

Dietary overlap and partitioning among three sympatric carcharhinid sharks

Bree J. Tillett^{1,2,3,*}, Mark G. Meekan^{2,4}, Iain C. Field^{1,2,5}

¹Research Institute for the Environment and Livelihoods, Charles Darwin University, Casuarina, Northern Territory 0810, Australia

²Australian Institute of Marine Science, Arafura Timor Research Facility, Casuarina, Northern Territory 0810, Australia

³Tropical Rivers and Coastal Knowledge Research Hub, Charles Darwin University, Casuarina, Northern Territory 0810, Australia

⁴Australian Institute of Marine Science, UWA Oceans Institute (MO 96), 35 Stirling Highway, Crawley, Western Australia 6009, Australia

⁵Graduate School of the Environment, Macquarie University, Sydney, New South Wales 2109, Australia

ABSTRACT: We used stomach content and stable isotope analyses to compare diet as a proxy for ecological similarity among 3 tropical sharks, *Carcharhinus leucas*, *C. amboinensis* and the Endangered *Glyphis* spp. Our analyses suggested that all 3 predators consumed mainly teleost fishes but also preyed on crustaceans, cephalopods and reptiles. Diets varied spatially and through ontogeny. Some juvenile *C. leucas* sourced prey from the same food web as juvenile *C. amboinensis*, although prey sources varied among *C. leucas* nurseries. Dietary overlap existed between sympatric adult *C. leucas* and *C. amboinensis*, and both species occupied higher trophic positions than juvenile conspecifics. Although sample sizes for *Glyphis* spp. were small, our results suggested previously undescribed dietary partitioning between this genus and the sympatric *C. leucas*.

KEY WORDS: Niche · Co-existence · *Carcharhinus* spp. · Sharks · North Australia

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INTRODUCTION

As top-order predators, sharks are thought to have a major role in structuring energy flows and trophic ecologies of the ecosystems in which they occur (Heithaus et al. 2008). The shallow, coastal environments of the tropics are characterized by high species diversity of carcharhinid sharks, many of which appear morphologically very similar (Speed et al. 2012). Whether this superficial appearance of similarity does indeed reflect an equivalence of trophic roles is largely unknown and may have important implications for resilience of these ecosystems, due to increasing human impacts on shark populations both directly through fisheries and indirectly through phenomena such as global warming and habitat loss

(Stevens et al. 2000, Stevens 2002, Salini et al. 2007, IUCN 2010).

Trophic interactions that are associated with ecological function are one tool for comparing ecological similarity among species (Bruno & O'Connor 2005, Griffin et al. 2008). Typically, these can be quantified by stomach content and stable isotope analyses (Estrada et al. 2003, Bethea et al. 2004, Domi et al. 2005, Maia et al. 2006). The former provides a 'snapshot' of recently ingested prey, allowing identification and in some cases other additional biological information (e.g. sex, size, age) of prey, but such analyses are limited by stomach size and evacuation rate (Hyslop 1980, Cortes 1997). Stable isotope analyses overcome these problems as prey are identified from chemical signatures ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, the ratios of

heavier to lighter isotopes of these elements; ^{13}C : ^{12}C ; ^{15}N : ^{14}N) assimilated into tissue, thus providing a long-term record of digested prey (Logan & Lutcavage 2010). As these isotopes move through the food web, the ratio of heavier to lighter isotopes shifts (fragmentation), changing the predator's isotopic value to that of its prey, with a slight enrichment (Post 2002). As $\delta^{13}\text{C}$ isotopes remain relatively unchanged they serve as a good indicator of prey source, whereas $\delta^{15}\text{N}$ isotopes enrich at 3.4‰ per trophic level, enabling calculation of trophic position of the study animal (Peterson & Fry 1987, Estrada et al. 2005).

Here, stable isotope and stomach content analyses are used to examine trophic similarity of 3 sharks that inhabit tropical estuarine and freshwater ecosystems: the bull shark *Carcharhinus leucas* Valenciennes, 1839; pigeye shark *C. amboinensis* Müller & Henle, 1839; and river sharks *Glyphis* spp. Müller & Henle, 1839. Bull and pigeye sharks are both described as having a short, broad and blunt snout, small eyes, no inter-dorsal ridge and triangular saw-edged teeth (Compagno 1984, Cliff & Dudley 1991a,b). Furthermore, both sharks are common in shallow coastal tropical and sub-tropical waters (Last & Stevens 2009). This morphological similarity combined with overlapping distributions has led to common misidentifications and the assumption of ecological similarity (Cliff & Dudley 1991a), although evidence for complex patterns of habitat use is beginning to question whether these 2 species are truly ecologically similar (Brunnschweiler et al. 2010, Carlson et al. 2010, Karl et al. 2011, Knip et al. 2011a,b, Tillett et al. 2012). Juvenile *C. amboinensis* prefer shallow coastal embayments (Knip et al. 2011a), whereas juvenile *C. leucas* inhabit freshwater nurseries (Heupel et al. 2010). As juvenile *C. leucas* mature, individuals increase the frequency and duration of marine excursions until they reach maturity, after which time they reside permanently in marine environments, sympatric with adult *C. amboinensis* (Simpfendorfer et al. 2005, Yeiser et al. 2008, Tillett et al. 2011b). The International Union for the Conservation of Nature (IUCN) has classified *C. leucas* as Near Threatened (IUCN 2010), whereas the frequent misidentifications of *C. amboinensis*, (mostly as *C. leucas*) in fishery catches have impeded estimates of population status, resulting in IUCN classification of this species as Data Deficient (IUCN 2010). *Glyphis* spp. also occupy freshwater nurseries, although the habitats used during the remainder of their life-cycle (i.e. duration spent in freshwater and habitat changes post-maturity) are unclear (Stevens et al. 2005, Last &

