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ORIGINAL PAPER

Integrated approach to assess the spatio-temporal foraging dynamics of a temperate marine predator, the copper shark (*Carcharhinus brachyurus*)

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Abstract

Large-bodied sharks can be critical for coupling disparate habitats and food webs, which is considered central for ecosystem stability. Understanding the role of sharks and their associated predator–prey relationships across spatial scales is also integral to the development of multi-species ecosystem models. A combined stomach content ($n = 212$) and multi-tissue stable isotope (fast [liver; $n = 101$] vs slow turnover [muscle; $n = 108$]) approach was used to investigate the feeding ecology of the copper shark (*Carcharhinus brachyurus*) in the temperate waters of Southern Australia. Sharks were sampled from fishery catches over 3 years, during the austral spring–summer seasons and across three distinct regions. Stomach content analysis identified the copper shark as a generalist predator that consumes a diverse prey base dominated by *Sepia novaehollandiae, Sepioteuthis australis*, and *Sardinops sagax* (36%, 21%, and 18% IRI). Regional differences in diet composition were evident, although no size- or sex-based variation was identified. Isotope mixing models and regional food web bi-plots also revealed that *S. sagax* was the most important prey species, but temporal variation in diet was observed that matched known movements. The copper shark was estimated to be a primary piscivore, feeding at trophic level 4.49. Data on the feeding behaviour of copper sharks will provide vital inputs into future ecosystem-based fishery models and guide conservation and management of this important marine predator in temperate Southern Australian coastal waters.

Keywords Copper shark · Bronze whaler · Stable isotopes · Stomach content analysis · Diet · Feeding

Introduction

The ability of predators to move among discrete habitats and shift foraging between locally abundant prey is central to coupling energy flow among distinct food webs components. Such species that migrate or undertake broad-scale movements effectively link food webs throughout their range promoting ecosystem stability (Rooney et al. 2006; Rooney and McCann 2012). For example, the mobility of ground-dwelling predators in the grasslands of the Kenyan savanna is known to couple the canopy and understory (Pringle and Fox-Dobbs 2008), while sika deer (*Cervus nippon*) link fringe agricultural land and dense natural scrub in forests and grasslands (Takada et al. 2002). Similarly, in the aquatic realm, killer whales (*Orcinus orca*) predating on sea-otters link inshore and pelagic habitats with indirect effects on kelp forest community structure (Estes et al. 1998) and energy flow and linkages between contrasting habitats in freshwater lakes and streams are widely reported (Post et al. 2000; Schindler and Scheuerell 2002; Seminoff et al. 2007; Drew 2018).

Large-bodied sharks are considered highly mobile, upper-trophic level predators that consume a diverse range of prey species (Cortes 1997; Hussey et al. 2015). As a result of wide-ranging movements, large sharks have the potential to act as vectors, coupling energy among discrete habitats through exploiting locally abundant prey species

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in both vertical and horizontal dimensions. A study of predators at Palmyra Atoll, for example, found that blacktip reef sharks (*Carcharhinus melanopterus*) and grey reef sharks (*C. amblyrhynchos*) derived varying proportions of prey resources from lagoon, fore reef, and pelagic habitats (McCauley et al. 2012). At the macro scale, tiger sharks (*Galeocerdo cuvier*) have been shown to undertake seasonal broadscale movements between contrasting habitats to target prey, namely nesting green turtles (*Chelonia mydas*) around Raine Island, Australia (Fitzpatrick et al. 2012) and fledging albatross off French Frigate shoals, Hawaii (Meyer et al. 2010). Quantifying dynamic predator–prey interactions in the context of spatio-temporal variation in predator foraging behaviour is consequently necessary to accurately inform ecosystem-based models, but is often not considered (Pikitch et al. 2004; Barnett et al. 2010; Goldsworthy et al. 2013; Drew 2018).

Until recently, the diet or feeding ecology of sharks was mostly assessed through stomach content analysis (SCA) (Cortes 1997). This method offers a high degree of taxonomic precision through identifying prey consumed, but can be limited by variable digestion rates. As a result, SCA essentially provides a snapshot of recently consumed prey items (Cortés 1997; Hyslop 1980). In addition, a large number of sampled stomachs are required to accurately quantify diet over the spatial and temporal range of a target species (Hussey et al. 2011). This scale of sampling can be confounded by field logistics and associated costs, a high percentage of empty stomachs (e.g. Huveneers et al. 2007) and moral issues with sampling sufficient animals of non-exploited species or those that may be considered imperilled (Shiffman et al. 2012).

Advances in biochemical approaches, e.g. stable isotope (SIA) and fatty acid analyses, have provided alternative techniques to assess the diet and feeding ecology of species over varying temporal scales (Beckmann et al. 2013; Drew 2018; Meyer et al 2019). These approaches allow an assessment of diet through non-lethal and cost-effective methods, but lack the dietary resolution of SCA (Hussey et al. 2012). Consequently, a combined SCA and biochemical approach is now viewed as the most comprehensive method to assess diet (Hussey et al. 2011; Layman et al. 2012; Petta et al. 2020). For SIA, the rate of isotope incorporation into tissues has been shown to vary widely among tissue types dependent on metabolic turnover (Pinnegar and Polunin 1999). As a result, temporal shifts in feeding ecology can be investigated through analysing isotope values in tissues with different metabolic turnover rates (Kim et al. 2012; Logan and Lutcavage 2010; MacNeil et al. 2005). Isotopic incorporation rates can be as short as three to six months for blood (Kim et al. 2012) and liver (MacNeil et al. 2005) to over a year for muscle tissue (Kim et al. 2012; Matich et al. 2011; Pinnegar and Polunin 1999; Tieszen et al. 1983).

The copper shark (C*archarhinus brachyurus*) is a large-bodied, long-lived (> 30 years) (Drew et al. 2016; Drew 2018) neritic species that is widely distributed throughout the temperate waters of the northern and southern hemispheres (Last and Stevens 2009). To date, the feeding ecology of copper sharks has been investigated in the South West Atlantic (Argentina) and the South West and South East Indian Ocean (South Africa and Australia, respectively; Smale 1991; Lucifora et al. 2009; Dudley and Cliff 2010; Rogers et al. 2012; Drew 2018). In Argentina, SCA identified an ontogenetic shift in the diet of copper sharks with an increase in small-bodied elasmobranchs in the diet of adult sharks. However, small pelagic teleosts such as engraulidae and atherinopsidae species were identified as key prey items for both juveniles and adults (Lucifora et al. 2009). Off South Africa's Eastern Cape, copper sharks consumed a broad range of prey, with the Cape Hope squid (*Loligo reynandii*) being the most important prey item for both adult and juvenile sharks (Smale 1991). In contrast, the diet of copper sharks captured in beach protection nets in northern KwaZulu-Natal, 1000 km north of the Eastern Cape, was dominated by African sardines (*Sardinops occellatus*), a result of sharks seasonally exploiting this prey during their annual northward migration (Dudley and Cliff 2010). Similar to other regions, SCA identified that copper sharks in Southern Australia are generalist feeders, with a preference for small pelagic teleosts and cephalopod species (Rogers et al. 2012). Long-range movements of copper sharks > 1000 km have been identified in Southern Australia from acoustic telemetry and tag-recapture data, highlighting the potential for this species to couple resources across multiple habitats within their range (Rogers et al. 2011; Drew 2018; Drew et al. 2019) similar to conspecifics in South Africa. Currently, in Southern Australia little information exists on how copper sharks feeding ecology varies seasonally and regionally, and how spatio-temporal variations in prey abundance may promote movement patterns of this large-bodied predator.

To address this knowledge gap, an integrated assessment of the spatio-temporal foraging ecology of the copper shark off southern Australia, combining SCA and SIA approaches was undertaken. Specifically, sharks (and prey) were sampled at three regional sites in inshore and offshore waters (northern Gulf St. Vincent, eastern Spencer Gulf, and southern Spencer Gulf; Fig. 1) spanning distances between sampling regions of > 1000 km to address the following objectives: (i) quantify feeding behaviour based on SCA; (ii) estimate seasonal and spatial prey contributions to diet using SIA (using fast and slow turnover rate tissues); (iii) quantify seasonal variation in niche width; and (iv) characterise trophic position. These findings provide a comprehensive assessment of the feeding ecology of the copper shark in temperate waters off Southern Australia and highlight the

