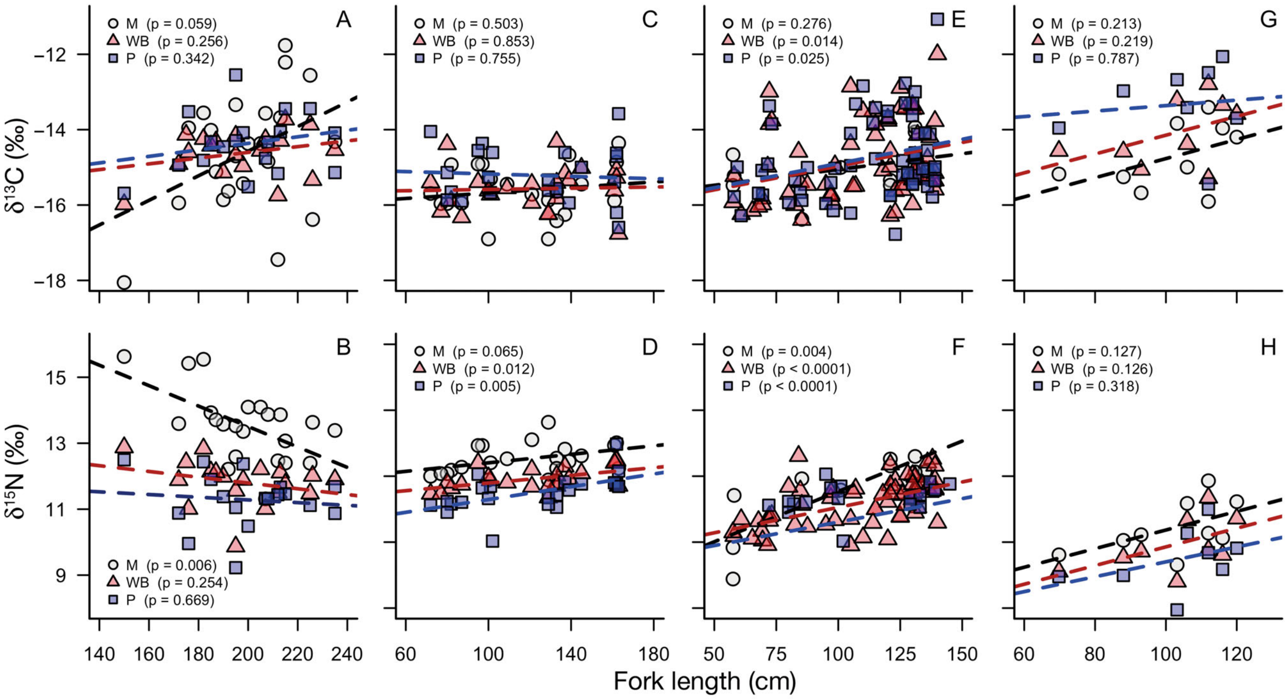

Fig. 6. Relationship between isotopic values ($\delta^{13}C$ and $\delta^{15}N$) and fork length of reef-associated sharks, central Great Barrier Reef of Australia. Panels indicate isotope values of muscle (M), whole blood (WB) and plasma (P) samples from the (A,B) bull shark *Carcharhinus leucas*, (C,D) silvertip shark *C. albimarginatus*, (E,F) grey reef shark *C. amblyrhynchos* and (G,H) whitetip reef shark *Triaenodon obesus*. Dashed lines indicate regression slopes for muscle (black dashed line), whole blood (red dashed line) and plasma (blue dashed line)