Stevens 2009). The lack of sound biological data and the exploitation of many populations across the range of this species have contributed to its classification as Endangered (IUCN 2010).

Northern Australia is considered one of the last population strongholds for *Glyphis* spp. (Taniuchi et al. 1991), and stocks of both *Carcharhinus leucas* and *C. amboinensis* in this region are also less impacted by human activity than in most other parts of the species' ranges (Field et al. 2009). As such, northern Australia provides one of the few places where trophic partitioning among these sharks can be studied without the strong confounding effects of anthropogenic influences such as fishing and habitat degradation.

This study assesses dietary similarity by determining (1) the degree of long-term dietary overlap between *Carcharhinus leucas* and *C. amboinensis*; (2) whether prey identity and the number of prey species consumed differs between juvenile *C. leucas* and *C. amboinensis*; (3) whether prey identity and number increases with maturity of *C. amboinensis*; (4) whether any increase in prey diversity correlates with adults occupying higher trophic positions and whether this differs between *C. leucas* and *C. amboinensis* (Werry et al. 2011, Hussey et al. 2011) and; (5) whether any dietary differences are further partitioned by gender. Lastly, (6) we provide preliminary dietary analysis for endangered *Glyphis* spp.

MATERIALS AND METHODS

Sample collection

Samples were collected by observers working with the Northern Territory Offshore Net and Line (NTONL) and Barramundi fisheries (NTBarr) operating along the Northern Territory (Australia) coastline in 2009 and by fishery-independent surveys conducted in Van Diemen Gulf, Daly River and Darwin Harbour from 2009 to 2010 (Fig. 1). Stomachs were removed from 48 (43 juvenile and 5 adult) *Carcharhinus leucas*. Juveniles were collected from Blue Mud Bay (n = 5), Van Diemen Gulf (n = 15), Daly River (n = 15), and oceanic locations (n = 8). All adult *C. leucas* were collected north of the Tiwi Islands. Stomachs were also removed from 106 (79 juvenile and 27 adult) *C. amboinensis*. Juvenile *C. amboinensis* were collected from Darwin Harbour (n = 30), Fog Bay (n = 41) and oceanic locations (n = 8). Similar to adult *C. leucas*, all adult *C. amboinensis* were collected north of the Tiwi Islands. Five juvenile *Glyphis* spp. sharks

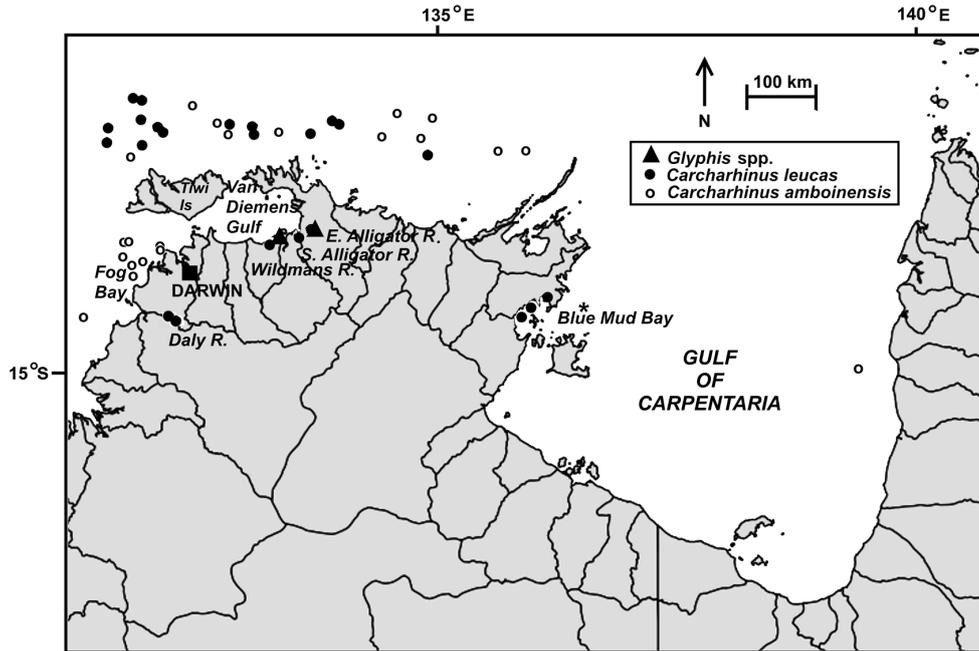


Fig. 1. Capture locations for pigeye sharks *Carcharhinus amboinensis* (n = 106; 79 juvenile and 27 adult), bull sharks *C. leucas* (n = 48; 43 juvenile and 5 adult) and northern river sharks *Glyphis* spp. (n = 5; 5 juvenile and 0 adults) across northern Australia

