REPORT

Trophic ecology of reef sharks determined using stable isotopes and telemetry

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Abstract Establishing the ecological role of predators within an ecosystem is central to understanding community dynamics and is useful in designing effective management and conservation strategies. We analysed differences in the trophic ecology of four species of reef sharks (*Carcharhinus melanopterus*, *Carcharhinus amblyrhynchos*, *Triaenodon obesus* and *Negaprion acutidens*) at Ningaloo Reef, Western Australia, by analysing tissue stable isotopes (δ^{15}N and δ^{13}C). We also monitored animals using acoustic telemetry to determine long-term residency patterns in a bay at the southern end of the reef, Coral Bay. Overall, mean δ^{13}C was similar among species, ranging between -10.9 and -11.8‰, suggesting a food-web dependency on coastal producers. Classification and regression tree analysis identified an effect of species on δ^{15}N that separated *C. amblyrhynchos* and *C. melanopterus* from *N. acutidens* and *T. obesus*. For *C. amblyrhynchos* and *C. melanopterus*, animals were also divided by size classes, with smaller sharks having lower average δ^{15}N than larger animals; this suggests that δ^{15}N increases with size for these two species. Juvenile *C. melanopterus*, juvenile *N. acutidens* and adult *T. obesus* had trophic levels of 3.7, for juvenile *C. amblyrhynchos* and adult *C. melanopterus* it was 4, and adult *C. amblyrhynchos* had a value of 4.3. Trophic-level estimates for *C. melanopterus* and *C. amblyrhynchos* corroborate previous conclusions based on diet studies. We found no evidence for a difference in isotopic composition between resident and non-resident sharks. The lack of variation in isotopic composition was consistent with high mean residency of these species recorded using acoustic telemetry, which was 79% (± 0.09 SE) of days monitored for *T. obesus*, followed by *N. acutidens* ($57 \pm 19.55\%$), *C. amblyrhynchos* ($54 \pm 13\%$) and *C. melanopterus* ($33 \pm 8.28\%$). High δ^{13}C composition in reef sharks and long-term residency behaviour suggest that coastal marine reserves might provide effective conservation refuges for some species.

Keywords Elasmobranch · Food web · Acoustic monitoring · Residency · δ^{13}C · δ^{15}N

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Introduction

Identifying an animal's trophic role within an ecosystem is a prerequisite for any understanding of larger processes of community dynamics. Sharks are often classified as apex predators within marine ecosystems, although there is much dietary variation among species (e.g. Cortés 1999). This plasticity in feeding habits, combined with ontogenetic and spatiotemporal changes in diet, has complicated our understanding of the ecology of this diverse group (Wetherbee and Cortés 2004). As predators exerting top-down influences on communities (Stevens et al. 2000; Myers and Worm 2003; Bascompte et al. 2005; Shepard and Myers 2005; Myers et al. 2007), coupled with declines in populations (Ferretti et al. 2010), sharks have become the focus of recent management and conservation research (Field et al. 2009; Speed et al. 2010). However, the role individual species play within communities is still often unclear, which makes predictions of the consequences of their removal from ecosystems tenuous at best. To redress this problem, long-term studies (months to years), obtaining species-specific dietary and behavioural information, is essential.

The trophic position and role of sharks have been determined primarily using two methods: (1) description of stomach contents (e.g. Cortés 1999) and (2) comparison of stable isotope composition (mainly δ^{15}N) of sharks and their prey (Estrada et al. 2003). The former technique typically requires capture and killing of the animal and can only provide a snapshot of recently consumed items. In contrast, stable isotope analysis provides information on assimilated rather than just consumed prey items, thus reflecting long-term feeding behaviour (Estrada et al. 2003), albeit providing a lower taxonomic resolution of diet than traditional dietary studies. Furthermore, samples for isotope analysis can be collected using non-lethal methods via the removal of a small sample tissue. For these reasons, the use of stable isotopes in elasmobranch research has increased over the past decade (e.g. Fisk et al. 2002; Estrada et al. 2003; Domi et al. 2005; MacNeil et al. 2005; Revill et al. 2009; Papastamatiou et al. 2010a; Borrell et al. 2011; Matich et al. 2011; Vaudo and Heithaus 2011). However, the use and application of this technique has been questioned recently due to a poor understanding of diet-tissue discrimination factors in sharks (Hussey et al. 2010a) and a general lack of baseline stable isotope data (Post 2002), both of which are necessary for accurate determination of trophic positions.

Some of the variability in isotopic values among individuals of the same species has been explained by combining isotopic analyses with other types of sampling. For example, telemetry has been used in conjunction with analysis of stable isotopes to provide information on long-term movement patterns of birds (Suryan and Fischer 2010), fish (Cunjak et al. 2005), and also reef sharks (Papastamatiou et al. 2010a). Generally, these studies show that individual variability in stable isotopic composition can be attributed to diet changes arising from differences in migration and residency patterns. Similarly, spatial variation in fish stable isotope composition has been investigated by sampling species across environmental gradients (Stephenson et al. 2001; Revill et al. 2009), although this approach appears to be useful only at regional scales (100 s of km) rather than at the smaller scale of a single coral reef (1–10 s km) (Papastamatiou et al. 2010a; Wyatt et al. 2010). Small-scale temporal changes (monthly, seasonal) in isotopic composition are difficult to resolve for sharks because complete turnover of blood and muscle tissue can take up to 7 and 24 months, respectively (Logan and Lutcavage 2010). This means that the stable isotope composition of large sharks that undergo broadscale migrations over protracted periods (100–1,000 km, e.g. white sharks, *Carcharodon carcharias*—Bonfil et al. 2005, whale sharks *Rhincodon typus*—Eckert et al. 2002) reflects the incorporation of sources with different stable isotope composition.