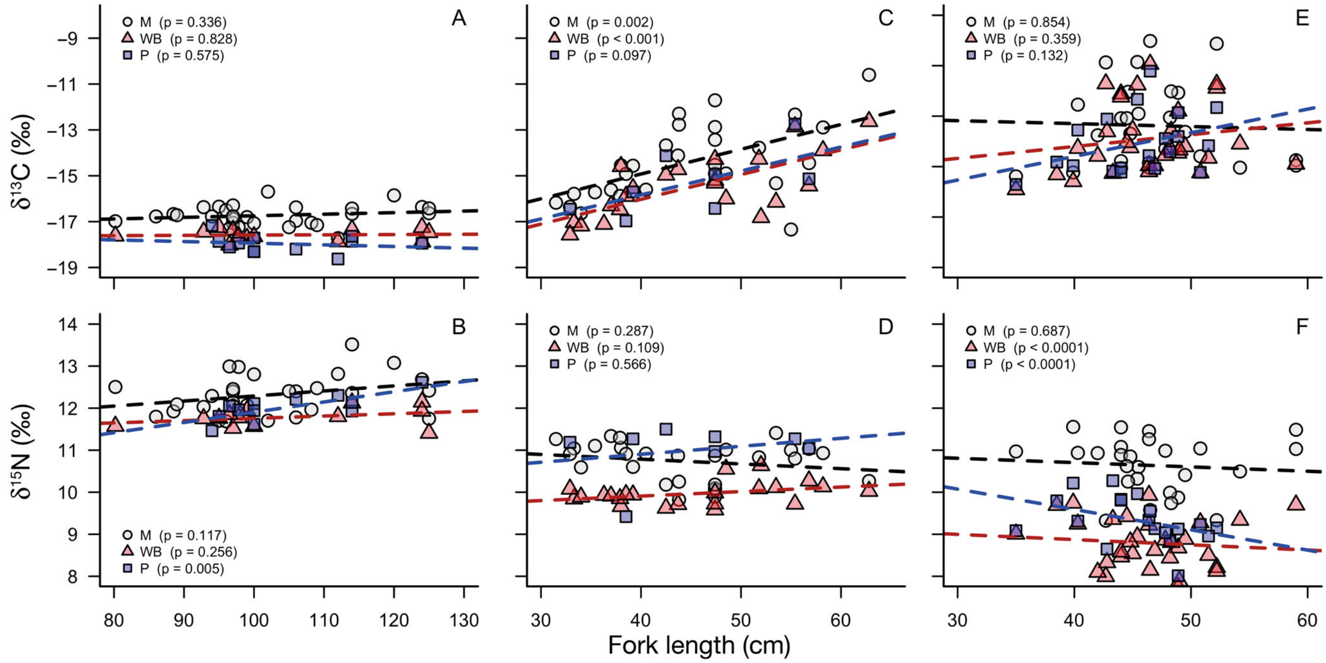

Fig. 7. Relationship between isotopic values ($\delta^{13}C$ and $\delta^{15}N$) and fork length of reef-associated teleosts, central Great Barrier Reef of Australia. Panels indicate isotope values of muscle samples from the (A,B) Spanish mackerel *Scomberomorus commerson*, (C,D) common coral trout *Plectropomus leopardus* (E,F) and redthroat emperor *Lethrinus miniatus*. See Fig. 6 for definitions

sharks had similar $\delta^{15}N$ to common coral trout and grey reef and silvertip sharks at more recent time periods relative to sampling, and were also feeding on isotopically similar prey items. These tissues from bull sharks had $\delta^{13}C$ values that reflected some of the central GBR coral reef primary producers, including macroalgae (−17.9 ± 1.5, mean ± SD; N = 15), hard corals such as *Pocillopora damicornis* (−16.3 ± 0.5; N = 10) and *P. verrucosa* (−15.3 ± 0.8; N = 13) and bulk plankton (−20.8 ± 0.8; N = 20) (M. Espinoza unpubl. data). The smaller isotopic niche of bull sharks from whole blood and plasma also suggests that individuals are feeding from and/or competing for similar resources with smaller predators when present in reefs. The large isotopic niche of the muscle suggests more variability in prey selection across longer time periods, likely when away from reef habitats. Alternatively, it could be a reflection a high degree of variability in past movement patterns and occupancy of regions with different isotope baselines.

The extent to which bull sharks may be competing (directly or indirectly) with large reef predators such as grey reef and silvertip sharks is unknown. Grey reef and silvertip sharks exhibit high reef fidelity and low to intermediate levels of reef connectivity within the central GBR (Espinoza et al. 2015a,b,c, Heupel & Simpfendorfer 2015). In contrast, bull sharks typically spend less than 20% of their time on reefs near their tagging site, and up to 51% of the tagged population from the central GBR undertook long-range migrations (Espinoza et al. 2016). Interestingly, Espinoza et al. (2016) demonstrated that a portion of the population was detected year-round, and all migrating individuals (except 1) returned to the central GBR reefs, which means that at least some bull sharks may have stronger interactions with resident reef predators. Based on movement studies of bull sharks within the GBR (Heupel et al. 2015, Espinoza et al. 2016) and the results presented in this study, some individuals, and/or individuals at key times when on the reef, are likely overlapping with several reef predators, and therefore, trophic interactions may be more complicated than might be implied by body size alone.

