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Expanded trophic complexity among large sharks

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ABSTRACT

To understand the effects of predator removal in marine ecosystems requires accurate estimates of trophic position and trophic structure that have been difficult to obtain to date. For example, most sharks are classified as diet generalists that feed around trophic position 4, but this classification contradicts observations of diverse feeding behaviour among large species, suggesting that trophic structure has been oversimplified among upper trophic level species. To test this assumption, bulk $\delta^{15}N$ and $\delta^{13}C$ values of 13 shark species constituting the large shark assemblage off southern Africa were integrated into (i) a hierarchical Bayesian model, accounting for body size and variable sample sizes among species, and (ii) a dietary $\delta^{15}N$-dependent enrichment model to quantify individual and assemblage-wide trophic position and structure. Compound specific isotopic analysis of amino acids (CSIA-AAs) for a subset of species was used to verify results. Although discrepancies occurred between methods, overall these data confirm that large sharks, including several globally threatened species, feed at markedly higher trophic positions and across a broader trophic range than is currently assumed. This identifies a lower degree of functional equivalence among the assemblage. Such complex trophic structure among large sharks suggests that cascading effects from species-specific removals in food webs may be weaker but more pervasive than currently assumed. Reassignment of the trophic structure of large marine predators has important consequences for any potential regulatory and stabilizing roles in marine food webs.

1. Introduction

The removal of top predators and purported adverse effects on ecosystem structure, function, and resilience among ecosystems is widely debated. In terrestrial systems, trophic cascades, defined as 'predator regulated top down control of community structure with conspicuous indirect effects transferring to lower linkages', are well documented (McLaren and Peterson, 1994; Crooks and Soule, 1999; Terborgh et al., 2001) and the occurrence of trophic cascades in low diversity marine systems is widely accepted (Paine, 1966; Strong, 1992; Shurin et al., 2002). However among more diverse marine environments, where a higher number of complex trophic linkages exist, the impact of predator removal remains largely unknown. With documented declines in marine predator populations (Christensen et al., 2003; Myers and Worm, 2003; Estes et al., 2011) and current concern over the status of global fish stocks (Pauly et al., 1998; Pauly et al., 2005; Worm et al., 2009), there is a heightened interest in the strength of top down control and its pervasiveness in the marine environment (Heithaus et al., 2008).

The occurrence and strength of predator removal effects within food webs are dependent on the functional equivalence within a given ecosystem. Functional equivalence assumes that guilds of species have similar effects on community or ecosystem processes, such as equivalent ecological or trophic roles, and are frequently defined by functional groups (Loreau, 2004; Petchey and Gaston, 2006). Removal of a species where functional equivalents co-occur is presumed to minimise interference to food web structure, buffering potential trophic cascades as species are assumed interchangeable and compensatory (Yachi and Loreau, 1999; Loreau, 2004).

Among whole marine ecosystem models, large predatory fishes are typically defined as generalist feeders, implying that species within the group have similar regulative effects on lower trophic position (TP) species in the food web (TP range 4.1–4.5; Cortés, 1999). Yet empirical evidence suggests that many species viewed as functional equivalents and assigned a priori into functional groups is often inaccurate (Chalcraft and Resetarits, 2003a,b; Loreau, 2004; Resetarits and Chalcraft, 2007), including for large sharks (Matich et al., 2011;

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Heithaus et al., 2013; Hussey et al., 2014a). In contrast, species with similar life histories and morphology can have diverse functional roles that generate complex intra-guild functional diversity (Chalcraft and Resetarits, 2003a,b; Resetarits and Chalcraft, 2007; Heithaus et al., 2013).

Potential misclassification of functionally diverse species into a single trophic group raises concerns about studying top-down predator effects and fisheries exploitation (Pauly et al., 1998; Branch et al., 2010; Christensen and Pauly, 1992; Williams and Martinez, 2004) using the conventional designation of large predators as largely secondary consumers (primary piscivores; TP = 4). For example, while some large sharks feed predominantly on small schooling zooplanktivorous fish, other species feed preferentially on piscivorous fish (Dudley and Cliff, 1993; Wetherbee and Cortes, 2004; Dudley et al., 2005), elasmobranchs (Cliff et al., 1990; Cliff and Dudley, 1991a, 1991b), and marine mammals (Tricas and McCosker, 1984; Hussey et al., 2011). Consequently, even with high levels of omnivory, large sharks would feed above TP 4, with species potentially feeding upwards of TP 6, revealing more complex trophic structuring than the current 'generalist' feeding paradigm (Hussey et al., 2014a,b; Fig. 1).