were also collected from Van Diemen Gulf. All stomachs were stored frozen. In addition, 10 to 15 thoracic vertebrae were removed for stable isotope analyses from a subsample of juvenile *C. leucas* resident in the Daly River (n = 15) and Van Diemen Gulf (n = 20) freshwater nurseries, adult *C. leucas* (n = 20) occupying oceanic regions, juvenile and adult *C. amboinensis* (n = 20 for both cohorts) residing in Fog Bay and oceanic regions, respectively, and juvenile *Glyphis* spp. occupying the East Alligator River (n = 5); these were stored frozen. Maturity was determined from previously described age structure and growth dynamic assessments (Tillett et al. 2011a). Sex, total weight (TW) total length (TL) and fork length (FL) were collected when possible from dead specimens. Small individuals (<1 m TL) were inspected for the presence of umbilical scars as an indication of time since birth.

Stomach content analyses

Once thawed, stomach contents were flushed with freshwater and placed on a sorting tray. Prey contents including hard structures such as otoliths, skulls, vertebrae and stingray barbs were identified to the finest taxonomic resolution possible. Contents with shear cuts associated with filleting such as directly behind the skull at the base on the vertebral

column were identified as fishery discards. Frequency of occurrence was determined as the proportion of each species identified relative to the total number of identified species.

Prey species accumulation curves were calculated for *C. leucas* and *C. amboinensis* based on random addition of individuals and 1000 permutations. No statistical analyses were performed on gut samples of *Glyphis* spp. or between adult *C. leucas* and *C. amboinensis* due to low sample sizes. Broad differences between prey taxa consumed by (1) juvenile *C. leucas* and *C. amboinensis*, (2) juvenile and adult *C. amboinensis* and (3) between genders were compared using permutational multivariate analysis of variance, PERMANOVA (presence/absence data) on Bray-Curtis similarity matrices (PRIMER-6, Plymouth Marine Laboratory, PML) using 9999 permutations. To overcome low sample sizes, p-values based on unrestricted permutations of raw data and Monte Carlo sampling were compared. Where significant differences between main effects were identified, similarity of percentages analyses, SIMPER (PRIMER-6, PML) described which prey taxa differed between groups (presence/absence). Additionally, ANOVA and generalised linear modelling (GLM) was applied in R cran (R Development Core Team 2011) to determine any finer-scale differences in the number of prey species consumed among the above groups. Akaike's information criterion cor-

rected for small sample size (AIC_c) ranked models based on their bias-corrected distance from the true model (Burnham & Anderson 2002, Link & Barker 2006). Here, the relative weight of evidence for each model given the model set was its AIC_c weight ($wAIC_c$) ranging from 0 (no support) to 1 (complete support) (Burnham & Anderson 2002). Each model's goodness of fit was also estimated using the percentage of deviance explained (%DE) relative to the null (intercept-only) model. For consistency, within these analyses unknown prey types such as teleosts that could not be identified were classified as separate species within taxa, e.g. as unknown teleosts. Standard Shannon-Wiener diversity indices were also calculated between juvenile *C. leucas* and *C. amboinensis* and also between juvenile and adult *C. amboinensis*.

Stable isotope analyses

Frozen vertebrae of sharks were defrosted prior to stable isotope analyses and excess tissue, neural and haemal arches were excised to expose the centra. Individual centra were separated and any connective tissue removed by washing in Milli-Q water. Polished centra were left to air-dry causing any remaining tissue to become brittle and peel away. To ensure only diet post-parturition was analysed, cores of juvenile vertebrae corresponding to *in vitro* development (post the angle of change) were removed and the remaining material was ground and oven dried (~0.8 mg). Adult vertebrae were sectioned (~2 mm) using a low-speed isomet diamond saw at approximately 240 rpm with a 250 g load weight. Potential contaminants were removed by grinding sections on wet and dry paper and rinsing again in Milli-Q water. Sections were viewed under a Leica DM 400B compound microscope, and the outer growth band representing the final year before capture was removed, ground and oven dried (~0.8 mg). Samples were then combusted in an EA 3000 elemental analyser (Eurovector) and sample gases delivered to an Isoprime mass spectrometer (GV Instruments) for isotope analysis of C and N. Working standards were liquids calibrated against IAEA CH6, CH7, N1 and N2, and had elemental composition that matched the samples (44% C and 11% N). Average $\delta^{13}C$ values for sharks were plotted against average $\delta^{15}N$ values and compared between age cohorts, species and habitats. Layman metrics were also calculated using the SIAR package in R cran (R Development Core Team 2011) and compared between shark species and maturity stages (Layman