Spanish mackerel and grey reef and silvertip sharks occupied similar trophic positions, assuming similar baseline $\delta^{15}N$. However, $\delta^{13}C$ across tissues revealed that Spanish mackerel had a more specialized pelagic feeding behaviour (i.e. narrow isotopic niche breadth and high unique isotopic area), which suggests these species do not share similar prey or foraging grounds. Spanish mackerel feed primarily on small pelagic fishes such as anchovies, sardines, threadfin herring and silversides (Collete & Nauen 1983). In contrast, the diets of grey reef and silvertip sharks consist of a wide range of reef fishes (e.g. surgeon fish, butterfly fish, cowfish), and to a lesser extent cephalopods and crustaceans (Wetherbee et al. 1997, Frisch et al. 2016), all of which are benthic and reef-associated. Therefore, although $\delta^{15}N$ values are comparable, these predator species are reducing competition by utilizing different habitats for feeding.

Small resident sharks and teleosts, including the whitetip reef shark, common coral trout and redthroat emperor, occupied the lowest relative trophic positions of the predators studied based on $\delta^{15}N$. Whitetip reef sharks and redthroat emperor both exhibit similar diet selection based on stomach content studies, particularly for benthic prey such as crustaceans, echinoderms, molluscs and small fishes (Randall 1977, Carpenter 2001). In contrast, adult common coral trout feed on a variety of reef-associated fishes, but pelagic sources are predominant, particularly damselfishes and fusiliers (St. John 2001, Frisch et al. 2014, Matley et al. 2018). Therefore, despite their close association with benthic reef structure, common coral trout have similar $\delta^{13}C$ to grey reef sharks, reflecting pelagic feeding. Furthermore, high intake of secondary and tertiary consumers by large resident and opportunistic sharks may have resulted in similar $\delta^{15}N$, at least at a short-term scale (i.e. plasma), indicating similar trophic interactions and prey sources.

Isotopic niche breadth remained relatively constant across tissues for large and small resident sharks, suggesting consistent feeding through time. Unlike the large opportunistic and wide-ranging bull shark (Brunnschweiler & Barnett 2013, Daly et al. 2014, Heupel et al. 2015), large and small reef shark species in the GBR are often detected on a single reef year-round and/or commonly disperse relatively short to intermediate distances before returning to their tagging reef (Barnett et al. 2012, Espinoza et al. 2015a, Heupel & Simpfendorfer 2015). The consistency of niche breadth and relative isotopic niche space across tissues for resident sharks suggests similar resources (within-species) are accessed at reefs throughout a relatively large temporal scale. In contrast, the isotopic niche breadth of small resident teleosts was larger over shorter periods, particularly for plasma of coral trout. This is not surprising considering the polyphagous diet of coral trout, which consists of more than 20 families (St. John 2001, Matley et al. 2018). Over longer time periods (e.g. in muscle), the isotopic variation between individuals likely decreased as more prey were incorporated in the tissue reducing the effect of rare prey or outlying isotopic values. This may also help explain why coral trout were feeding at an equivalent trophic level to larger predators such as sharks (except whitetip reef shark) only in plasma tissue, although inter-reef isotopic variability or seasonal dietary changes are also possible reasons. The large isotopic overlap also means that in recent periods, individuals were feeding on similar resources, and that seasonal variation in feeding was greater than individual-level variation.

The size of individuals was an important factor influencing stable isotope values of shark and teleost predators. Sex did not have any effect, suggesting that both males and females share similar diets and foraging grounds. When comparing δ^{15}N with species size, there was a clear positive trend for all tissues indicating larger species are foraging at higher trophic levels. This trend is likely more pronounced after accounting for narrowing discrimination as trophic positions increase (Hussey et al. 2014). At the species level, size–δ^{15}N relationships were more variable across tissues, but significant positive relationships were found in Spanish mackerel and grey reef and silvertip sharks. For example, all tissues of grey reef sharks had a significant positive size–δ^{15}N relationship across the range sampled (58–140 cm FL). A similar relationship was present for silvertip sharks (size range: 72–163 cm FL), although we did not detect any statistical difference in muscle samples. Similar patterns have been reported in other shark and teleost predators (Lowe et al. 1996, McElroy et al. 2006, Usmar 2012, Pereira et al. 2015), suggesting ontogenic changes in prey consumption. Prey consumption changes associated with growth/development are common in sharks (Lowe et al. 1996, Bethea et al. 2007) and teleosts (Pereira et al. 2015). As individuals grow older/larger they are less restricted by learned/physiological constraints such as swimming ability or gape-width, leading to greater ability to capture larger prey (Dean & Motta 2004, Bethea et al. 2007, Espinoza et al. 2012). Surprisingly, the regression of bull shark muscle tissue indicated a significant negative relationship between δ^{15}N and size (size range: 150–235 cm FL). This unexpected trend may be attributed to feeding in a variety of food webs with different baseline δ^{15}N sources when individuals move between inshore/estuarine and coral reef habitats on the east coast of Australia (Werry et al. 2011, Heupel et al. 2015, Espinoza et al. 2016). This idea is supported, in part, by the large variation in muscle δ^{13}C throughout the size range.