Sharks associated with coral reefs generally have small home ranges (<100 km^2; McKibben and Nelson 1986; Papastamatiou et al. 2009), although large movements (>100 km) have been observed occasionally (Heupel et al. 2010). Many of these species are classified as apex predators and are thought to occupy similar niches (e.g. Papastamatiou et al. 2006). Gut content analyses have generally placed reef sharks around the trophic level of 4 (ranging from 3.9 to 4.2) (Cortés 1999), and the diets of the most common sharks on Indo-Pacific reefs, the blacktip reef *Carcharhinus melanopterus*, grey reef *Carcharhinus amblyrhynchos* and whitetip reef *Triaenodon obesus* sharks, have been documented in a number of studies (Randall 1977; Stevens 1984; Stevens and McLoughlin 1991; Salini et al. 1992; Wetherbee et al. 1997; Papastamatiou et al. 2006). However, there is little information available on the geographic or ontogenetic changes in diet of many species of reef sharks (but see Stevens 1984; Wetherbee et al. 1997). Ontogenetic changes in the diet of some species of sharks have been documented (Wetherbee and Cortés 2004), although it is unknown whether such shifts are common in reef sharks.

At Ningaloo Reef, Western Australia, four species of reef sharks (*C. melanopterus*, *C. amblyrhynchos*, *T. obesus* and juvenile *Negaprion acutidens*) aggregate in an inshore bay at the southern end of the reef (Speed et al. 2011). The abundance and diversity of sharks within this site provides ideal conditions for the study of intra- and inter-specific differences in trophic ecology through the combined use of stable isotopes (δ^{15}N and δ^{13}C) and telemetry.

We hypothesised that: (1) *C. melanopterus*, *C. amblyrhynchos* and *T. obesus* adults occupy a trophic position of around 4, as estimated in the previous dietary studies, (2) juvenile sharks occupy a lower trophic position than adults due to ontogenetic differences in diet, (3) within a species, there is no gender difference in trophic position, (4) no differences occur across years in $\delta^{15}N$ and $\delta^{13}C$ values in any species and (5) no differences occur in isotopic values between 'resident' and 'non-resident' individuals due to restricted movements of these species.

Methods

Study site

Our study site was at the southern end of Ningaloo Reef in Western Australia (−23° 08′ 41″, 113° 45′ 53″) (Fig. 1). This area encompasses Skeleton and Bill's Bays, where reef sharks are known to aggregate (Speed et al. 2011). Bill's Bay is a relatively shallow (1–3 m) lagoon, approximately 2.5 × 5 km in dimension to the north of the township of Coral Bay.

Data collection and processing

We sampled at Skeleton Bay in November and December of 2008 and 2009, and August 2010. We caught sharks from the beach using hand lines with baited barbless hooks. We used a variety of baits, which included frozen squid (*Loligo opalescens*), frozen octopus (unknown species) and frozen pilchards (*Sardinops neopilchardus*), as well as fresh reef fish frames that were discarded from local commercial fishers. Fishing times were generally from late morning

Fig. 1 Map of study site. Study site showing acoustic receivers (*filled circle*), approximate mean maximum detection range of receivers (*dashed line*), reef (*line with square*), sand (*open square*) and land (*filled square*)

until early evening, when shark aggregations were most common (Speed et al. 2011). Upon capture, we kept sharks in a holding tank on the beach and placed them upside down to induce tonic immobility. We put Jumbo Rototags (Dalton Supplies, Henley-on-Thames, United Kingdom) in the first dorsal fin of all animals and recorded sex, and total length (L_T) in cm. Life stage (i.e. juvenile or adult) was determined by (L_T) and previous size-at-age measurements (Last and Stevens 2009). We considered individuals to be adults if the L_T was greater than 130 cm in *C. amblyrhynchos*, 95 cm in *C. melanopterus*, 220 cm in *N. acutidens* and 112 cm in *T. obesus* (Speed et al. 2011). We also tagged some sharks with acoustic transmitters by making a 2-cm incision above the cloaca for acoustic tag insertion (V13-1H (153 dB), V16-5H (165 dB) and V16-5x (165 dB) Vemco©, Halifax, Canada) into the peritoneal cavity. We took a tissue sample (<1 g) from the white muscle of the gut wall for stable isotope analysis prior to closing the incision with absorbable sutures (Ethicon©). We repeated this procedure for animals that were not implanted with acoustic tags, although we made a smaller incision (1 cm). We used no anaesthetics during the procedure. This process took on average 12.3 (±0.4 SE) minutes from capture to release of animals (Speed et al. 2011). The release of sharks in good condition was paramount because we sampled in a marine sanctuary and were also interested in the long-term behavioural patterns of these animals. Sharks tagged with acoustic transmitters were monitored with an acoustic array of nine receivers (VR2w Vemco©, Halifax, Canada) that had mean maximum detection ranges of approximately 300 m (Fig. 1) (Speed et al. 2011).