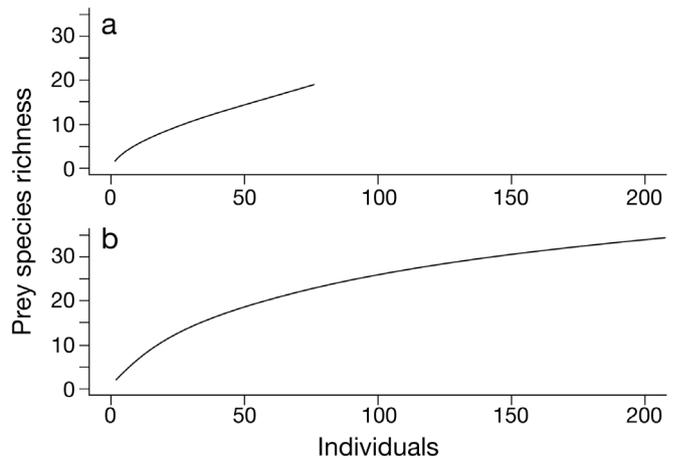


Fig. 2. Prey species accumulation curves for (a) *Carcharhinus leucas* (n = 48) and (b) *C. amboinensis* (n = 106) based on random addition of individuals and 1000 permutations

et al. 2007). Limited sample sizes prevented further comparisons between juvenile *C. leucas* and *C. amboinensis* or between juvenile *C. leucas* nurseries.

RESULTS

Stomach content analyses

An asymptote in the prey species accumulation curve indicated that increasing the sample size of stomachs of *Carcharhinus amboinensis* would have been unlikely to identify many new prey species. However, an obvious asymptote did not occur for *C. leucas* suggesting that we under-sampled the range of prey of this species (Fig. 2). Analysis of stomach contents showed that *C. leucas* and *C. amboinensis* fed on a diverse array of prey taxa (Fig 3, Table 1). Both juvenile *C. leucas* and *C. amboinensis* ate crustaceans, other elasmobranchs and teleost fish (Table 2); however, the diet of *C. amboinensis* also included cephalopods and a wider range of teleost fishes than found in the stomachs of *C. leucas* (Table 3). Debris (e.g. rocks, sand, leaves) were common in *C. leucas* stomachs and both this species and *C. amboinensis* consumed fishery discards, although these were more common in the stomachs of the latter species (Table 3). Prey taxa i.e. crustaceans, teleosts etc. consumed by juvenile and adult *C. amboinensis* did not differ (Table 2); however, significant differences in the number of prey species were identified ($p = 0.012$; ANOVA). Juvenile *C. amboinensis* fed on a greater proportion of crustaceans and had fewer empty stomachs than adults (Fig. 3,

Table 1), although the prey taxa did not differ between genders of this species (Table 2). The number of prey species also differed between juvenile *C. leucas* and *C. amboinensis* ($p = 0.0001$; ANOVA) and between juvenile and adult *C. amboinensis* ($p = 0.012$; ANOVA), although again, not between genders ($p = 0.880$; ANOVA and $p = 0.639$; ANOVA respectively for each group). Generalised linear models supported these results (Table 4). Interestingly, although sample sizes were small, only teleosts were found in the guts of *Glyphis* spp. Shannon-Wiener diversity indices for *C. amboinensis* and *C. leucas* were 2.625 and 2.076 respectively, and 2.660 and 2.227 for juvenile and mature *C. amboinensis* respectively.

Stable isotope analyses

Mean values of $\delta^{15}\text{N}$ were higher for adults than juveniles of both *C. leucas* and *C. amboinensis*. Conversely, mean values of $\delta^{13}\text{C}$ were similar between juveniles and adults within species. Mean $\delta^{13}\text{C}$ values were also similar for juvenile *C. leucas* and *C. amboinensis*, despite the former inhabiting freshwater and the latter occupying estuarine nurseries. However, mean $\delta^{13}\text{C}$ values differed for juvenile *C. leucas* among freshwater nurseries and between juvenile *C. leucas* and *Glyphis* spp. that occupied the same nurseries. Juvenile *C. leucas* had the broadest range of mean $\delta^{13}\text{C}$ values (Fig. 4). *C. leucas* occupied the widest niche space with the most diversity present in juveniles. Conversely, trophic diversity of juvenile and adult *C. amboinensis* was similar (Table 5).