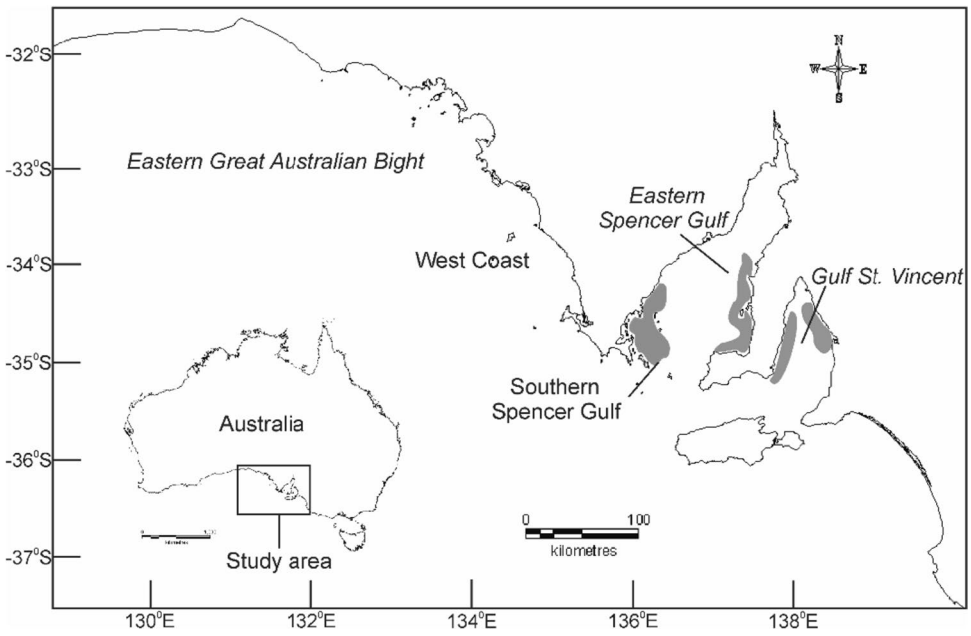

Fig. 1 Map of the coastline off Southern Australian, (inset) Australia. The three sampling regions for both sharks and prey, southern Spencer Gulf, eastern Spencer Gulf and Gulf St. Vincent are shaded in dark grey

importance of considering the spatial and seasonal occurrence of predators and prey to define the ecological role of mobile predators.

Methods

Sample collection

Copper sharks were sampled during the Austral autumn–spring (September 2009 and March 2014) through a combination of fisheries-dependent (South Australian Marine Scalefish Fishery and recreational fishers) and -independent sampling (scientific longlines) (Drew 2018). For the commercial fishery, sharks were sampled from longlines consisting of floating rope or monofilament mainlines with 1.2–1.7 mm stainless steel leaders with up to 200–400 16/o steel circle hooks attached to the mainline with a stainless steel clip. Mainlines were up to 8 km long and marked at each terminal end with 20 to 70 cm diameter rubber floats. Anchors at each end of the mainline were used to minimise drifting of gear during sets (Drew 2018). Hooks were spaced along the mainline at intervals of 10–20 m apart with small floats every two hooks. For the recreational fishery, sharks were caught using suspended baits under balloons, heavy tackle (30–80 lb line) and leaders of 1.5–1.7 mm nylon-coated wire attached to 12/0 or 14/0 J-style hooks (Drew 2018). Finally, scientific longlines were deployed using similar gear to that of the commercial longlines, but with a reduced number of hooks (~ 110 hooks) and a mainline of 1.1–1.7 km in length (Drew 2018). All sampling of sharks targeted the three focal study regions within South Australian Gulf waters: Gulf St. Vincent (GSV), eastern Spencer Gulf (ESG), and southern Spencer Gulf (SSG) (Fig. 1) (Drew 2018).

On capture, the sex of each shark was determined by the presence (male) or absence (female) of claspers. Length measurements were recorded to the nearest centimetre and included total length (TL), pre-caudal length (PCL), and trunk length (TKL). Linear regressions of TL on FL, PCL, and TKL were determined using data pooled across sexes. When TL could not be measured, e.g. due to fisher processing sharks before TL could be recorded ($n = 79$), TL was estimated using the regression for the next largest measurement, which was mostly PCL (Drew et al. 2016). For all captured sharks, the full stomach was removed and stored frozen. For a subset of sharks, sampled within the three regions (GSV, ESG, and SSG) and across the size range of individuals encountered, tissue samples were taken for SIA (Table 1) (Drew 2018). Approximately, 5 g of muscle and liver tissue were sampled; muscle samples were collected posterior of the cranium, where the head is separated from the carcass by commercial fishers and liver samples were collected from the lower section of the right or left lobe. Samples were either frozen immediately after collection or placed on ice until they were stored in a − 20 °C freezer. In conjunction with the sampling of predators, known prey species of copper sharks identified from SCA were sampled from the defined habitats (seagrass, reef, benthic, and pelagic) within each study region (Drew 2018). In addition, three representative baseline species (*Crassostrea gigas*, *Melicertus latisulcatus,* and *S. sagax*) were sampled from each region to delineate unique regional isotopic baselines. Muscle tissue from prey and baseline species were sampled

Table 1 Sample numbers used for stomach content analysis (SCA) and stable isotope analysis (SIA) of the copper shark (*Carcharhinus brachyurus*)

	Region	Total	Male	> 120	< 120	Female	> 120	< 120
SCA	GSV	105	38	15	23	67	29	38
	ESG	64	29	11	18	35	13	22
	SSG	43	18	11	7	25	19	6
	Total	212	92	37	48	120	61	66
SIA-muscle	GSV	33	14	4	10	19	4	15
	ESG	34	16	4	12	18	11	7
	SSG	34	15	12	3	19	16	3
	Total	101	45	20	25	56	31	25
SIA-liver	GSV	34	14	4	10	20	5	15
	ESG	36	17	12	5	19	11	8
	SSG	38	17	14	3	21	18	3
	Total	108	48	30	18	60	34	26

Muscle = muscle tissue samples for SIA; Liver = liver tissue samples for SIA; GSV = Gulf St. Vincent; ESG = eastern Spencer Gulf; SSG = southern Spencer Gulf; > 120 = no. of sharks greater than 120 cm TL; < 120 = no. of sharks less than 120 cm TL

from commercial fishing vessels, fish markets, scientific surveys, and recreational fishers (Drew 2018).

Stomach content

Stomachs were completely thawed prior to analysis and washed in running water using 0.5 mm sieves, and total contents weighed to the nearest 0.01 g. Prey identification was based on intact and remaining hard items, including cephalopod beaks, fish otoliths, and internal and external skeletal material, combined with general shape and anatomical features of the prey (Drew 2018). Where possible, prey were identified to the lowest taxon using reference guides (Lu and Ickeringill 2002; Gommon et al. 2008). Contents identified as bait via prominent hook marks or knife cuts were excluded from the analysis. The number of empty stomachs together with the number of stomachs containing only bait were recorded and expressed as a percentage of the total number examined (vacuity index, %V) (Drew 2018). Prey items were categorised into broad functional prey groups: chordata; crustacean; benthic cephalopod; unidentified cephalopod; benthic teleost; large pelagic teleost; small pelagic teleost; unidentified teleost; and elasmobranchs). Each item was weighed to the nearest 0.01 g. Cephalopod beaks were grouped into pairs, then weighed and recorded as one individual (Drew 2018).

Cumulative prey curves were generated for the number of prey items and functional prey groups identified to assess if the quantity of stomachs collected was adequate to describe the diet of copper sharks (Ferry and Cailliet 1996). The order in which stomachs were analysed was randomised ten times and the number of new prey items counted for each randomisation (Espinoza et al. 2015; Huveneers et al. 2007; Rogers et al. 2012). The mean number of prey items (± standard deviation, SD) and functional prey groups (± SD) per stomach were plotted against the number of stomachs sampled and a three-parameter von Bertalanffy growth curve fitted to the data, with L_{inf} representing the theoretical maximum number of species or functional prey groups that copper sharks consumed. If the estimated L_{inf} was less than the observed number of species or functional prey groups, it was considered that an adequate number of stomachs had been obtained to describe the total diet (Drew 2018).

The contribution of different prey items or functional prey groups to the diet of copper sharks was determined by the per cent numerical importance (%N) (Hyslop 1980), per cent frequency of occurrence (F%) (Hynes 1950; Hyslop 1980), and per cent weight (W%) (Hyslop 1980; Pillay 1952). Using these three indices, the index of relative importance (IRI) (Pinkas 1971), expressed as a percentage (IRI%) (Cortés 1997), was calculated for comparison with previously published diet studies (Rogers et al. 2012).

Analysis of copper shark diet by sex and size and among regions (GSV, ESG, and SSG) was performed using permutational multivariate analysis of variance (PERMANOVA, 4999 permutations) (Primer version 7.0.6). Prior to running the PERMANOVA, IRI prey values for each stomach were fourth-root transformed and a Bray–Curtis similarity resemblance matrix constructed. Region and shark sex and size were included in the analysis as categorical variables: GSV, ESG, and SSG; male and female; and < 120 cm TL or > 120 cm TL, the size at which differences in movement patterns are known to occur that could potentially influence the prey consumed in different regions (Drew 2018; Drew et al. 2019). If either sex or size were identified to have a significant effect among regions, a pairwise test using 4999 permutations was undertaken. Similarity of percentages (SIMPER) analysis was used to determine the prey species

that contributed the most to the similarities and dissimilarities between significant variables.