We stored muscle samples for stable isotope analysis below −20°C. We then dried samples at 60°C to constant weight prior to being weighed into tin capsules and analysed with a isotope ratio mass spectrometer (20–20 IRMS, Europa, Crewe, United Kingdom) coupled with an elemental analyser (ANCA-GSL, Europa, Crewe, United Kingdom). We interspaced reference materials of known elemental composition and isotopic ratios with the tissue samples for calibration (USGS41 [$\delta^{15}N$ = 47.6‰, $\delta^{13}C$ = 37.6‰] and USGS40 [$\delta^{15}N$ = −4.5‰, $\delta^{13}C$ = −26.4‰]). Nitrogen isotope ratios ($\delta^{15}N$) are reported in parts per thousand (‰) deviation from N^2 in air, while carbon isotope ratios ($\delta^{13}C$) are ‰ deviation from Pee Dee Belemnite (V-PDB) using the following equation:

$$\delta X = \left[\frac{R_{Sample}}{R_{Standard}} - 1\right] 1000 \qquad (1)$$

where X is ^{13}C or ^{15}N, and R is the corresponding ratio $^{13}C{:}^{12}C$ or $^{15}N{:}^{14}N$ (Peterson and Fry 1987). All samples had low C:N ratios (mean 2.77 ± 0.21 SD); therefore, we did not correct values for the potential effects of lipids (cf. Hussey et al. 2010b; Vaudo and Heithaus 2011).
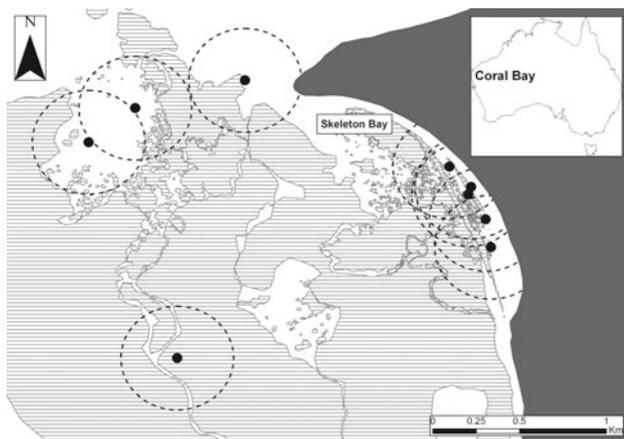
Inter- and intra-specific differences

For *C. melanopterus* and *C. amblyrhynchos*, we examined the relationship between size and $\delta^{13}C$ and $\delta^{15}N$ using linear regression. We applied classification and regression tree analyses (CART) (De'ath and Fabricius 2000) to explain the extent to which sharks' $\delta^{13}C$ and $\delta^{15}N$ depends on species, animal size (L_T), sex, maturity and sampling year. We used CART analysis because it is a non-parametric test that accommodates unbalanced designs, reduced sample sizes, missing values and outliers (De'ath and Fabricius 2000). We did this analysis using the TREES package on S-PLUS 2000® (MathSoft, Cambridge, MA, USA). We only included individuals tagged in November 2008 and 2009 in the CART analysis due to only two specimens being collected in August 2010.

Trophic positions

We estimated the trophic position of each species and size class using the following equation:

$$\text{Trophic position} = 2 + \left(\delta^{15}N_{consumer} - \delta^{15}N_{base}\right)/\Delta\delta^{15}N \tag{2}$$

where '2' is the trophic position of the primary consumers as a baseline, $\Delta\delta^{15}N$ is $\delta^{15}N$ trophic fractionation, $\delta^{15}N_{base}$ is the baseline $\delta^{15}N$ for the local food web and $\delta^{15}N_{consumer}$ is the $\delta^{15}N$ of the target species (Cabana and Rasmussen 1996; Vander Zanden and Rasmussen 1999; Post 2002). We used $\delta^{15}N$ values of the herbivorous fishes *Acanthurus triostegus* ($n = 22$), *Naso unicornis* ($n = 14$) and *Zebrasoma scopas* ($n = 12$) collected from three sites around Ningaloo Reef (one of which was Bill's Bay) as a $\delta^{15}N$ baseline (G. Hyndes, Edith Cowan University, unpubl. data). We averaged the data between the three species. We chose these primary consumers as indicators of the $\delta^{15}N$ of the base of the local food web because they are long-lived and inhabit the same area as the four target species of sharks, enabling the temporal and spatial integration of the isotopic composition of local producers (Cabana and Rasmussen 1996; Vander Zanden and Rasmussen 1999; Post 2002). The average $\delta^{15}N$ of these species was $7.9 \pm 0.2‰$ ($\pm$SE) ($n = 48$), and this value was considered as indicative of trophic position '2'. We used trophic fractionation values of 2.3‰ (average $\Delta\delta^{15}N$ value for sharks held in captivity (Hussey et al. 2010b)).