DISCUSSION

Our results confirm the diversities of intra- and inter-specific ecologies in coastal sharks. Juvenile *Carcharhinus leucas* and *C. amboinensis*, much like other coastal carcharhinids, fed on a wide selection of prey, consuming mostly teleosts, but also cephalopods, crustaceans and reptiles (Salini et al. 1992,

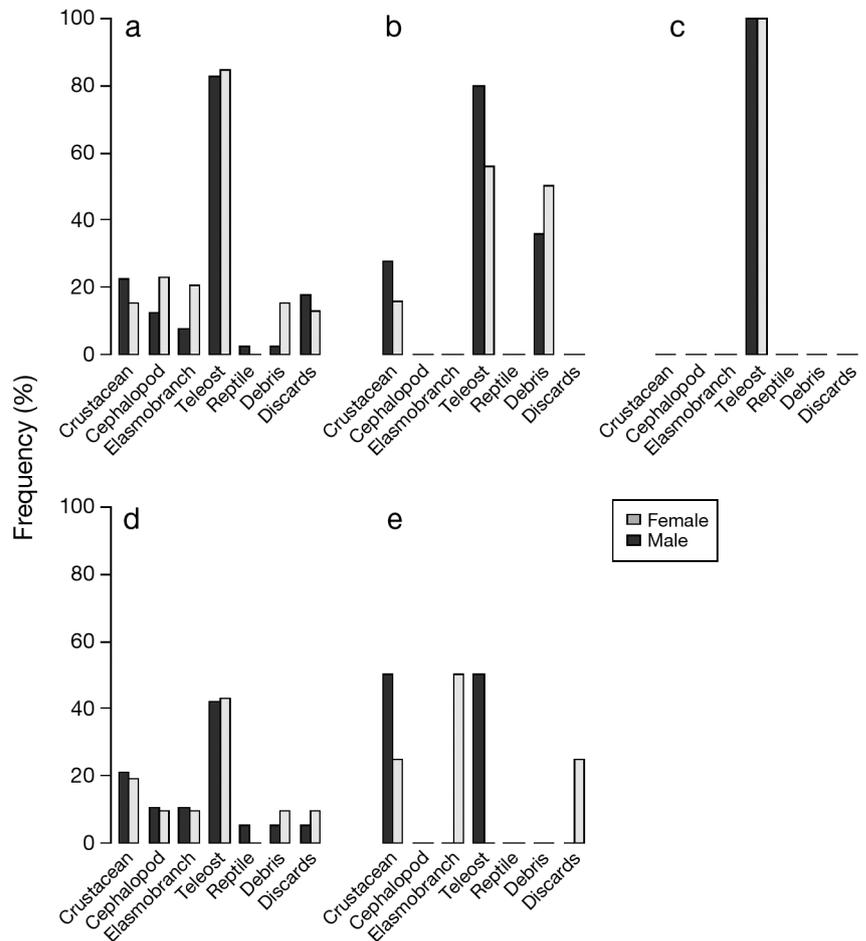


Fig. 3. Frequency of general prey types in juvenile (a) pigeye sharks *Carcharhinus amboinensis* ($n = 79$; 39 females and 40 males), (b) bull sharks *C. leucas* ($n = 43$; 18 females and 25 males) and (c) northern river sharks *Glyphis* spp. ($n = 5$; 3 females and 2 males), and adult (d) pigeye ($n = 27$; 15 females and 12 males) and (e) bull sharks ($n = 5$; 3 females and 2 males)

Bethea et al. 2004, McElroy et al. 2006, Last & Stevens 2009, Newman et al. 2010). Stomach content analyses of *C. leucas* collected from south-east Queensland and north-west Australian estuaries have reported similar diets for this species (Stevens & McLoughlin 1991, Thorburn & Rowland 2008, Werry et al. 2011). Stomach content analysis of *Glyphis* spp. identified a diet solely composed of teleosts. However, the breadth of $\delta^{13}\text{C}$ values in tissues, which provide a long-term record of digested prey, indicated a broader diet, possibly from a different food web than either juvenile *C. leucas* or *C. amboinensis*. This comparison must be treated with caution, since only 5 individuals of *Glyphis* spp. were sampled. Such dietary variation displayed in these 3 species is commonly associated with 'generalist predators' (Ellis & Musick 2007, Lopez et al. 2009); however, recent research has shown that for *C. leucas*, despite a

Table 1. Percent frequency of occurrence (% FO) of prey items in stomach contents for bull sharks *Carcharhinus leucas*, pigeye sharks *C. amboinensis* and northern river sharks *Glyphis* spp. All prey items were identified to the lowest possible classification; n = number of stomachs containing prey item; empty: no prey items found C