Stable isotope

Tissue samples for all predator, prey, and baseline species were freeze-dried for 48–96 h at – 50 °C and lipid extracted using chloroform and methanol (2:1) to remove lipids following the techniques described in Bligh and Dyer (1959). For sharks, muscle tissue samples were not water washed (Carlisle et al. 2017), but it was expected that lipid extraction would remove most urea (Hussey et al. 2012; Li et al. 2016). For liver, water washing has recently been shown to have minimal effect on $\delta^{15}N$ values (Pahl et al. 2020). The relative abundances of carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) were determined by a Thermo Finnigan Delta^{Plus} mass spectrometer (Thermo Finnigan, San Jose, CA, USA) coupled to an elemental analyser (Costech, Valencia, CA, USA). Ratios of heavy to light isotopes were expressed in δ notation according to the following equation: $\delta X = [(R_{Sample} / R_{Standard}) - 1] \times 1000$ (‰), where X is ^{15}N or ^{13}C, R_{sample} is the ratio of heavy to light isotope in the sample, and $R_{standard}$, the ratio of heavy to light isotope in the reference standard. Pee Dee Belemnite and atmospheric N_2 were used as standard reference materials for carbon and nitrogen, respectively. Laboratory and National Institute of Standards and Technology (NIST) standards were analysed every 12 samples to determine analytical precision. 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 $\delta^{13}C$ and 0.11‰ and 0.11‰ for $\delta^{15}N$.

Non-metric multidimensional scaling plots were created using copper shark $\delta^{13}C$ and $\delta^{15}N$ values by region and sex for liver and muscle tissue independently. A PERMANOVA (4999 permutations) was then performed on muscle and liver stable isotope data using a non-transformed Euclidean distance resemblance matrix, with region and sex as fixed factors. Size was excluded from the analysis given insufficient samples numbers per size class (Table 1). If significant differences were identified, a pairwise test using 4999 permutations was conducted as above for the SCA analyses.

To assess ontogenetic variation in muscle and liver $\delta^{13}C$ and $\delta^{15}N$ values of copper sharks as a measure of changes in foraging location and diet, respectively, isotope data were plotted against total body length and linear regression performed (Drew 2018). To examine individual and regional isotopic variation among sharks, inter-tissue variation in isotope values was estimated by calculating $\delta^{13}C$ and $\delta^{15}N$ residual values between muscle and liver for each individual (i.e. paired samples). Prior to this, the isotope values for muscle and liver were standardised to account for fractionation. Muscle tissue data were adjusted by the mean of the individual prey adjusted trophic enrichment factors (TEFs) calculated from the slope coefficients in Hussey et al. (2014) and Caut et al. (2009) ($\Delta^{15}N = 2.83$ and $\Delta^{13}C = 0.83$), while liver tissue was adjusted by a fixed value of 1.5 for $\delta^{15}N$ and 0.22 for $\delta^{13}C$ (Hussey et al. 2011). A single factor ANOVA was used to test for statistical differences between tissues for each isotope ($\delta^{13}C$and $\delta^{15}N$) for each region.

To visually assess the relative trophic role of copper sharks relative to potential sympatric prey consumed in each sampling region, bi-plots of mean (±) SD $\delta^{13}C$ and $\delta^{15}N$ values of predator and prey were examined (Drew 2018). The isotope values of all prey items were adjusted to account for the TEF of shark muscle and liver tissue as described above.

Bayesian isotopic ellipse area (SEAb) and corrected ellipse area for small sample sizes (SEAc), a measure of isotopic niche space, were estimated using the R package SIBER (Stable Isotope Bayesian Ellipses in R) (Jackson et al. 2011). Ellipse areas were calculated for muscle and liver tissue data independently and for each region to examine spatio-temporal variation in isotopic niche space (Drew 2018). Differences in niche among regions were considered significant if the 95% credible interval ellipses did not overlap.

The proportional contribution of primary prey items and functional prey groups to the diet of copper sharks was estimated for both tissue types across all regions using the R package SIAR (Stable Isotope Analysis in R) (Jackson et al. 2011). The SIAR isotope mixing model incorporates uncertainties within the consumer and prey isotope data and that of TEFs (± SD). All prey selected as source contributions for the isotope mixing models were identified as primary prey from stomach content data (Rogers et al. 2012 and Drew 2018). To determine if prey source isotope values were unique, i.e. to identify isotopically appropriate sources for the mixing models, A PERMANOVA was performed on the non-transformed stable isotope values of prey using a Euclidean distance matrix. Species with overlapping isotope values were categorised into biologically relevant groups based on species type and primary residing habitat resulting in five distinct prey categories: cephalopods, reef teleosts, small pelagic teleosts, herbivorous and omnivorous species, and carnivorous species.

To examine the trophic role of copper sharks within the three gulfs, the trophic position (TP) was calculated using both a scaled $\Delta^{15}N$ approach (TP_{scaled}; Hussey et al. 2014) and the traditional additive method of Vander Zanden and Rasmussen (1999; $TP_{addititive}$). The scaled approached was calculated as follows:

$$\mathrm{TPscaled} = \frac{\log(\delta^{15}\mathrm{Nlim} - \delta^{15}\mathrm{Nbase}) - \log(\delta^{15}\mathrm{Nlim} - \delta^{15}\mathrm{Ntp})}{k} + \alpha, \quad (1)$$

$$\mathrm{TPadditive} = \alpha + \frac{(\delta^{15}\mathrm{Nconsumer} - \delta^{15}\mathrm{Nbase})}{\Delta n}, \quad (2)$$

where (1) δ^{15}N lim is the dietary δ^{15}N value at which ^{15}N incorporation and ^{15}N elimination are equal; δ^{15}N base is the δ^{15}N value of a known baseline consumer; $\delta^{15}N_{tp}$ is the consumer isotope value at a given trophic position; α is the tropic position of the baseline organism; and (2) δ^{15}N consumer is the δ^{15}N value of the consumer of interest, and Δn is the trophic enrichment factor of 2.3‰ for muscle tissue according to Hussey et al. (2011). Three independent baseline consumers (*C. gigas* TP = 2.5, *M. latisulcatus* and *S. sagax* TP = 3) and a combination of the three baselines were used to estimate the TP of copper sharks using muscle tissue to limit bias associated with using a single baseline species (Frisch et al. 2016).

Results

Stomach content analysis

A total of 306 stomachs from copper sharks were sampled from three regions within coastal South Australia (Fig. 1), with 212 containing prey items (Table 1; vacuity index = 31%). Fifty-two different prey items were identified and categorised into nine functional prey groups (Table 2). Prey diversity was high and ranged from small crustaceans to large pelagic teleosts and demersal elasmobranchs. The cumulative prey curve for the lowest taxonomic level did not reach an asymptote (Fig. 2a), but the cumulative prey curve for the functional prey groups indicated that a sample size of 212 stomachs was sufficient to describe the diet of copper sharks at this taxonomic level (Fig. 2b). The von Bertanlanffy curve fit to both the lowest taxonomic level and functional prey group data was in agreement with the cumulative prey curves with L_{inf} estimates of 58.1 and 8.1, respectively, with the latter value less than the observed number of functional prey groups.

The most important prey species contributing to diet of copper sharks (with IRI% > 10%) were the cephalopods, *S. apama, S. novaehollandiae* and *S. australis*, and the small pelagic teleost, *S. sagax* (Table 2). Additional important prey were benthic teleosts including flathead (*Platycephalus* spp.) (IRI% = 0.78) the Southern Garfish (*Hyporhamphus melanochir*) (IRI% = 0.41) and snook (*Sphyraena novaehollandiae*) (IRI% = 0.24). The small pelagic teleosts, Australian Salmon (*Arripis truttaceus*) (IRI% = 0.93) and maray (*Etrumeus teres*) (IRI% = 0.29) also contributed to the diet of copper sharks (Table 2). Rays species including the southern eagle ray (*Myliobatis australis*) and Rajidae sp. were identified in the diet, but in relatively low numbers (IRI% = 0.11 and 0.14, respectively).

Non-metric multidimensional scaling revealed no visual clustering or separation of diet between sexes, sizes, or regions (Supplementary material, Fig. S1). PERMANOVA, however, found a significant difference in overall diet by region ($p < 0.001$), but not by sex ($p = 0.11$) or size ($p = 0.06$). PERMANOVA pairwise tests by region estimated that both GSV and ESG were significantly different from SSG ($p = 0.001$ for both regions, respectively), but were not significantly different from each other ($p = 0.15$). SIMPER analysis showed the diet of copper sharks from GSV and ESG were the most similar (dissimilarity = 85.5%), while copper sharks from SSG had diet that was moderately distinct from the other two regions (dissimilarity = 89.5% from ESG and 90.1% from GSV). Differences in diet between SSG and ESG/GSV regions were mostly driven by fish, and cephalopods with both *S. sagax* and *S. novaehollandiae* contributing ~ 32% of the difference.