Size had limited influence on δ^{13}C in smaller, resident sharks indicating similar sources of prey independent of growth. In both grey reef and silvertip sharks, larger individuals, and often males, are known to disperse greater distances from their tagging reef than smaller individuals (Espinoza et al. 2015b,c). Espinoza et al. (2015b) suggested a male-biased dispersal strategy may be advantageous to increase genetic diversity/population connectivity, and increase foraging opportunities while reducing competition with larger females. However, based on the δ^{13}C from resident species, sharks might disperse to other areas for reproduction, but may forage close to their home reef or alternatively feed on similar prey that is found on multiple reefs and has similar isotope values between reefs. Meanwhile, the trend of higher δ^{13}C in larger individuals for muscle and whole blood of common coral trout suggests an ontogenetic shift to pelagic prey. Typically, the shift from benthic to pelagic prey occurs close to maturity (e.g. ~35 cm; St. John 2001); however, given the extended time for slow-turnover tissues to incorporate diet (Matley et al. 2016a), this trend may still be apparent in larger common coral trout caught in this study.

It is relevant to note that trophic position of predators could not be reliably calculated using isotope values from baseline organisms at each reef and sampling period. Although converting δ^{15}N to TP would have provided context for comparing our study to other studies, we chose not to because: (1) there was a lack of appropriate samples at every reef; (2) as all samples were sharks of relatively similar sizes, conversion of δ^{15}N to TP would produce the exact same relative values as δ^{15}N; and (3) it would lead to a large reduction in sample size. This reduces confidence in the ability to group and compare individuals or species. Given the spatial extent in which these predators move, this was logistically difficult to accomplish and interpret. To be conservative, general trends have been discussed based on isotope patterns grouped among nearby reefs. Also, isotopic variation of fusiliers, a small planktivorous fish sampled at several reefs, differed by less than 0.2‰ (see Table 1); therefore, reef-wide variation is likely not significant and isotopic differences in predators are mainly driven by diet. Bull sharks may be the exception given their continental-scale migrations (Heupel et al. 2015). Similarly, variation in diet–tissue discrimination among predator species could not be accounted for in this study, and may be in part responsible for interspecific differences in isotopic values (Hussey et al. 2010, Olin et al. 2013, Matley et al. 2016a). Nevertheless, the effect is likely minimal since trophic levels of predatory groups conformed as expected based on known feeding ecology.

Predators are increasingly recognized as key elements in food web connectivity and stability because of their ability to link the fluxes of nutrients and energy within (Papastamatiou et al. 2015, Williams et al. 2018) and between spatially separated ecosystems (Rooney et al. 2006, Schmitz et al. 2010). For example, grey reef sharks at Palmyra Atoll were estimated to transfer approximately 90 kg of nitrogen per day to the reef from offshore sources, further suggesting that sharks might play an important role in bottom up control (Williams et al. 2018). Wider ranging predators such as the bull shark not only can facilitate nutrient transfer within coral reefs, but also might connect offshore reef habitats from the GBR with inshore bays and estuaries in southern Queensland and New South Wales (Espinoza et al. 2016). Therefore, further studies integrating stable isotopes with telemetry data of mobile predators that occupy high trophic positions, and that are capable of exploiting a wide range of resources across habitats, may increase our understanding of the role they play in bottom up control, food web structure and stability (Rooney & McCann 2012).