Prey item	<i>C. leucas</i> (n = 48)		<i>C. amboinensis</i> (n = 106)		<i>Glyphis</i> spp. (n = 5)	
	% FO	n	% FO	n	% FO	n
Crustaceans						
Unknown order	2.08	1	–	–	–	–
Stomatopoda	–	–	–	–	–	–
Squillidae	–	–	2.83	3	–	–
Decapoda unknown family	12.50	6	2.83	3	–	–
Palaemonidae unknown genera	10.42	5	–	–	–	–
<i>Macrobrachium rosenbergii</i>	2.08	1	–	–	–	–
Penaeidae	–	–	–	–	–	–
<i>Penaeus</i> spp.	–	–	8.49	9	–	–
Copepoda	4.17	2	8.49	9	–	–
Cephalopods						
Unknown order	–	–	11.32	12	–	–
Teuthida	–	–	–	–	–	–
Loliginidae	–	–	0.94	1	–	–
Ommastrephidae	–	–	0.94	1	–	–
Sepiolida	–	–	–	–	–	–
Sepiolidae unknown genera	–	–	2.83	3	–	–
<i>Sepia</i> spp.	–	–	5.66	6	–	–
Octopoda	–	–	0.94	1	–	–
Elasmobranchs						
Unknown order	2.08	1	10.38	11	–	–
Carcharhiniformes	–	–	–	–	–	–
Carcharhinidae	2.08	1	1.89	2	–	–
Myliobatiformes	–	–	–	–	–	–
Dasyatidae	2.08	1	1.89	2	–	–
Teleost fishes						
Perciformes unknown family	62.50	30	76.42	81	60	3
Acanthuridae	–	–	0.94	1	–	–
Carangidae	2.08	1	4.72	5	–	–
Centropomidae	4.17	2	–	–	–	–
Gobiidae	2.08	1	2.83	3	–	–
Teraponidae	–	–	3.77	4	–	–
Labridae	–	–	0.94	1	–	–
Polynemidae	–	–	0.94	1	–	–
Pomacanthidae	–	–	0.94	1	–	–
Scombridae	2.08	1	2.83	3	–	–
Sparidae	–	–	0.94	1	–	–
Ambassidae	–	–	–	–	–	–
<i>Ambassis</i> sp.	2.08	1	–	–	–	–
Ariidae	–	–	–	–	–	–
<i>Arius</i> sp.	10.42	5	0.94	1	40	2
Leiognathidae	–	–	–	–	–	–
<i>Leiognathus</i> sp.	2.08	1	9.43	10	–	–
Psettodidae	–	–	–	–	–	–
<i>Psettodes</i> sp.	–	–	0.94	1	–	–
Sciaenidae	–	–	–	–	–	–
<i>Austroneba</i> sp.	–	–	5.66	6	–	–
<i>Johinus</i> sp.	–	–	7.55	8	–	–
<i>Nibe</i> sp.	–	–	1.89	2	40	2
<i>Otolithes</i> sp.	–	–	4.72	5	–	–
Clupeiformes	–	–	–	–	–	–
Chirocentridae	–	–	0.94	1	–	–
Elopiformes	–	–	–	–	–	–
Megalopidae	–	–	0.94	1	–	–
Synbranchiformes	–	–	–	–	–	–
Synbranchidae	2.08	1	–	–	–	–
Tetraodontiformes	–	–	–	–	–	–
Tetraodontidae	–	–	0.94	1	–	–

Table 1 (continued)

Prey item	<i>C. leucas</i> (n = 48)		<i>C. amboinensis</i> (n = 106)		<i>Glyphis</i> spp. (n = 5)	
	% FO	n	% FO	n	% FO	n
Anguilliformes			–	–		
Anguillidae			–	–		
<i>Anguilla</i> sp.	2.08	1	–	–	20	1
Osteoglossiformes						
Osteoglossidae			–	–	–	–
<i>Scleropages</i> sp.	2.08	1	–	–	–	–
Reptiles						
Squamata						
Elapidae	–	–			–	–
<i>Aipysurus</i> sp.	–	–	1.89	2	–	–
Fishery discards	2.08	1	14.15	15	–	–
Debris	37.50	18	9.43	10	–	–
Empty	16.67	8	13.21	14	–	–

Table 2. Permutational multivariate analysis of variance, PERMANOVA (presence/absence data) on Bray-Curtis similarity matrices using 9999 permutations. (a) Differences in prey taxa between juvenile *Carcharhinus leucas* (n = 43) and juvenile *C. amboinensis* (n = 79); (b) differences in prey taxa consumed between juvenile (n = 79) and adult *C. amboinensis* (n = 27). df: degrees of freedom; SS: sum of squared error; MS: mean of squared error; $\alpha = 0.05$; N: number of unique permutations. Significant p-values are in **bold**

Source	df	SS	MS	Pseudo-F	p (Permutation)	N	p (Monte Carlo)
a) Species	1	11416	11416	13.32	0.083	12	0.034
Sex	1	1820.7	1820.7	2.20	0.331	12	0.259
Species \times Sex	1	839.98	839.98	0.43	0.745	9954	0.731
b) Maturity	1	6689.1	6689.1	5.84	0.257	412	0.084
Sex	1	1131	1131	1.36	0.494	12	0.435
Sex \times Maturity	1	1136.4	1136.4	0.52	0.666	9960	0.662

Table 3. Similarity of percentage (SIMPER) analyses between juvenile *Carcharhinus leucas* (n = 43) and pigeye *C. amboinensis* (n = 79) sharks

Prey type	Mean abundance		Dissimilarity		% contribution	% cumulation
	<i>C. leucas</i>	<i>C. amboinensis</i>	Mean	SD		
Teleost	0.79	0.81	12.06	0.64	22.72	22.72
Debri	0.42	0.05	11.87	0.81	22.36	45.08
Crustacean	0.26	0.18	9.38	0.68	17.67	62.75
Empty	0.12	0.09	7.44	0.45	14.01	76.76
Cephalopod	0.00	0.16	4.64	0.41	8.74	85.50
Fishery discards	0.00	0.15	3.87	0.41	7.29	92.79

broad diet for the species as a whole, the diet of each individual is less varied, suggesting that the species are individual dietary specialists, rather than true generalists (Matich et al. 2011).