Bulk nitrogen isotopes in animals' tissues provide an empirical tool for calculating TP and trophic structure in aquatic systems that have generated novel insights into ecosystem dynamics (Fry, 1988; Madigan et al., 2012; Hussey et al., 2014a,b). However the approach can be confounded by its dependence on the isotopic value of a reliable baseline organism (Cabana and Rasmussen, 1996) and variable isotopic discrimination between predator and prey groups (Caut et al., 2009) that may bias TP estimates. Alternatively, recent compound specific nitrogen isotope analysis of individual amino acids (CSIA-AAs) provides an autogenous, within-web measure of the system baseline (source) and consumer TP for each individual consumer (McClelland and Montoya, 2002; Popp et al., 2007). Differences between trophic AA δ^{15}N values (which enrich during trophic processing) and source AA δ^{15}N values (which show minimal fractionation) can be used to calculate TP, negating the need for independent baseline organism nitrogen isotope values. This increases confidence in estimates for the isotopic structuring of aquatic assemblages and provides an absolute measure of TP (Chikaraishi et al., 2009).

Using a combined bulk tissue and CSIA-AA nitrogen isotopic approach, we show that members of a large shark assemblage feed across a higher and broader trophic range than is conventionally assumed. Specifically we identify more complex species-specific roles and lower levels of functional equivalence among so-called generalist predators.

2. Materials and methods

2.1. Sampling and stable isotope analysis

Thirteen species of sharks (n = 271) comprising the 'large shark assemblage' off southern Africa, and including common large sharks and IUCN threatened species, were sampled from captures in beach protection nets along the KwaZulu-Natal (KZN) coast between 2005 and 2009 (for details see Dudley et al., 2005). All samples were processed for bulk stable isotope analysis, δ^{15}N and δ^{13}C values, following standard procedures (see electronic online supporting materials, Method S1). A subset of seven species (n = 18), spanning the range from low to high bulk δ^{15}N values were selected for CSIA-AA and prepared as described in Popp et al. (2007) (see electronic online supporting materials, Method S2).

2.2. Trophic position and trophic structure (BULK SIA–TP_{SIA})

As ontogenetic diet shifts to higher TP prey have been widely reported in large sharks, resulting in ^{15}N enrichment with size (Estrada et al., 2006; Hussey et al., 2011; Rabehagasoa et al., 2012), we developed a Bayesian hierarchical model to estimate species-level δ^{15}N values ($\delta^{15}N_{species}$) given individual body size (precaudal length — PCL). Specifically, we modelled δ^{15}N values for individual *i*, as;

$$\delta^{15}N_{is} \sim N\left(\mu_{is}, \varepsilon_s\right) \quad (1)$$

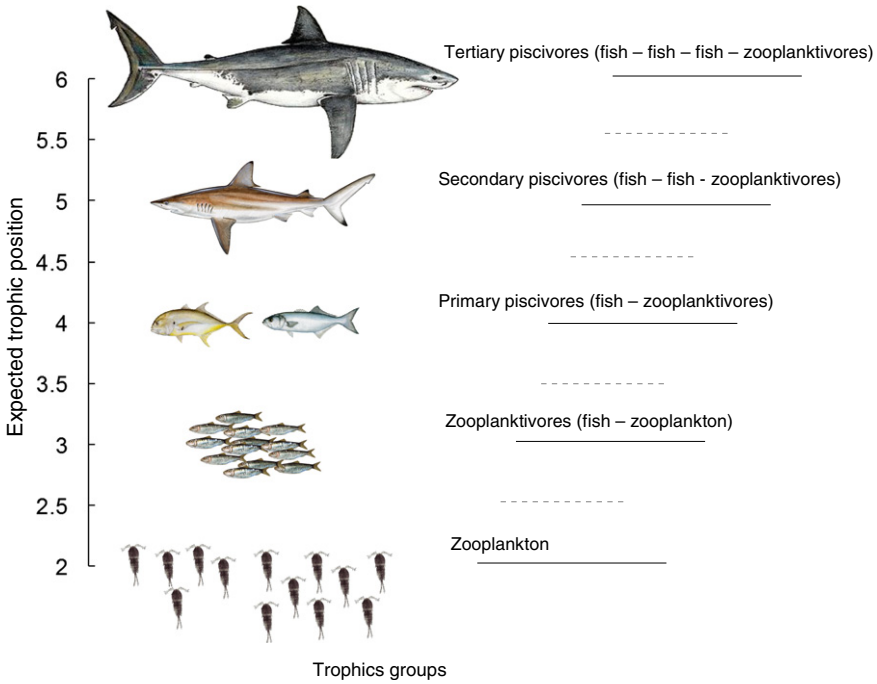

Fig. 1. Expected marine food web with extended upper trophic levels to accommodate known feeding behaviours of the large shark assemblage.