Stable isotope analysis (δ^{13}C and δ^{15}N)

A total of 101 muscle and 108 liver samples were collected from copper sharks (Table 1; $n = 108$ individuals) and mean (± SD) isotope values calculated for 34 potential prey items (Table 3). Estimated mean δ^{13}C and δ^{15}N values for both copper shark muscle and liver tissue showed large variation, with the highest evident in both muscle and liver δ^{13}C values for the ESG (Fig. 3). Liver tissue δ^{15}N values were consistently lower than those in muscle across all three sampling sites and liver δ^{13}C values were moderately depleted when compared to muscle (Fig. 3). nMDS plots showed no separation or clustering of δ^{13}C and δ^{15}N values for either region or sex (Supplementary material, Fig. S2a); the δ^{13}C and δ^{15}N values of muscle were similar between sexes ($p = 0.092$ and 0.176, respectively) and among regions ($p = 0.078$). In contrast, nMDS plots of liver δ^{13}C and δ^{15}N values showed minor separation among regions, but no distinct clustering was apparent between sexes (Supplementary material, Fig. S2b); liver δ^{13}C and δ^{15}N were found to differ among all regions ($p = 0.002$), with ESG the most different to GSV and SSG ($p = 0.014$ and 0.005, respectively). When examining total length of copper sharks versus δ^{13}C and δ^{15}N values, there was high variation in the smallest sharks and some evidence of increasing δ^{15}N values between individuals measuring ~ 150 cm to 180 cm, but no significant linear relationship was observed for either muscle or liver tissue (Fig. 4a–d).

Equally, no significant differences in inter-tissue isotopic comparisons were observed among regions for either δ^{13}C or δ^{15}N (Table 4, Fig. 5). The mean δ^{13}C residual values for each region were similar ($p = 0.083$), with the

Table 2 Prey items identified through stomach content analysis and calculated diet indices contributing to the total diet of the copper shark (*Carcharhinus brachyurus*).

	N	%N	F	%F	W (grams)	%W	IRI	%IRI
Chordata								
Unidentified ascidian	1.00	0.17	1.00	0.47	9.00	0.04	0.10	0.00
Crustaceans								
Brachyura	1.00	0.17	1.00	0.47	5.00	0.02	0.09	0.00
Isopoda	7.00	1.22	7.00	3.29	1.51	0.01	4.04	0.14
Jasus edwardsii	1.00	0.17	1.00	0.47	26.95	0.13	0.14	0.00
Ovalipes australiensis	2.00	0.35	2.00	0.94	16.36	0.08	0.40	0.01
Portunus pelagicus	3.00	0.52	3.00	1.41	180.57	0.85	1.94	0.07
Benthic Cephalopod								
Octopus berrima	7.00	1.22	6.00	2.82	10.13	0.05	3.58	0.12
O. maorum	1.00	0.17	1.00	0.47	0.20	0.00	0.08	0.00
Sepia apama	60.00	10.47	46.00	21.60	1024.32	4.83	330.43	11.30
S. braggi	1.00	0.17	1.00	0.47	5.10	0.02	0.09	0.00
S. novaehollandiae	139.00	24.26	74.00	34.74	1276.57	6.02	1051.87	35.98
Sepioteuthis australis	77.00	13.44	50.00	23.47	2567.91	12.11	599.65	20.51
Unidentified cephalopod	5.00	0.87	4.00	1.88	19.83	0.09	1.81	0.06
Unidentified sepia	6.00	1.05	6.00	2.82	206.99	0.98	5.70	0.19
Small pelagic teleost								
Arripis georgianus	3.00	0.52	3.00	1.41	38.61	0.18	0.99	0.03
A. truttaceus	10.00	1.75	9.00	4.23	988.08	4.66	27.06	0.93
Engraulis australis	1.00	0.17	1.00	0.47	5.36	0.03	0.09	0.00
Etrumeus teres	21.00	3.66	2.00	0.94	1105.88	5.21	8.34	0.29
Sardinops sagax	95.00	16.58	34.00	15.96	3616.98	17.05	536.86	18.37
Scomber australasicus	3.00	0.52	3.00	1.41	164.14	0.77	1.83	0.06
Unidentified small pelagic teleost	12.00	2.09	10.00	4.69	449.40	2.12	19.78	0.68
Benthic teleost								
Achoerodus gouldii	1.00	0.17	1.00	0.47	1660.87	7.83	3.76	0.13
Aldrichetta forsteri	4.00	0.70	4.00	1.88	410.24	1.93	4.94	0.17
Chelmonops curiosus	1.00	0.17	1.00	0.47	34.50	0.16	0.16	0.01
Chrysophrys auratus	4.00	0.70	4.00	1.88	538.22	2.54	6.08	0.21
Genypterus tigerinus	1.00	0.17	1.00	0.47	555.04	2.62	1.31	0.04
Girella zebra	1.00	0.17	1.00	0.47	373.55	1.76	0.91	0.03
Haletta semifasciata	1.00	0.17	1.00	0.47	76.34	0.36	0.25	0.01
Hyporhamphus menanochir	11.00	1.92	9.00	4.23	196.34	0.93	12.02	0.41
Mugilidae sp.	1.00	0.17	1.00	0.47	17.60	0.08	0.12	0.00
Parequula melbournensis	2.00	0.35	2.00	0.94	23.46	0.11	0.43	0.01
Platycephalus bassensis	1.00	0.17	1.00	0.47	219.48	1.03	0.57	0.02
Platycephalus sp.	7.00	1.22	7.00	3.29	1204.44	5.68	22.68	0.78
Pseudocaranx georgianus	1.00	0.17	1.00	0.47	19.15	0.09	0.12	0.00
Scolecenchels australis	7.00	1.22	7.00	3.29	138.81	0.65	6.17	0.21
Scorpis aequipinnis	1.00	0.17	1.00	0.47	12.03	0.06	0.11	0.00
Sillago schomburgkii	1.00	0.17	1.00	0.47	58.40	0.28	0.21	0.01
Sillago sp.	5.00	0.87	5.00	2.35	267.34	1.26	5.01	0.17
Sphyraena novaehollandiae	5.00	0.87	4.00	1.88	608.40	2.87	7.03	0.24
Syngnathidae Sp	1.00	0.17	1.00	0.47	3.29	0.02	0.09	0.00
Thamnaconus degeni	1.00	0.17	1.00	0.47	12.94	0.06	0.11	0.00
Upeneichthys vlamingii	3.00	0.52	3.00	1.41	264.31	1.25	2.49	0.09
Large pelagic teleost								
Thunnus sp.	1.00	0.17	1.00	0.47	201.18	0.95	0.53	0.02
Unidentified teleost								
Unidentified teleost	39.00	6.81	39.00	18.31	1347.88	6.35	240.98	8.24

Table 2 (continued)

	N	%N	F	%F	W (grams)	%W	IRI	%IRI
Elasmobranch								
Myliobatis tenuicaudatus	3.00	0.52	3.00	1.41	388.00	1.83	3.31	0.11
Orectolobus sp.	1.00	0.17	1.00	0.47	118.52	0.56	0.34	0.01
Parascyllium Sp	1.00	0.17	1.00	0.47	35.72	0.17	0.16	0.01
Rajidae sp.	4.00	0.70	4.00	1.88	327.21	1.54	4.21	0.14
Squatina australis	1.00	0.17	1.00	0.47	11.89	0.06	0.11	0.00
Trygonorrhina fasciata	1.00	0.17	1.00	0.47	89.65	0.42	0.28	0.01
Unidentified elamsobranch	4.00	0.70	4.00	1.88	263.27	1.24	3.64	0.12
Urolophus sp.	1.00	0.17	1.00	0.47	12.81	0.06	0.11	0.00

N number counted; %N numerical importance; F frequency item occurred; %F percentage of frequency of occurrence; W = total weight of items; %W = percentage of weight; IRI = index of relative importance; %IRI = percentage of index of relative importance

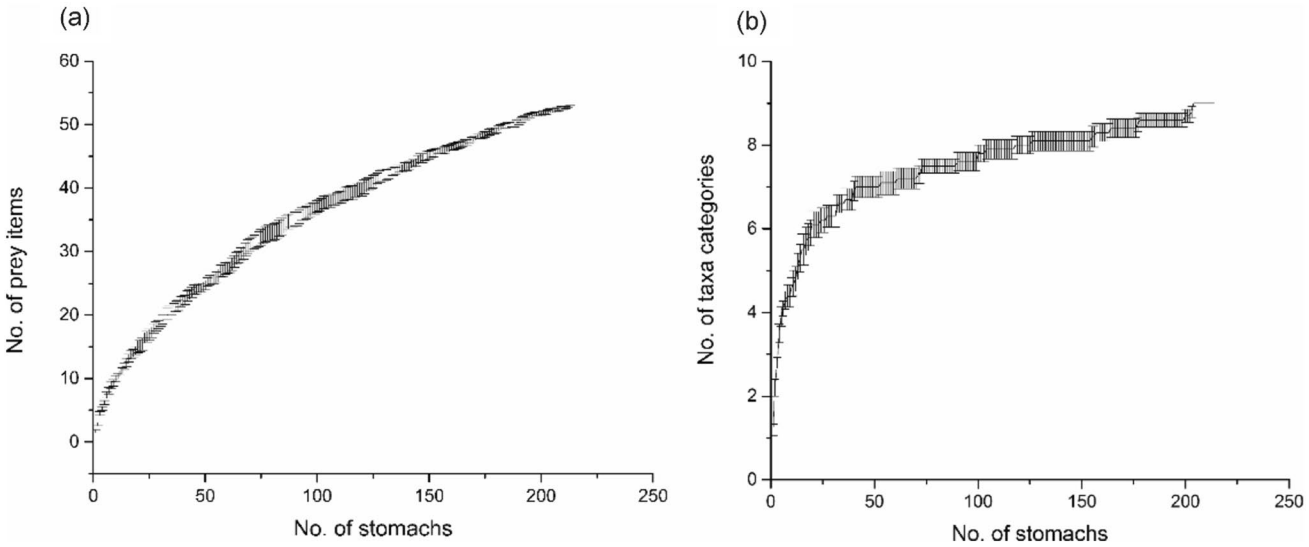

Fig. 2 Cumulative prey curves showing the relationship between the number of stomachs and the number of identifiable prey items (**a**) and functional prey groups (**b**). Error bars represent the standard error of the means

largest variation observed for SSG (Table 4, Fig. 5a). For $\delta^{15}N$ residuals, GSV showed the largest variation, but again the mean trend among regions was not significant ($p = 0.087$; Fig. 5b).