Competition for prey (indicated by the degree of $\delta^{13}\text{C}$ overlap) between *C. leucas* and *C. amboinensis* should increase through ontogeny because these species move from separate nurseries to shared adult habitat (Compagno 1984). However, $\delta^{13}\text{C}$ results

from the current study suggest that dietary overlap, or at least competition for prey from similar trophic levels could occur at the juvenile stage and then continue into adult life. There was also some evidence that this result was dependent on sampling location, as we found large differences in prey source (as indicated by $\delta^{13}\text{C}$ values) among juveniles of *C. leucas* inhabiting different freshwater nurseries. These differences may be due to the occupation of different salinity environments within each river nursery (Heupel & Simpendorfer 2008, Ortega et al. 2009) and relative inputs from terrestrial carbon sources (Fry & Sherr 1984). Dietary overlap between similar-sized sharks was reported by Bethea et al. (2004) although it was hypothesized that these species may have reduced competition by partitioning resources in time or space. In our study, differences in prey taxa and species consumed by juvenile *C. leucas* and *C.*

Table 4. Information-theoretic ranking of models testing the effects of species, sex and maturity on prey species diversity. (a) Prey species diversity between juvenile *Carcharhinus leucas* (n = 43) and juvenile *C. amboinensis* (n = 79); (b) prey species diversity between juvenile (n = 79) and adult (n = 27) *C. amboinensis*. LL: maximum log-likelihood; k: number of model parameters; AIC_c: Akaike's information criterion corrected for small samples; ΔAIC_c: differences between the current and top-ranked model AIC_c; wAIC_c: AIC_c weights; % DE: percent deviance explained

	Model	LL	k	AIC _c	ΔAIC _c	wAIC _c	%DE
a)	Diversity ~ Species	-186.67	2	377.44	0.00	0.668	11.23
	Diversity ~ Sex	-197.91	2	389.93	12.48	0.001	0.13
	Diversity ~ Species + Sex	-186.66	3	379.52	2.09	0.235	11.25
	Diversity ~ Species × Sex	-186.49	4	381.33	3.89	0.096	11.55
b)	Diversity ~ Maturity	-169.44	2	342.99	0.00	0.625	5.22
	Diversity ~ Sex	-172.10	2	348.31	5.31	0.044	0.16
	Diversity ~ Sex + Maturity	-169.31	3	344.86	1.86	0.246	5.46
	Diversity ~ Sex × Maturity	-169.29	4	346.99	3.99	0.085	5.48

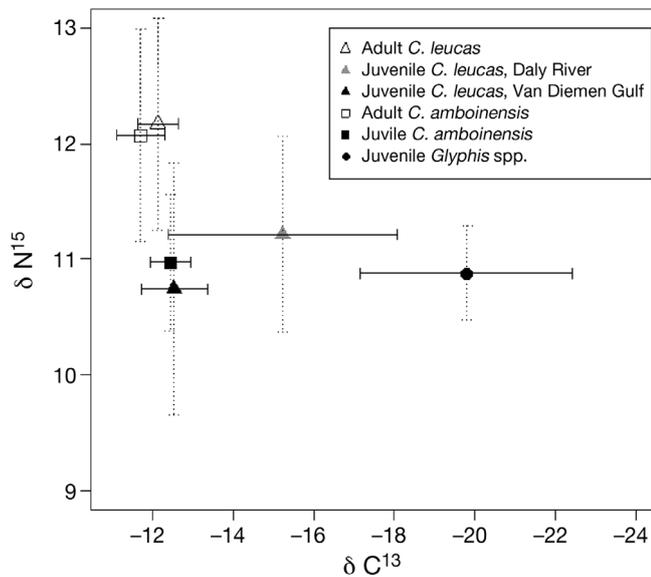


Fig. 4. Mean and variation of vertebral $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ concentrations from adult bull sharks *Carcharhinus leucas* (n = 20), adult pigeye sharks *C. amboinensis* (n = 20), juvenile bull sharks (sampled from 2 nurseries; n = 15 and n = 20 for Daly River and Van Diemen Gulf, respectively), juvenile pigeye sharks (n = 20), and juvenile northern river sharks *Glyphis* spp. (n = 5)

amboinensis identified from stomach contents could indicate temporal or spatial partitioning of diet within these food webs although it is unclear if this also occurs between sympatric adults.