Residents versus non-residents

We compared shark isotopic composition for *C. melanopterus and C. amblyrhynchos* between 'resident' and 'non-resident' animals with a CART analysis to test the hypothesis that the two groups feed in different areas (inside vs. outside Coral Bay). We combined detections from all nine receivers and considered an individual present in Coral Bay if detected more than once by the array in a day. Residency to Skeleton Bay (an aggregation site within the Coral Bay area) has previously been reported (Speed et al. 2011); however, residency to the entire Coral Bay area has not. Given that receivers were not completely overlapping in detection ranges, it was possible that an individual could have been present within the array but not within the range of any of the receivers. Due to the small size of the study area (~ 4.5 km^2), and known daily movement patterns and home range sizes of reef sharks (e.g. McKibben and Nelson 1986; Papastamatiou et al. 2009; Heupel et al. 2010; Field et al. 2011), it is unlikely that the above scenario occurred often, and therefore, this would have had minimal impact on the overall results. For the purpose of this analysis, we considered a shark to be a resident if it was detected by receivers in more than 50% of the number of days of possible detections (i.e. number of days from tagging to end of study). We only included individuals tagged in 2008 in this analysis because they were monitored for >1 year, which would have captured any seasonal differences in residency patterns.

Using both stable isotopes and acoustic telemetry provides information on differing time periods (i.e. pre-sampling for isotopes and post-tagging for movement); therefore, we made an assumption that pre- and post-tagging behaviour was consistent. Given that all species of reef sharks have displayed residency and site fidelity across several years (Speed et al. 2011), it is reasonable to assume that pre-tagging behaviour is consistent with post-tagging behaviour, provided that no ontogenetic or environmental changes have influenced movement. This is a logical assumption due to the slow growth and development of sharks, coupled with the maximum tissue turnover rate for stable isotopes of up to 2 years (Logan and Lutcavage 2010). Furthermore, we observed no anomalies in environmental conditions in Coral Bay during the period of investigation, which might have affected movement patterns.

Results

Inter- and intra-specific differences in isotopic composition

We analysed muscle samples from 71 sharks across four species (*C. melanopterus* [$n = 53$], *C. amblyrhynchos* [$n = 10$], *T. obesus* [$n = 4$] and *N. acutidens* [$n = 4$]) for stable isotope composition (Electronic Supplemental Material, ESM Appendix 1). *Carcharhinus amblyrhynchos*

had the highest mean $\delta^{15}N$ (13.1 $\pm$ 0.2‰ $\pm$ SE), followed closely by *C. melanopterus* (12.6 $\pm$ 0.1‰), and *N. acutidens* (11.9 $\pm$ 0.6‰) and *T. obesus* (11.9 $\pm$ 0.3‰) (Table 1). Accordingly, CART analysis identified an effect of species on $\delta^{15}N$, which separated *C. amblyrhynchos* and *C. melanopterus* from *N. acutidens* and *T. obesus* (Fig. 2). For *C. amblyrhynchos* and *C. melanopterus*, a secondary divide occurred due to size, with sharks smaller than 103 cm L_T having on average lower $\delta^{15}N$ than larger animals, and for the larger group, a third split indicated that animals larger than 158 cm L_T had the highest $\delta^{15}N$ of all individuals (Fig. 2). The largest size grouping was comprised solely of *C. amblyrhynchos*. This suggests that for *C. amblyrhynchos* and *C. melanopterus*, $\delta^{15}N$ increases with size, although regression analysis did not identify evidence for a relationship (*C. melanopterus* [$R^2 = 0.06$, $F_{1, 51} = 3.27$, $P = 0.076$], Fig. 3a; *C. amblyrhynchos* [$R^2 = 0.38$, $F_{1, 8} = 4.97$, $P = 0.056$], Fig. 3b). The CART model only explained 33% of the variability, meaning that there might be other variables influencing animal $\delta^{15}N$. There was no evidence for an effect of year of tagging, sex or maturity on shark $\delta^{15}N$ because none of these variables was responsible for any branch separation in the tree (Fig. 2).

We found the highest $\delta^{13}C$ in *C. melanopterus* (-10.9 ± 0.2‰), followed by *T. obesus* (-11.0 ± 0.2‰), *N. acutidens* (-11.0 ± 0.7‰) and *C. amblyrhynchos* (-11.8 ± 0.5‰) (Table 1). We observed a higher variability for each species in $\delta^{13}C$ than in $\delta^{15}N$, with the greatest range in $\delta^{13}C$ occurring in adult *C. amblyrhynchos* (-15.05 to -10.15‰) (Fig. 4). As a result, we detected no separations by CART analysis for $\delta^{13}C$ in any species or size classes. We found a weak, positive relationship between $\delta^{13}C$ and size in *C. melanopterus* ($P = 0.032$; Fig. 3c). We found no evidence for a relationship between $\delta^{13}C$ and size in *C. amblyrhynchos* ($P = 0.548$; Fig. 3d). We did not examine relationships between $\delta^{13}C$ and $\delta^{15}N$ with total length in other species due to an insufficient number of samples.