The mean isotope values for muscle and liver tissue from copper sharks sampled in the northern gulf waters regions of GSV and ESG closely align with prey items from benthic and seagrass habitats (Fig. 6). Conversely, sharks sampled from the southern gulf and more pelagic environment strongly align with the pelagic prey species sampled (Fig. 6).

Copper sharks occupied a large isotopic niche area (SEAc), with the isotopic niche area based on liver tissue (2.4–3.7) being slightly larger than for muscle tissue (2.0–2.4) (Fig. 7a, b). There was little regional variation in isotope niche width for either muscle or liver tissue (Fig. 7a, b) with large overlap in Bayesian standard ellipses (Fig. 7a, b).

The estimated proportional contribution of categorised prey groups to the diet of all size copper sharks combined based on muscle tissue isotope values, found that small pelagic fishes contributed the most to the diet (~ 70%), followed by the herbivorous and omnivorous fish group (~ 20%), while the two groups of cephalopods and reef fishes had low contributions of ~ 5–10%, and carnivorous teleost contributed < 5% (Fig. 8a). In contrast, mixing model results for liver tissue found that the dominant prey were herbivorous and omnivorous fish (~ 45%) and small pelagic teleosts (~ 45%), with minor contributions by the cephalopods, reef fishes and carnivorous teleost (Fig. 8b).

Trophic position of copper sharks estimated using a combination of baseline species was 4.5 ± 0.38 and 5.4 ± 0.42

Table 3 Mean stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) for copper shark (*Carcharhinus brachyurus*) tissue (muscle and liver) by region and for prey (muscle)

Species	SIAR group	No. of samples	Tissue	Region	$\delta^{13}C$	SD	$\delta^{15}N$	SD
Carcharhinus brachyurus		33	Muscle	GSV	− 17.18	0.71	13.94	1.01
C. brachyurus		34	Muscle	ESG	− 17.21	0.99	13.03	0.86
C. brachyurus		34	Muscle	SSG	− 17.83	1.35	13.60	0.80
C. brachyurus		34	Liver	GSV	− 17.60	1.08	12.16	1.21
C. brachyurus		36	Liver	ESG	− 17.84	1.03	11.43	0.94
C. brachyurus		38	Liver	SSG	− 18.53	1.41	11.92	0.84
Melicertus latisulcatus	*	5	Muscle	GSV	− 17.56	0.80	9.43	1.60
Notolabrus tetricus	Reef	5	Muscle	GSV	− 21.04	0.63	12.33	0.76
Scorpis aequipinnis	Reef	5	Muscle	GSV	− 20.89	1.32	12.46	1.14
Meuschenia hippocrepis	Reef	5	Muscle	GSV	− 19.97	0.62	10.84	0.54
Sepioteuthis australis	Cephalopod	5	Mantle	GSV	− 14.29	1.69	10.66	0.44
Sphyraena novaehollandiae	Carnivorous	5	Muscle	GSV	− 16.85	2.31	12.65	1.11
Chrysophrs auratus	Carnivorous	5	Muscle	GSV	− 17.38	1.70	11.86	1.16
Hyporhamphus menanochir	H and O	5	Muscle	GSV	− 14.81	2.38	8.86	0.98
Sillaginodes punctatus	H and O	3	Muscle	GSV	− 16.65	0.67	10.88	0.05
Sepioteuthis australis	Cephalopod	3	Muscle	GSV	− 18.20	0.65	10.44	1.18
Posidonia angustifolia	*	5	Seagrass	GSV	− 9.33	0.76	6.25	0.48
Crassostrea gigas	*	5	Muscle	GSV	− 18.58	0.23	4.55	0.32
M. latisulcatus	*	5	Muscle	ESG	− 18.50	0.46	7.16	1.07
N. tetricus	Reef	5	Muscle	ESG	− 20.44	0.70	11.54	0.73
C. gigas	*	5	Muscle	ESG	− 19.83	0.28	4.09	0.29
S. australis	Cephalopod	5	Muscle	ESG	− 16.14	1.34	11.44	0.46
S. novaehollandiae	Carnivorous	5	Muscle	ESG	− 17.36	0.48	11.66	0.65
Arripis georgianus	H and O	5	Muscle	ESG	− 16.12	1.14	11.76	0.82
H. menanochir	H and O	5	Muscle	ESG	− 17.80	1.50	11.97	1.85
S. punctatus	H and O	5	Muscle	ESG	− 16.28	0.78	10.71	0.67
Acanthaluteres brownii	Reef	5	Muscle	ESG	− 17.00	0.71	6.12	0.18
Platycephalus speculator	Carnivorous	3	Muscle	ESG	− 17.28	0.09	11.78	0.42
P. bassenis	Carnivorous	2	Muscle	ESG	− 16.86	0.41	11.68	0.12
Posidonia species	*	5	Seagrass	ESG	− 11.48	0.37	3.17	1.24
Haletta semifasciata	H and O	5	Muscle	ESG	− 15.07	1.14	8.54	0.39
Meuschenia scaber	H and O	5	Muscle	ESG	− 17.78	0.48	8.56	0.33
M. freycineti	H and O	6	Muscle	ESG	− 16.10	1.11	9.31	0.45
Notolabrus parilus		2	Muscle	ESG	− 15.82	0.34	11.72	0.02
A. truttacea	Carnivorous	5	Muscle	ESG	− 18.95	0.41	13.71	1.08
C. gigas	*	5	Muscle	SSG	− 20.10	0.32	4.28	0.20
Sardinops sagax	Small pelagic	5	Muscle	WC	− 19.95	0.16	11.38	0.70
Nototodarus gouldi	Cephalopod	4	Muscle	WC	− 17.27	0.28	11.77	0.86
S. sagax	Small pelagic	5	Muscle	SSG	− 20.69	0.16	9.73	0.40
P. conatus	Carnivorous	5	Muscle	SSG	− 19.09	0.11	14.58	0.34

The SIAR group identifies the prey category that the prey item was grouped in based on habitat type/functional diet; H and O = herbivorous and omnivorous; GSV = Gulf St. Vincent; ESG = eastern Spencer Gulf; SSG = southern Spencer Gulf; SD = standard deviation

*Denotes baseline species for tropic position estimates

for the scaled and constant TP approaches, respectively (Table 5). Trophic position estimates using the additive approach were consistently higher than those estimated using the scaled approach when using individual and combined baseline species (Table 5).

Discussion

Understanding the feeding and trophic ecology of predators can identify critical predator–prey relationships, revealing insights into habitat use, broad-scale

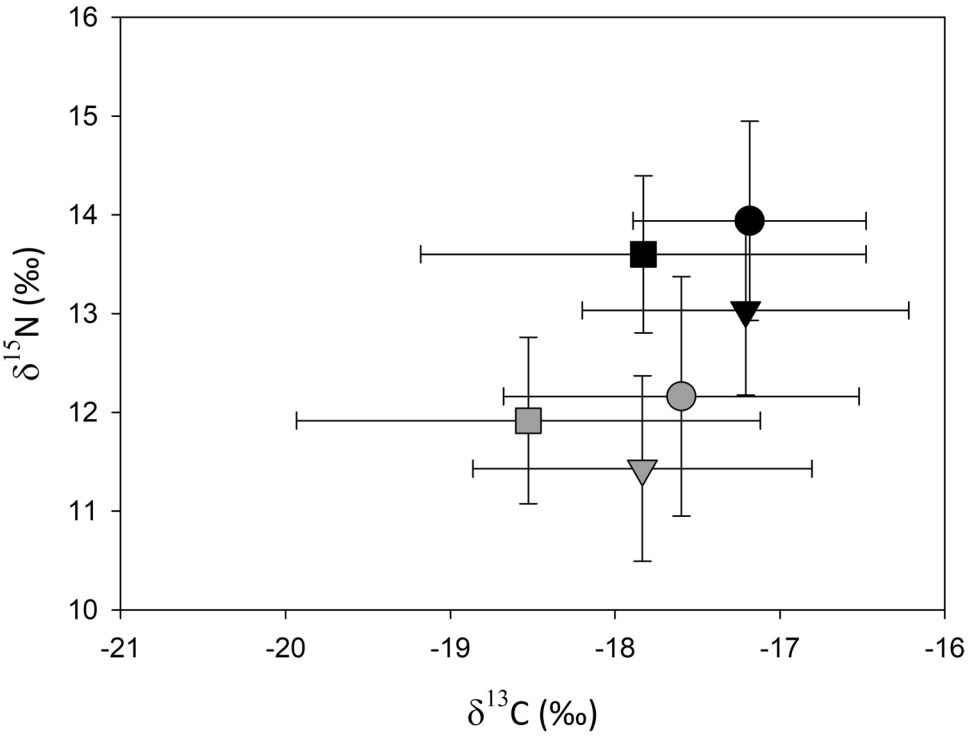

Fig. 3 Bi-plots of mean copper shark (*Carcharhinus brachyurus*) $\delta^{15}N$ and $\delta^{13}C$ values (with standard deviation) for muscle (black) and liver (grey) tissue sampled from the three study regions. Circles represent Gulf St. Vincent; triangles represent eastern Spencer Gulf; squares represent southern Spencer Gulf (see Fig. 1)