We also found significant differences in diet between juvenile and adult *C. amboinensis*. Changes in diet with maturity are common in sharks, probably reflecting the improved foraging experience and prey acquisition skills of adults relative to juveniles (Lowe et al. 1996, Estrada et al. 2006, McElroy et al. 2006, Estupinan-Montano et al. 2009). Adults are more likely to overcome defences of prey than juveniles and have a larger mouth gape, increasing the size and diversity of prey available (Pyke 1984). We found evidence for increased consumption of larger-sized (and typically higher trophic-order) prey with maturity as isotope analyses suggested that adult *C. leucas* and *C. amboinensis* occupied a higher trophic position than juveniles of the same species, similar to results reported for *C. leucas* in earlier work by Werry et al. (2011). Furthermore, the diversity of prey also increased with maturity in *C. amboinensis*.

The apparent similarities in diet we found between male and female *C. leucas* and *C. amboinensis* were

Table 5. Summary of isotopic metrics. n: total number of individuals sampled; TA: total niche space occupied; CD: mean distance to centroid; MNND: mean nearest neighbour distance; SDNND: standard deviation of nearest neighbour distance

	n	$\delta^{15}\text{N}$ range	$\delta^{13}\text{C}$ range	TA	CD	MNND	SDNND
<i>Carcharhinus leucas</i>	55	9.8	5.9	28.74	1.843	0.353	0.329
<i>Carcharhinus amboinensis</i>	40	2.3	4.2	6.05	0.990	0.252	0.155
<i>Glyphis</i> spp.	5	5.6	1.1	2.6	2.255	0.803	0.285
Juvenile <i>C. leucas</i>	35	9.3	3.9	22.87	2.119	0.445	0.305
Adult <i>C. leucas</i>	20	2.5	4.3	5.34	1.034	0.411	0.359
Juvenile <i>C. amboinensis</i>	20	2	2	2.58	0.699	0.294	0.128
Adult <i>C. amboinensis</i>	20	2	2.8	3.31	0.829	0.312	0.214

not unexpected, since few studies have recorded differences in diets between sexes in other carcharhinids (McElroy et al. 2006, Lopez et al. 2009, Cabrera-Chávez-Costa et al. 2010, Markaida & Sosa-Nishizaki 2010). This similarity occurred despite the sex-specific use of habitats driven by traits such as female philopatry, which has recently been identified in *C. leucas* (Tillett et al. 2012) or the possibility of female mate avoidance (Feldheim et al. 2001, 2004, Portnoy et al. 2010, Verissimo et al. 2011). It would seem reasonable to assume that the greater energetic cost associated with egg production and gestation in females might result in preferences for prey yielding higher net energy profits, and thus differences in diet between males and females. Examination of this idea will require detailed quantification of the caloric value of diets of males and females during the time of mating and/or gestation.

The evidence we found for dietary partitioning within populations supports the conclusion of Matich et al. (2011) that individual specialisation may be an important feature of trophic dynamics in large marine predators. For juveniles, such small-scale partitioning may reflect differences in both anthropogenic pressures (e.g. fishing, dams or alteration of freshwater flow) and natural processes (e.g. catchment productivity) among estuarine systems. Because of the limited mobility of this life history stage, with juvenile *C. leucas* remaining in the same nursery habitats for up to 4 years, any differences in food availability driven by these processes are likely to be expressed in diets.

Our results suggest that resource partitioning occurs between the juveniles of sympatric *C. leucas* and endangered *Glyphis* spp. sharks in the Northern Territory (Peverell et al. 2006). Unlike the broad range of prey taken by juvenile *C. leucas*, only teleost fishes were identified in the stomachs of *Glyphis* spp. sharks. Although this result must be treated with caution, since it is based on a relatively small sample size, isotopic analyses also support the suggestion that juvenile *C. leucas* and *Glyphis* spp. feed on different food sources or that the food webs that support them are based on different primary producers. This result may reflect the fact that the nursery habitats shared by these species (freshwater rivers just upstream from estuaries) are very limited in spatial extent throughout much of northern Australia, thus providing strong selection for these species to partition resources.

Our study confirms the value in comparing both long-term and short-term analyses of diet to understand the ecological role of coastal sharks. Despite a similar dependence on coastal ecosystems, we found

differences in diet both in the short-term and through ontogeny that suggests partitioning of resources by sharks within these habitats. As such, we caution against pooling morphologically similar species *C. leucas* and *C. amboinensis* in ecosystem assessments. Similarly, our preliminary assessment of the diet of *Glyphis* sharks shows that their diet differs from that of sympatric *C. leucas*, suggesting that their ecological role will differ, at least within the juvenile habitat. Overall, $\delta^{13}\text{C}$ values reported here were similar to those quantified for other coastal sharks (Speed et al. 2012), reiterating the dependency of sharks on these near-shore environments and the importance of these habitats for conservation of these apex predators.

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