Trophic positions

When using a trophic fractionation of 2.3‰, juvenile *C. melanopterus*, juvenile *N. acutidens* and adult *T. obesus*
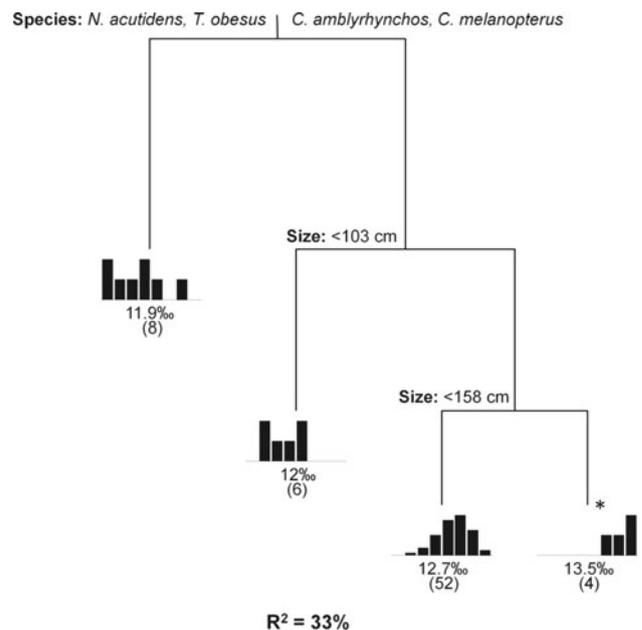
Fig. 2 Classification and regression tree of shark $\delta^{15}N$ values. Isotope values were compared with species, size, sex, maturity and year of tagging. Histograms of $\delta^{15}N$ distribution for each group (ranging from 10 to 14‰) are presented below the terminal nodes, and mean $\delta^{15}N$ and sample size (in *brackets*) are also indicated. *Asterisk* indicates that this group was comprised solely of *C. amblyrhynchos*

had trophic levels of 3.7, juvenile *C. amblyrhynchos* and adult *C. melanopterus* had a trophic level of 4 and adult *C. amblyrhynchos* had trophic levels of 4.3 (Table 2).

Residents versus non-residents

Of the 53 sharks we sampled for stable isotope analysis in 2008, we also successfully monitored 34 with acoustic transmitters. Average residency times were highest for *T. obesus* (79 $\pm$ 0.07% of days monitored), followed by *N. acutidens* (57 $\pm$ 19.55%), *C. amblyrhynchos* (54 $\pm$ 13%) and *C. melanopterus* (33 $\pm$ 8.28%) (ESM Appendix 1). Overall, 47% ($n = 16$) of the tagged animals were considered resident (>50% days detected), compared to 53% that were non-resident (<50% days detected). Of the 20 *C. melanopterus* we tagged, 6 were resident and

Table 1 Summary of reef sharks sampled for stable isotope analysis and acoustic monitoring

Species	Mean TL (cm $\pm$ SE)	Sex ratio (m:f)	Isotope sample (n)	Mean $\delta^{13}C$ ($\pm$SE; CV%)	Mean $\delta^{15}N$ ($\pm$SE; CV%)	Acoustic tag (n)
Carcharhinus amblyrhynchos	147.5 ($\pm$6.7)	1:9	10	-11.8 ($\pm$0.5; 4.2)	13.1 ($\pm$0.2; 1.5)	10
Carcharhinus melanopterus	126 ($\pm$1.9)	16:37	53	-10.9 ($\pm$0.2; 1.8)	12.6 ($\pm$0.1; 0.8)	24
Negaprion acutidens	111 ($\pm$13.9)	3:1	4	-11.0 ($\pm$0.7; 6.4)	11.9 ($\pm$0.6; 5.0)	4
Triaenodon obesus	134 ($\pm$3.6)	4:0	4	-11.0 ($\pm$0.2; 1.8)	11.9 ($\pm$0.3; 2.5)	4

CV% is the coefficient of variation

Fig. 3 Relationship between δ^{15}N and δ^{13}C of *C. melanopterus* and *C. amblyrhynchos* and total length (L_T). *C. melanopterus* (**a**, **c**) and *C. amblyrhynchos* (**b**, **d**)
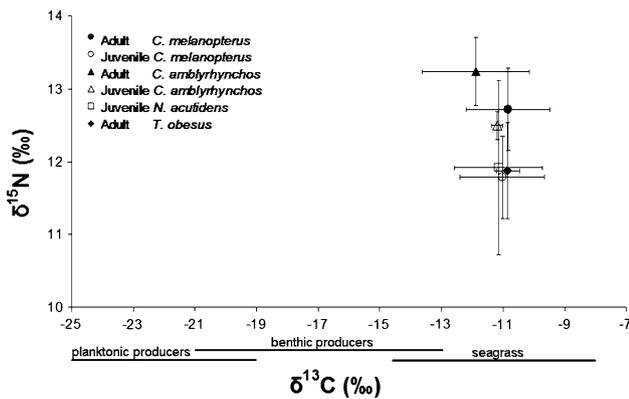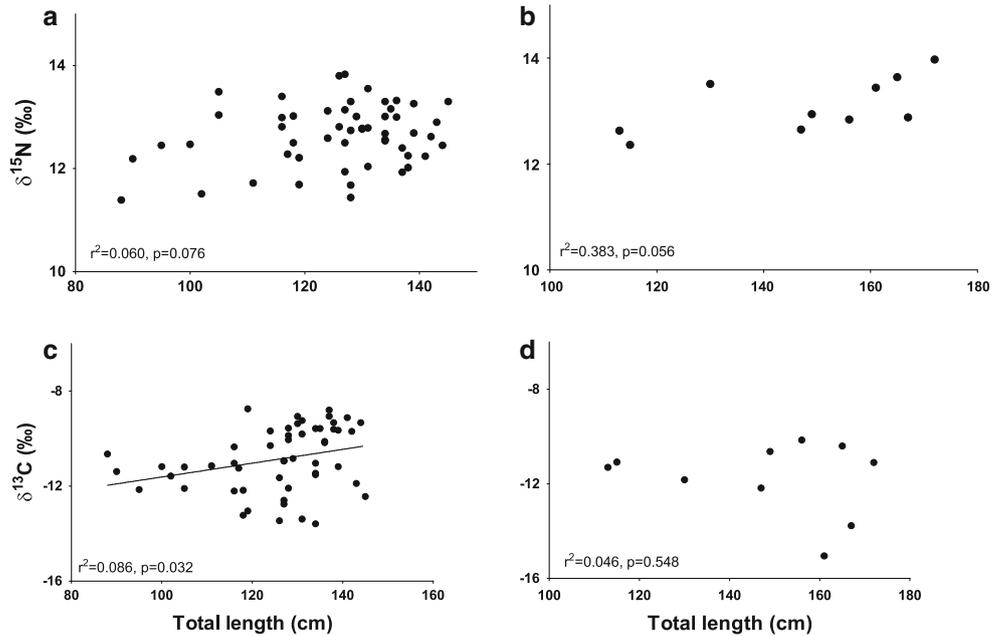
Fig. 4 Mean isotopic compositions of species and size classes of reef sharks. Mean δ^{13}C ($\pm$SD) for seagrass is from France (1995) and benthic and planktonic means ($\pm$SD) from Hemminga and Mateo (1996)