movements, and shifts in life history. Such information is integral for developing ecosystem models to understand the effects of changing predator or prey populations due to natural or anthropogenic events. The copper shark off Southern Australia was found to feed on a diverse prey base, based on SCA, but its diet was dominated by key species: *S. sagax* and locally abundant cephalopods (*S. novaehollandiae*, *S. australis*, and *S. apama*). In terms of temporal variation in diet examined using SIA, tissue-specific incorporation rates (i.e. muscle versus liver) revealed a seasonal shift in the proportion of prey originating from continental shelf waters (*S. sagax* dominant; muscle) to those from both coastal areas and shelf waters (herbivorous and omnivorous fish equally represented with *S. sagax*; liver). Stomach content and stable isotope data were mostly in agreement and support previous findings for copper sharks sampled in southern Australia, South Africa, and Argentina (Cliff and Dudley 1992; Lucifora et al. 2009; Smale 1991; Rogers et al. 2012). The high contribution of low trophic level prey species, such as *S. sagax,* irrespective of shark body length, resulted in a lack of ontogenetic variation and a trophic position estimate associated with lower order carnivorous teleosts. These data suggest that large copper sharks can exert direct top down pressure on lower trophic level species in their respective food webs.

Overall prey species diversity

Stomach content analysis revealed that copper sharks consumed 52 unique prey items with prey species ranging from benthic crustaceans to pelagic teleosts and small-bodied elasmobranchs, identifying feeding in diverse habitats (both spatially and vertically in the water column). While prey species diversity was high, both SCA and SIA identified several dominant species including small pelagic teleosts (*S. sagax*) and locally abundant cephalopods species (*S. australis, S. novaehollandiae*, and *S. apama*); isotope mixing models estimated that small pelagic teleosts and herbivorous and omnivorous teleosts were principal contributions to total diet. The importance of small pelagic teleosts to the diet of copper sharks highlights the ecological role that this shark species may play in regulating population dynamics of small pelagic teleosts in the coastal waters of Southern Australia (Goldsworthy et al. 2013).

The dominance of small pelagic teleosts and cephalopods in the diet of copper sharks has previously been identified in Southern Australia (Rogers et al. 2012), in the South West Atlantic (Lucifora et al 2005), and South West Indian Ocean (Cliff and Dudley 1992; Smale 1991). The dominance of the South African sardine (*Sardinops ocellatus*) in the stomach contents of copper sharks off South Africa was, however, biased by the high seasonal abundance of *S. ocellatus* during

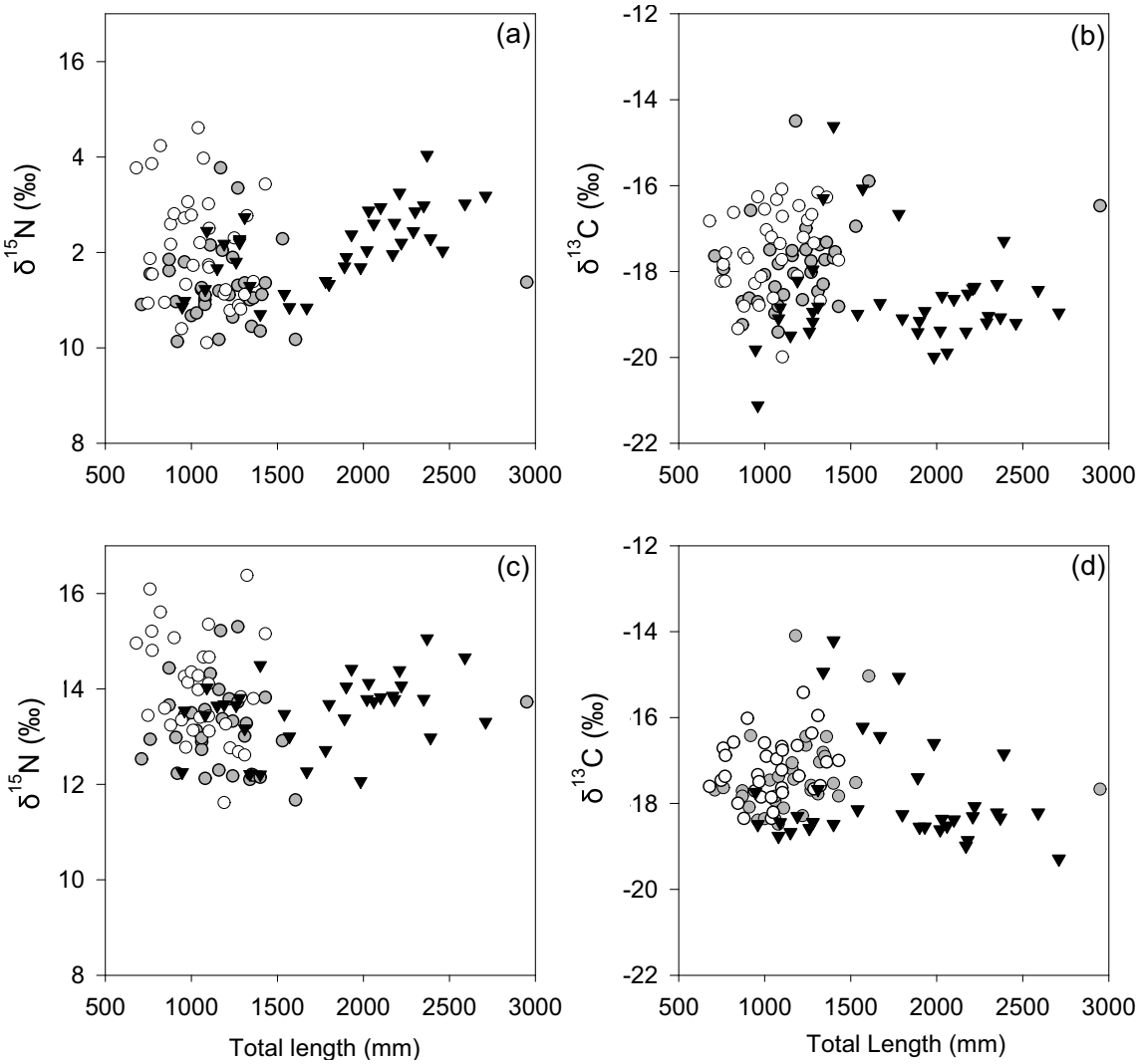

Fig. 4 Ontogenetic variation in $\delta^{15}N$ and $\delta^{13}C$ values by total length of copper sharks (*Carcharhinus brachyurus*). Top = muscle tissue; bottom = liver tissue. Left (**a, c**) = $\delta^{15}N$; right (**b, d**) = $\delta^{13}C$. White circles = Gulf St. Vincent; grey circles = eastern Spencer Gulf; and black triangles = southern Spencer Gulf

Table 4 The mean d13C and d15N values for inter-tissue variation in isotope values between sampling regions for muscle and liver tissue of the copper shark (*Carcharhinus brachyurus*)

	Muscle $\delta^{13}C$	Liver $\delta^{13}C$	$\Delta\ \delta^{13}C$	Muscle $\delta^{15}N$	Liver $\delta^{15}N$	$\Delta\ \delta^{15}N$
GSV	− 17.17	− 17.53	0.36	13.98	12.19	1.79
ESG	− 17.37	− 17.82	0.44	13.17	11.30	1.87
SSG	− 17.85	− 18.62	0.77	13.54	12.05	1.49
Total	− 17.47	− 17.99	0.52	13.56	11.85	1.72

Δ represents the difference in mean isotope values

winter (June–August) when this species migrates northward, which coincides with the majority of copper shark catches in beach protection nets (Cliff and Dudley 1992; Dudley and Cliff 2010). Another study undertaken off the Eastern Cape of South Africa, however, found that the Cape Hope squid (*Loligo reynandii*) dominated the diet of both juvenile and adult copper sharks (Smale 1991). These findings were based on sharks collected over a relatively short spatio-temporal scale, and consequently may reflect the selective targeting of prey by copper sharks at that point in time and in specific locations (Munroe et al. 2014). Similarly, our integrated approach suggests that copper sharks off Southern