5. CONCLUSIONS

Collectively, our findings highlight the importance of understanding linkages between the spatial and trophic ecology of reef-associated predators over different temporal scales, particularly in larger non-resident species that may have a high level of trophic interaction with common reef predators. Similar studies have shown that reef sharks tend to occupy similar trophic positions and functional roles to those of large-bodied teleosts (Frisch et al. 2016, Bond et al. 2018). This seemingly high level of trophic redundancy could explain the lack of evidence for shark-induced trophic cascades in coral reef studies (Frisch et al. 2016, Casey et al. 2017), as some reef sharks are likely acting as mesopredators rather than apex predators in coral reefs (Heupel et al. 2014, Roff et al. 2016a, Bond et al. 2018). However, given that stable isotope studies of reef-associated predators have typically used muscle samples (Speed et al. 2012, Daly et al. 2013, Frisch et al. 2016, Bond et al. 2018), investigating dietary changes in faster-turnover tissues remains crucial to a better understanding of the effect transient and/or seasonal predators could have on coral reef food webs.

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Appendix.

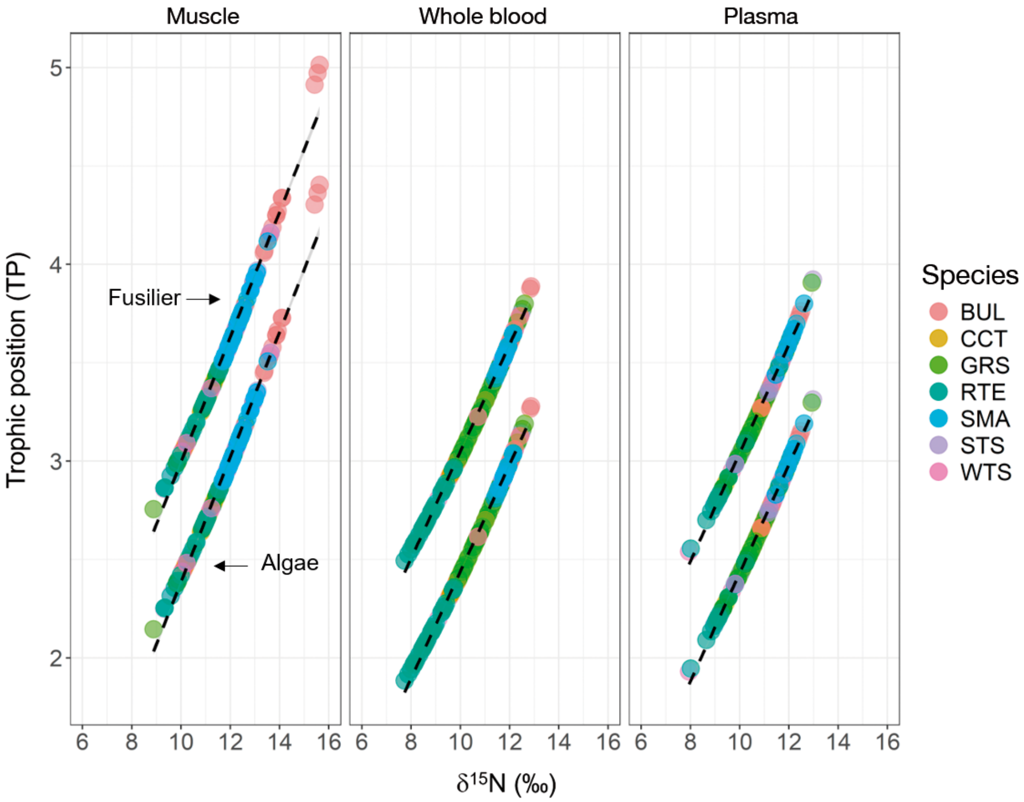

Fig. A1. Relationship between δ^{15}N and trophic position (TP) among the 3 tissues of sharks (BUL: bull shark *Carcharhinus leucas*, STS: silvertip shark *C. albimarginatus*, GRS: grey reef shark *C. amblyrhynchos*, WTS: whitetip reef shark *Triaenodon obesus*) and teleosts (SMA: Spanish mackerel *Scomberomorus commersoni*, CCT: common coral trout *Plectropomus leopardus*, RTE: redthroat emperor *Lethrinus miniatus*). Two different TP estimates were calculated using narrowing diet–tissue discrimination factors (see Hussey et al. 2014) based on yellowtail fusilier *Caesio cuning* (upper line; $TP_{baseline} = 3$) and algae *Halimeda* spp. (lower line; $TP_{baseline} = 1$), under the assumption that baseline δ^{15}N values were similar reef-wide. A linear regression is plotted over the estimates

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