Table 2 Trophic position estimates for species and size classes of reef sharks tagged at Ningaloo Reef

Species/size class	Trophic position (2.3‰)
Adult *Carcharhinus amblyrhynchos*	4.3
Juvenile *Carcharhinus amblyrhynchos*	4
Adult *Carcharhinus melanopterus*	4
Juvenile *Carcharhinus melanopterus*	3.7
Juvenile *Negaprion acutidens*	3.7
Adult *Triaenodon obesus*	3.7

14 non-resident, while 5 of the 8 tagged *C. amblyrhynchos* were resident. For *N. acutidens*, 3 of 4 tagged individuals were resident, and both *T. obesus* we tagged were residents. The resident *C. melanopterus* were detected regularly

throughout the year, while the non-residents were often absent from the study area between April 2008 and September 2009 (Fig. 5). Similarly, resident *C. amblyrhynchos* were detected throughout most of the monitoring period. The two *T. obesus* were not detected regularly between August and October 2009. Of the three *N. acutidens* that were resident, two were detected regularly throughout the study and one was often absent between June and November 2009. The CART analysis did not reveal any effect of residency status on shark δ^{13}C or δ^{15}N.

Discussion

Long-term residency and dependency on coastal food chains (i.e. high δ^{13}C) have potentially important implications for the effectiveness of current protection measures for reef sharks in north Western Australia. Other studies have also identified a link between elasmobranchs and coastal producers using stable isotope analysis (Papastamatiou et al. 2010a; Vaudo and Heithaus 2011), which highlights the importance of conserving inshore communities and habitats for higher-order predators in these regions. The use of telemetry with stable isotopes not only provides a means with which to identify links to coastal producers but also makes inferences about the species' reliance on this inshore habitat through long-term patterns of residency.

Bill's Bay is encompassed by Maud Sanctuary Zone (21.51 km²), which is one of many sanctuary zones at Ningaloo Reef (combined area of 883.65 km²) that prohibits any kind of fishing (DEC 2005). However, this

Fig. 5 Daily presence and absence of reef sharks at Coral Bay. A shark was considered present if it had >1 detection within the acoustic array per day
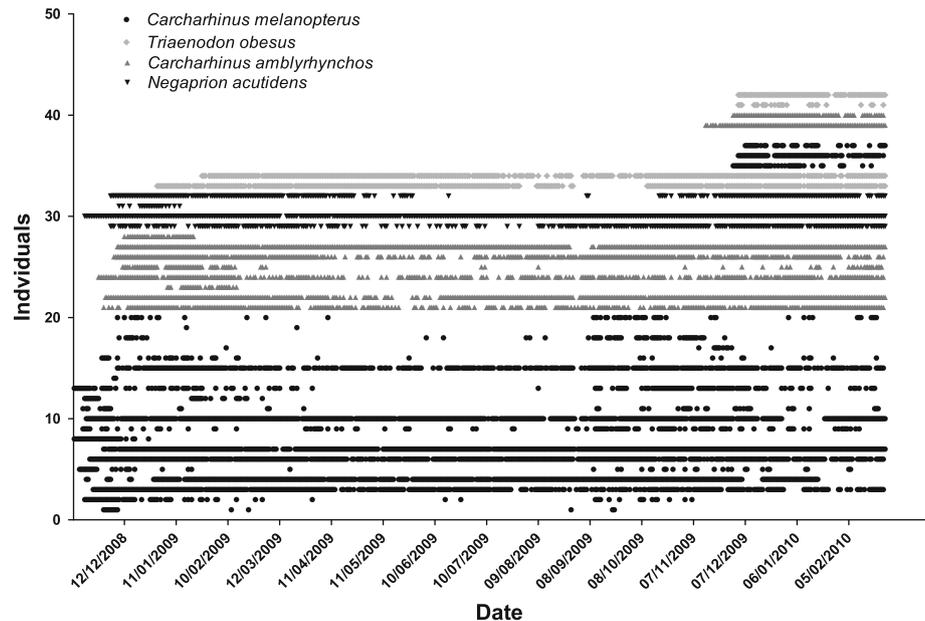
network of coastal sanctuary zones is interspersed with recreational and general use zones, in which a number of reef sharks tagged in concurrent studies have been caught by recreational fishers. The effectiveness of marine parks for reef sharks has been assessed in several recent studies, and there is considerable debate as to their efficacy in protecting sharks (Chapman et al. 2005; Heupel and Simpfendorfer 2005; Robbins et al. 2006; Heupel et al. 2009). Our study highlights that some species of reef sharks are highly resident to small areas of the coast and depend largely on coastal food chains. Therefore, any localised impacts in unprotected areas of coastline such as fishing, aquaculture or mining could impact reef sharks indirectly through habitat degradation, or directly through the removal of sharks or their prey (e.g. Jackson et al. 2001; Papastamatiou et al. 2010b; Ward-Paige et al. 2010).