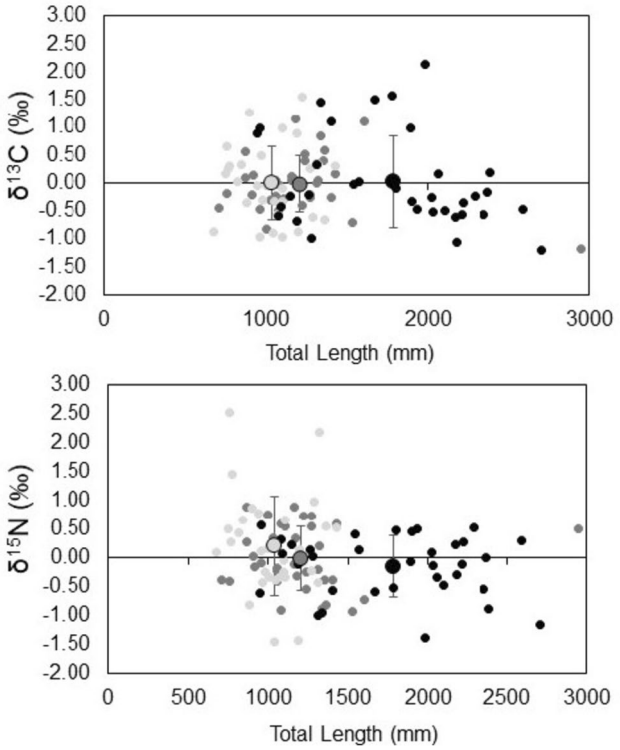

Fig. 5 Inter-tissue stable isotope ($\delta^{13}C$ and $\delta^{15}N$) residual variations for each individual copper shark. Plots are ordered in increasing total length within each region. a) The between tissue residual variation of each individual for $\delta^{13}C$; b) The between tissue residual variation of each individual for $\delta^{15}N$; light grey circle = GSV; light grey circle with black boarder = GSV mean residual value; medium grey circle = ESG; medium grey circle with black boarder = ESG mean residual; black circle = SSG; larger black circle = SSG mean residual

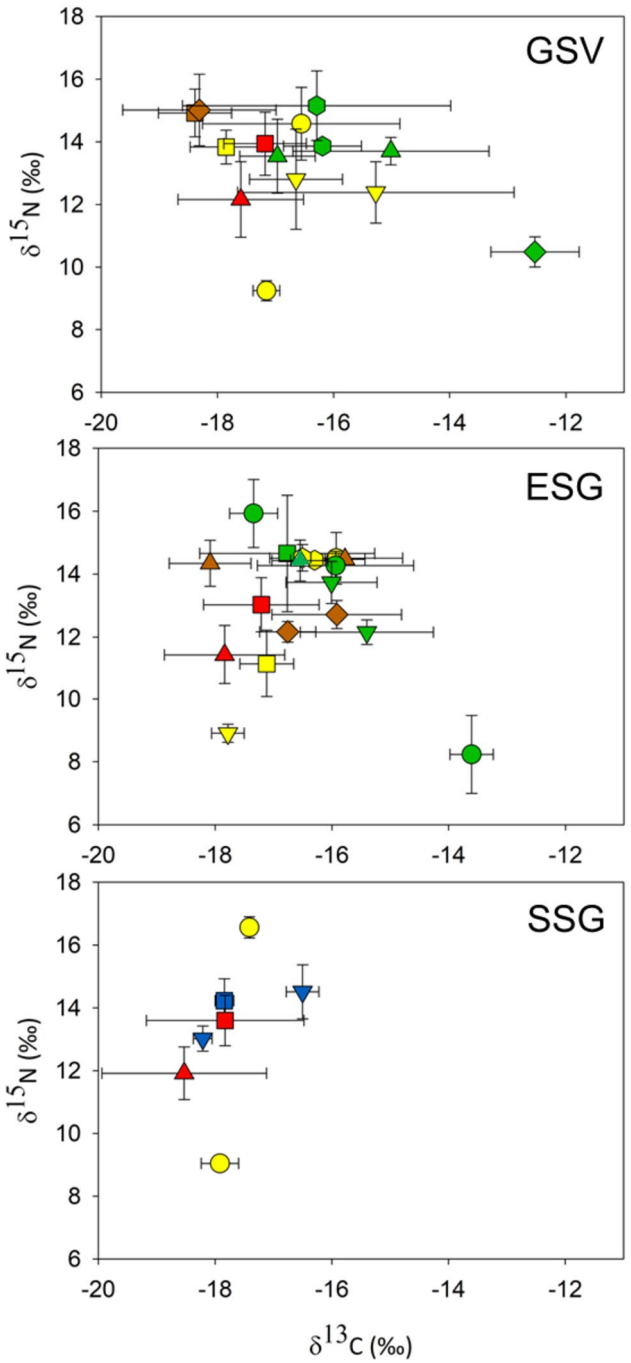

Fig. 6 Bi-plots of the mean stable isotope values with standard deviation of copper shark (*Carcharhinus brachyurus*) and sympatric prey species ($n = 34$; Table 3) for top = Gulf St. Vincent, middle = eastern Spencer Gulf, and bottom = southern Spencer Gulf. The colours represent the habitat that each prey item resides: yellow = benthic, green = seagrass, blue = pelagic, brown = reef. Red squares (muscle) and red triangles (liver) represent isotope data for copper sharks

Australia exploit seasonally or spatially abundant prey (relative to their abundance in the environment). This matches that observed for other shark species including tiger sharks, identifying that while sharks may appear to be generalist feeders, they often focus on key prey related to optimal foraging and meeting energetic requirements (Meyer et al 2010; Hammerschlag et al. 2016).

Regional variation in diet

Regional differences in prey species contributing to the diet of copper sharks were identified through SCA and liver tissue SIA, but not muscle SIA; the diet in the northern gulf regions ESG and GSV were moderately different from the southern gulf region of SSG. Both methods estimate diet over short timeframes, with SCA representing diet over the past few days and liver tissue representing prey assimilation over the previous 3–6 months (Logan and Lutcavage 2010; MacNeil et al. 2005). These regional variations in diet likely reflect regionally distinct prey fields, primarily driven by contrasting habitat types where sampling occurred. The two northern gulf regions have similar prey assemblages, with benthic and demersal teleosts species such as *Platycephalus* sp., *A. truttaceus*, *S. novaehollandiae*, and *sillago* sp.

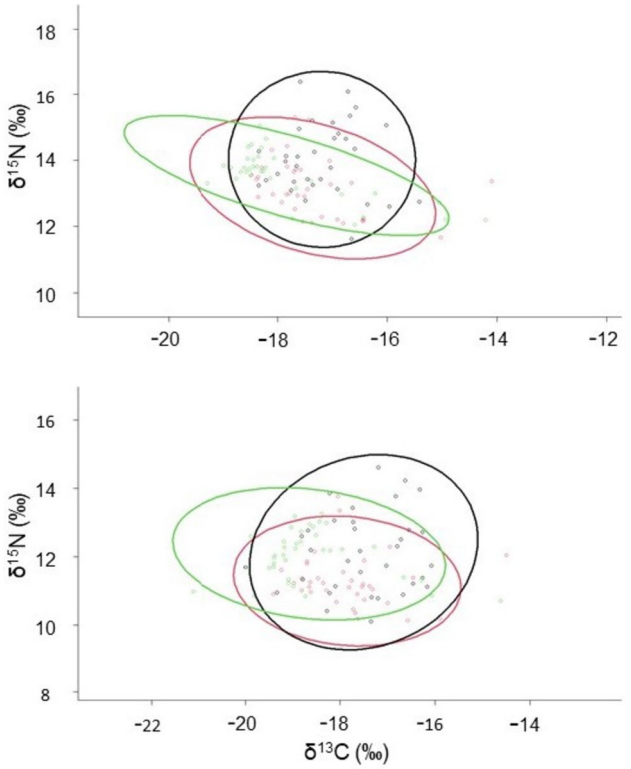

Fig. 7 Bayesian ellipses estimates of isotopic niche of copper sharks (*Carcharhinus brachyurus*) for muscle (top) and liver (bottom) tissue by region for all size/sex sharks combined. Standard Bayesian ellipses (SEA_b) encapsulating 95% of the data. Green = southern Spencer Gulf, black = Gulf St. Vincent and red = eastern Spencer Gulf