where

$$\mu_{is} = \delta^{15}N_{species} + \beta_1 PCL_i \quad (2)$$

included species-level intercepts ($\delta^{15}N_{species}$) and a fixed effect for PCL (β_1). The $\delta^{15}N_{species}$ effects were given a ~ N(γ, τ) prior that included weakly-informative hyperpriors for their overall mean and precision:

$$\gamma \sim U(5, 20) \quad (3)$$

$$\tau \sim \Gamma(0.001, 0.001). \quad (4)$$

The U(5, 20) prior expressed our knowledge about the bounds of $\delta^{15}N$ within the assemblage. The observation error (precision) was allowed to vary among species:

$$\varepsilon_s \sim \Gamma(0.001, 0.001) \quad (5)$$

and the prior on the PCL effect was uninformative

$$\beta_1 \sim N(0, 0.0001). \quad (6)$$

We also modelled bulk tissue $\delta^{13}C$ values using alternative U(−20,−5) priors in [Eq. (3)]. These Bayesian models were run in PyMC (Patil et al., 2000) using the Python programming language. Highest posterior density estimates of species-level expected values with 95% credible intervals (CIs) are presented in electronic supplementary materials Fig. S1.

Shark trophic position (a continuous trophic estimate) was calculated using bulk $\delta^{15}N$ isotopes incorporated into a recently-developed dietary $\delta^{15}N$-dependent $\Delta^{15}N$ enrichment model (Hussey et al., 2014a,b). This model reflects the observed negative linear relationship between dietary $\delta^{15}N$ values and diet discrimination ($\Delta^{15}N$) that implies a limit on $\delta^{15}N$ values ($\delta^{15}N_{lim}$) within food webs when the rates of ^{15}N and ^{14}N uptake balance those of elimination. Given consumer $\delta^{15}N$ values and estimates of $\delta^{15}N_{lim}$ and k, the rate at which the consumer isotope value at a given TP approaches $\delta^{15}N_{lim}$ can be used to estimate organismal TP (Hussey et al., 2014a,b). Zooplanktivorous whale sharks (*Rhincodon typus*) and devil rays (*Mobula* spp.) were used as the baseline species (TP = 3; see electronic online supporting materials, Method S3).

Because large sharks can be highly mobile and species-level $\delta^{15}N$ and $\delta^{13}C$ estimates are intrinsically linked by potential correlations in isotopic baseline conditions, we employed a bivariate linear regression model to ensure the ^{13}C enrichment was below the expected ~1‰ per TL step ($\delta^{15}N$ vs. $\delta^{13}C$; Leonard, 2011). If the increase in $\delta^{13}C$ values per TL averaged ≥ 1‰, the slope of this relationship would be greater than 2.3‰ (the expected per TL increase in $\delta^{15}N$ values). Therefore we estimated this slope parameter by zero-centering $\delta^{15}N$ and $\delta^{13}C$ on their means and regressing the centred values on one another using the leiv package (Leonard, 2011) in R (R Core Team, 2012).

2.3. Trophic position and trophic structure (CSIA-AA–TP_{CSIA})

To complement bulk nitrogen isotopes, TP structure and absolute TP estimates of the large shark assemblage were also calculated from groups of analytically quantifiable amino acids (AAs) based on their ^{15}N enrichment with each trophic step. This approach is effective because "trophic" AAs (valine, alanine, leucine, proline, aspartic acid, glutamic acid) enrich in ^{15}N with trophic transfer and "source" AAs (glycine and phenylalanine) are mostly unchanged with trophic shift (McClelland and Montoya, 2002). Here, isoleucine was excluded from the trophic AA group because it eluted closely with leucine, which compromised accurate $\delta^{15}N$ measurement.

The differences in $\delta^{15}N$ values between trophic and source AAs were used to calculate what is deemed an "internally normalized" estimate of TP (McClelland et al., 2003; Sherwood et al., 2011). First relative trophic structure of consumer TP_{CSIA} was calculated from the differences between weighted mean ($\bar{x}$) $\delta^{15}N$ values of trophic and source AAs for each species,

$$\bar{x} = \frac{\sum_{k=1}^{n} \frac{\delta^{15}N(k)}{\sigma^2}}{\sum_{k=1}^{n} \frac{1}{\sigma^2}} \quad (7)$$

where n is the number of AAs (source or trophic), σ is the standard deviation of $\delta^{15}N(k)$, between all three replicates run for sample k, and $\delta^{15}N(k)$ is the N isotopic composition for sample k. The weighted standard deviations for each set of AAs were calculated as:

$$s = \sqrt{\sum \frac{1}{\sigma^2}}. \quad (8)$$

Relative trophic position for each sample was calculated by subtracting weighted mean source $\delta^{15}N$ from weighted mean trophic $\delta^{15}N$ values,

$$TP = \bar{x}_{trophic} - \bar{x}_{source}. \quad (9)$$

Standard deviations were pooled for the final TP_{CSIA} ranking,

$$SD(TP) = \sqrt{{s_{trophic}}^2 - {s_{source}}^2}. \quad [10]$$

This technique can provide a more reliable estimate of the relative rank of consumer TP_{CSIA} than using only glutamic acid and phenylalanine alone (McCarthy et al., 2007).