Overall, we found little variation in isotope values among species and size classes. Trophic positions of species we sampled were similar to previous estimates based on stomach content analysis (Cortés 1999). Strong relationships between trophic positions based on $\delta^{15}N$ and dietary studies in other shark species have been identified previously from fisheries in India, with a few exceptions due to limited samples and predominance in juveniles (Borrell et al. 2011). Similarly, we found some differences between previous trophic position estimates for two of the species we sampled (N. acutidens and T. obesus). Negaprion acutidens (3.7) was markedly different from that examined by Cortés (1999) (4.1, $n = 271$), possibly because we only sampled juveniles and had a limited sample size ($n = 4$). Our estimate of trophic position for T. obesus (3.7) was also different to that examined by Cortés (1999) (4.2, $n = 31$), which also possibly reflects

our limited sample size (4 adult males) but might also have been due to differences in prey availability. While there was some evidence to suggest trophic differences in feeding among some species/size classes (i.e. C. amblyrhynchos compared to T. obesus; juvenile C. melanopterus to juvenile N. acutidens), the maximum difference represented only 0.6 trophic steps (4.3–3.7), which suggests considerable trophic overlap. Overlap in trophic position has also been observed in other inshore elasmobranch communities, many of which are able to coexist at the same trophic position by targeting different prey (Vaudo and Heithaus 2011). It is possible that while we observed evidence of trophic overlap among species at Coral Bay, each species may actually target different prey items with similar isotope compositions. Unfortunately, we were unable to compare our sharks' stomach contents with stable isotope values due to tagging priorities, and concerns for animal welfare had gastric lavaging been included in our sampling procedure.

The CART analysis results suggest that $\delta^{15}N$ increases with size for C. melanopterus and C. amblyrhynchos, as has been found for other carcharhinid sharks (Cortés 1999). Similarly, a recent isotopic study of elasmobranchs found a positive correlation between L_T and $\delta^{15}N$ (or trophic position) for both whale sharks (R. typus) and scalloped hammerheads (Sphyrna lewini) (Borrell et al. 2011). Interestingly, Papastamatiou et al. (2010a) found a positive relationship for C. melanopterus in only one of the two lagoon systems included in their study around Palmyra Atoll, Northern Line Islands, which they suggest might have been due to differences in the average body condition of animals between lagoons. Given that we used $\delta^{15}N$ to estimate trophic position, we expected that larger animals

would have higher $\delta^{15}N$ than smaller animals due to ontogenetic or feeding niche differences among species and size classes (Scharf et al. 2000; Estrada et al. 2006). Previous studies using stomach contents have found that adults feed more on larger prey items than juveniles in some species, which often include carnivores in higher trophic levels (e.g. Wetherbee and Cortés 2004; Bethea et al. 2006; McElroy et al. 2006). Indeed, adult *C. amblyrhynchos*, which were the largest animals we sampled, showed the greatest enrichment in $\delta^{15}N$ (13.1‰) when compared to *T. obesus*, which had the most depleted (11.9‰). Although *T. obesus* did not have the smallest L_T of animals sampled, low $\delta^{15}N$ might suggest a more benthic-specific diet of herbivores or invertivores, which is consistent with their relatively sedentary lifestyle (Randall 1977). Randall (1977) noted that the diet of *T. obesus* included many families of fish such as Holocentridae, Scarinae, Acanthuridae and Balistidae. In contrast, the diet of *C. amblyrhynchos* contains piscivores from families such as the Scombridae, Carangidae and Muraenidae (Stevens and McLoughlin 1991; Salini et al. 1992; Wetherbee et al. 1997; but see Papastamatiou et al. 2006). Low sample sizes in our study for *N. acutidens* and *T. obesus* mean that results should be interpreted with caution.