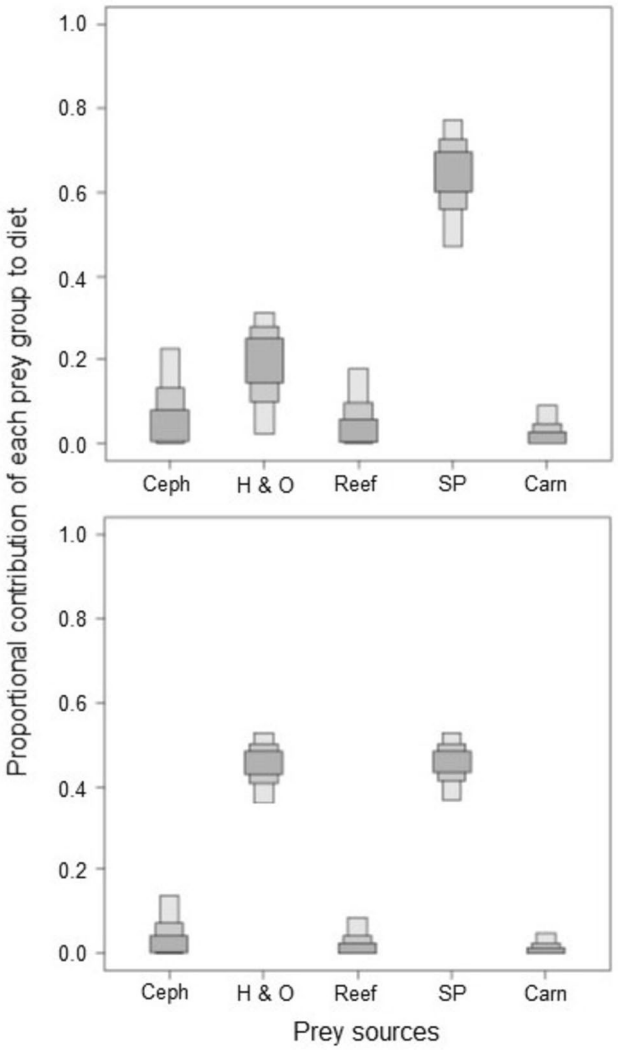

Fig. 8 SIAR mixing model estimates of prey contributions to the diet of copper sharks (*Carcharhinus brachyurus*) derived from muscle (top) and liver (bottom) tissue. X-axis prey sources are: Ceph = Cephalopods; Reef = reef fish species; SP = small pelagic teleosts; Carn = carnivorous teleosts; H & O = Herbivorous and omnivorous teleosts. Shaded bars represent the 95% (light grey), 75% (mid grey) and 50% (dark grey) credible intervals

contributing to the diet of copper sharks. In contrast, the southern gulf region of SSG prey assemblage included a high abundance of *S. sagax*. The sampling areas in the upper gulf regions of GSV and ESG are predominately shallow water (> 20 m) with large sea grass meadows, scattered sandy and mud benthos, and isolated patches of low-profile reef (Shepherd et al. 2008; Tanner 2005). Southern Spencer Gulf is characterised by deeper water (~ 50 m) with silt benthos and scattered sea grass and reef ecosystems (Shepherd et al. 2014). The prey species diversity was greater for the two upper gulf regions (GSV and ESG) containing numerous small pelagic, cephalopod, herbivorous, and carnivorous teleosts. The SSG region shares the same species diversity; however, small pelagic teleosts, i.e. *S. sagax*, are more abundant relative to the other two regions, and hence the reason that SSG supports Australia's largest small pelagic fishery (Ward et al. 2001). The marked difference in small pelagic teleost abundance explains the difference in the diet of copper sharks from SSG (Rogers et al. 2012). The high consumption of small pelagic teleosts in the SSG is additionally further supported by the lower mean $\delta^{15}N$ value for copper sharks in this region. The higher $\delta^{15}N$ values for the upper gulf regions are likely due to the foraging on higher trophic level consumers, such as cephalopods (*S. australis, S. novaehollandiae*, and *S. apama*) and carnivorous teleosts (*Platycephalus* sp., *S. novaehollandiae*), which was confirmed through SCA.

The isotope mixing model for regions combined shows difference in the diet of copper sharks according to the type of tissue used. The mixing model based on muscle identified small pelagic teleosts as the dominant prey group, while herbivorous and omnivorous teleosts were equally as important as small pelagic teleosts when based on liver tissue. The shorter isotopic assimilation rate of liver tissue suggests that during the 3–6 months prior to sampling, sharks consumed more herbivorous and omnivorous teleosts, which are in high

Table 5 Trophic position of the copper shark (*Carcharhinus brachyurus*) estimated from multiple baseline species

Baseline consumer	Trophic position	SD	Method
Melicertus latisulcatus	4.55	0.38	Scaled
Crassostrea gigas	4.84	0.38	Scaled
Sardinops sagax	3.98	0.38	Scaled
Combined	4.46	0.38	Scaled
M. latisulcatus	5.30	0.42	Constant DTDF
C. gigas	6.53	0.42	Constant DTDF
S. sagax	4.32	0.42	Constant DTDF
Combined	5.38	0.42	Constant DTDF

Combined is the mean TP estimate of all three baseline species for both the scaled and constant DTDF approaches. SD is the standard deviation of the means represented

abundances in the northern gulf and coastal waters. In contrast, muscle tissue in which diet is assimilated over a longer time frame indicated that copper sharks consumed a high proportion of small pelagic teleosts, which are most abundant offshore and in the southern gulf waters (Ward et al. 2001). These trends in varying prey contributions align with the increased seasonal abundances of this species within the gulf and coastal waters over the austral Spring–summer months (Sept–Apr). Movement studies based on acoustic telemetry have shown that copper sharks have their highest residency in GSV over the austral summer and relatively low residency throughout the remainder of the year (Drew et al. 2019). Furthermore, pop-up satellite archival satellite tags have also showed that large copper sharks (> 2 m TL) leave gulf waters when austral winter approaches (April–May) (Drew 2018).

Ontogenetic variation

There was no evidence of linear ontogenetic variation in the diet of copper sharks. The lack of a linear relationship between $\delta^{15}N$ and total length for both tissues contradicts the ontogenetic shift observed in the South West Atlantic, where the contribution of elasmobranchs in copper shark diet increased with body size (Lucifora et al. 2009). Although only a small number of sharks > 2.5 m TL were sampled in our study, ontogenetic changes would be expected to occur between small juveniles and sharks of ~ 2 m TL. Ontogenetic shifts with increasing body size is common for large-bodied shark species and has been shown in a broad range of species including tiger (*Galeocerdo cuvier*) (Lowe et al. 1996; Simpfendorfer et al. 2001), sevengill (*Notorynchus cepedianus*) (Ebert 2002), sandbar (*C. plumbeus*) (Ellis and Musick 2007; McElroy et al. 2006), and white sharks (*Carcharodon carcharias*) (Estrada et al. 2006). Copper sharks might challenge this paradigm. While the maximum size of the prey items consumed by copper sharks increases with body size, the minimum prey size remains relatively constant and small prey (i.e. pelagic teleosts) remain dominant across all size classes. The observed lack of linear ontogenetic variation and high contribution of small pelagic teleosts to their diet suggests that copper sharks may therefore have a direct top-down effect on lower trophic levels in temperate marine environments. Such lack of ontogenetic diet shift has previously been observed in a similar carcharhinid species, the silky shark (*C. falciformis*) in the eastern Pacific Ocean, which mostly feeds on Scombridae throughout all life history stages (Duffy et al. 2015).

Trophic position

Trophic position was estimated using the constant TEF and scaled TEF equations with multiple baseline species. The constant TEF estimations of TP for all baseline species individually and combined was higher (5.38) than estimates from the scaled TP equation, placing copper sharks at a similar TP to white sharks (*Carcharodon carcharias*). This is likely incorrect as the two species are known to feed on prey from differing trophic levels (Hussey et al. 2014). The scaled equation estimated a more conservative TP of 4.45, which is similar to that estimated for copper sharks from South Africa (Hussey et al. 2014) and according to Cortes (1999). The variability in TP estimations from a single baseline shown in this study highlights the importance of using multiple baseline species to reduce individual baseline species bias in SIA TP estimation. Trophic position estimates support the diet data, identifying the copper shark as primary piscivore.

Conclusion

In the present study, we investigated the diet and trophic ecology of copper sharks at multiple sites in coastal waters of southern Australia. We identified that the copper shark consumes a diverse prey base, but primarily feeds on small pelagic teleosts and locally abundant cephalopods irrespective of body length, resulting in limited ontogenetic diet variation. Regional variations in diet over short time frames were identified through SCA and SIA of liver (high turnover rate tissue), although diet was uniform across the sampling regions when considering muscle tissue (slow turnover rate tissue). Movement between regions and habitats (e.g. coastal vs. offshore) was inferred through changes in prey group contributions estimated from stable isotope mixing models and were in agreement with movement patterns derived using telemetry. Through combining two dietary analysis techniques and sampling over a large temporal and spatial scale, it was possible to discern temporal and spatial variability in foraging of the copper shark. The dietary information provided can be integrated in future ecosystem-based

models and improves our understanding of the feeding ecology of an important temperate marine predator.

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Author contributions All authors contributed to the study conception and design. Sample collection, material preparation, and sample processing were performed by MD, PR, and NH. Data analysis was performed by MD, CH, and NH. The first draft was written by MD, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Data availability The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest No conflicts of interest for all authors.

Ethical standards All applicable international, national, and/or institutional guidelines for sampling, care, and experimental use of organisms for the study have been followed and all animal handling complied with the animal ethics standards of Flinders University animal ethics guidelines (ethics approval #E360).

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