We found evidence of size-based differences in diet for *C. melanopterus* and *C. amblyrhynchos* based on increasing $\delta^{15}N$ with total length, although we did not find evidence for a relationship between life stages (i.e. 'juveniles' and 'adults') based on the CART analysis. However, ontogenetic shifts in diet have been observed in other elasmobranchs (Wetherbee and Cortés 2004), some of which were reef-associated sharks (Cortés and Gruber 1990; Lowe et al. 1996; Papastamatiou et al. 2006). It is possible that had we been able to sample young-of-the-year sharks, there might have been a greater difference in trophic positions occupied by different life stages. However, muscle samples of young-of-the-year often retain isotopic influences from the mother (e.g. Matich et al. 2010; Olin et al. 2011), which can complicate conclusions. Even though we only sampled late-stage juveniles and adults of *C. melanopterus* and *C. amblyrhynchos*, we observed differences in $\delta^{15}N$ between these groups, which suggest that larger individuals of these species are feeding higher in the food web.

In general, $\delta^{13}C$ was high in all four species (overall mean = -11.01 ± 0.16‰), suggesting a dependency on coastal producers in the area such as seagrass and macroalgae (e.g. *Halophila* sp., *Lobophora variegata* and *Sargassum oligocystym*) (G. Hyndes, Edith Cowan University, pers. comm.). High $\delta^{13}C$ values are generally associated with coastal habitats given that $\delta^{13}C$ is typically higher in inshore benthic food webs than in offshore plankton-based food webs (Fry et al. 1983; France 1995; Sherwood and

Rose 2005; Hill et al. 2006), and that producers associated with coastal habitats such as kelp and seagrass also have higher $\delta^{13}C$ than planktonic producers (Clementz and Koch 2001). A review of seagrass stable carbon isotopes found that the average value was -11.5 ± 3.2‰ (SD) (Hemminga and Mateo 1996) when compared to estuarine/marine benthic producers and planktonic producers, which were -17.4 and -22.3‰ (France 1995). More recently, a dependency on coastal producers such as seagrass ($\delta^{13}C$ -9.41 ± 1.32) was found in a number of species of elasmobranchs in Shark Bay, which is a large semi-enclosed bay situated approximately 270 km south of Coral Bay (Vaudo and Heithaus 2011). Papastamatiou et al. (2010a) also concluded that *C. melanopterus* in the Palmyra Atoll ultimately relied on coastal producers and, similar to our study, noted that this species had a wide range in $\delta^{13}C$. We found no evidence for an effect of year, age class, or sex on carbon isotope composition, so the reason for such individual variation in $\delta^{13}C$ values for *C. melanopterus* (-13.5 to -8.8‰) is unknown. We did not collect body condition information, which can potentially affect isotopic composition (Menard et al. 2007; Papastamatiou et al. 2010a), although body mass would have been correlated with L_T. The widest range of $\delta^{13}C$ values we observed was in *C. amblyrhynchos*, which was a magnitude larger than values found by Maljković and Côté (2011) for *C. perezi*, another reef-associated carcharhinid. It is possible that the narrow $\delta^{13}C$ range they found was partly due to food provisioning by tourism operators in the area. Wide variation in $\delta^{13}C$ for species at Coral Bay might be attributable to individual specialisation in diet, which has been previously identified in elasmobranch communities in north Western Australia (Vaudo and Heithaus 2011).

We found no difference between carbon or nitrogen stable isotope composition and residency status or size for any of the species studied. Although we did not measure home range sizes, previous research on coastal sharks up to 2.5 m total length has generally found that their movement is restricted to areas of <100 km² (Speed et al. 2010). Such a limited range of movement would imply that these species would be unlikely to travel to other coastal regions with differing baseline isotopic compositions; however, movement into deeper pelagic environments would be possible. Identification of inshore and offshore feeding through carbon stable isotope analysis has been identified previously in invertebrates, fish and seabirds (Hill et al. 2006; Sherwood and Rose 2005; Hobson et al. 1994). The lowest average $\delta^{13}C$ we found was in the widest-ranging species, *C. amblyrhynchos* (e.g. Heupel et al. 2010), which might indicate that some of its dietary source comes from offshore. In addition, if long-distance coastal movements did occur from time to time (e.g. Heupel et al. 2010), using $\delta^{13}C$ in muscle tissue to identify seasonal changes in

habitat use would probably be unsuccessful. This is due to the slow (up to 2 years) turnover rate of stable isotopes in elasmobranch tissues (Logan and Lutcavage 2010), which would lack the resolution necessary to identify short-term geographic changes in movement.

Overall, we found little evidence for differences in the trophic ecology of four coexisting species of reef sharks at Ningaloo Reef using stable isotope analysis and acoustic monitoring. There was some evidence of increasing $\delta^{15}N$ with size for *C. melanopterus* and *C. amblyrhynchos*, although we found no differences in isotope composition between sexes, age classes, year of sampling or residency status. High $\delta^{13}C$ of all species suggests a dependency on coastal food chains. More information is still required to sample across all age classes, particularly young-of-the-year, to determine the extent of ontogenetic differences in diet. Our study confirms the benefits of using a multi-disciplinary approach to studying the trophic ecology of species that are otherwise difficult to observe. The use of acoustic telemetry to complement isotope analysis was beneficial in determining differences in ecology between reef shark species at Ningaloo Reef, although it will also be necessary to quantify home range size and long-distance patterns of dispersal.

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