To estimate an absolute TP value for each species, consumer TP_{CSIA} was calculated from the isotopic value of phenylalanine and glutamic acid, following Hoen et al. (2014):

$$TP = \frac{\left(\delta^{15}N_{Glu} - \delta^{15}N_{Phe}\right) - \beta - TEF_{herbivore}}{TEF_{carnivore}} + 2. \quad (11)$$

Where the trophic enrichment factors (TEF) were:

$TEF_{carnivore} = 5.0 \pm 0.6$ (Dale et al., 2011)
$TEF_{herbivore} = 7.6 \pm 1.2$ (Chikaraishi et al., 2009).

The standard deviation is given in relation to the three replicates run for each sample, with weighted standard deviations for each set of AAs calculated as in Eq. (8). Standard deviations were pooled for the final TP_{CSIA} estimate,

$$\sigma^2_{TP} = \left(\frac{\partial TP}{\partial \delta^{15}N_{Glu}}\right)^2 \sigma^2_{\delta^{15}N_{Glu}} + \left(\frac{\partial TP}{\partial \delta^{15}N_{Phe}}\right)^2 \sigma^2_{\delta^{15}N_{Phe}} + \left(\frac{\partial TP}{\partial \beta}\right)^2 \sigma^2_{\beta} + \left(\frac{\partial TP}{\partial TEF_{herbivore}}\right)^2 \sigma^2_{TEF_{herbivore}} + \left(\frac{\partial TP}{\partial TEF_{carnivore}}\right)^2 \sigma^2_{TEF_{carnivore}}. \quad (12)$$

This method is considered to provide a more reliable estimate of absolute consumer TP_{CSIA} than using the same TEF for herbivores and carnivores (Germain et al., 2013; Hoen et al., 2014).

3. Results

Multiple species were found to be tropically distinct across the large shark assemblage, with clear differences among species in bulk $\delta^{15}N$ and

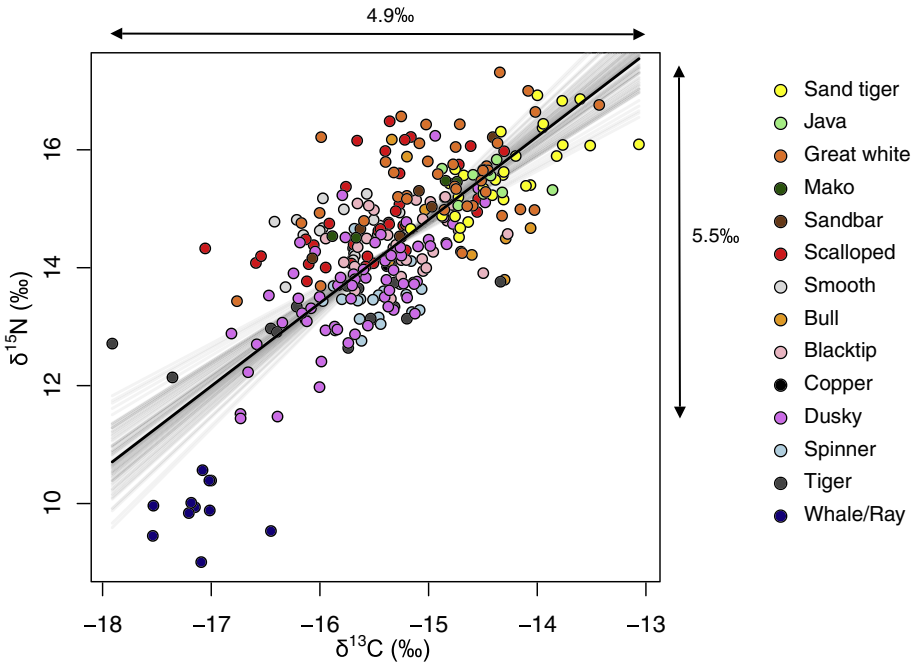

Fig. 2. Bi-plot of $\delta^{15}N$ and $\delta^{13}C$ values in 13 large predatory shark species and the zooplanktivorous whale shark (*Rhincodon typus*) and devil ray (*Mobula* spp.). Each species is a unique colour and data points are for individual animals per species. Arrows and associated values indicate the shark assemblage $\delta^{15}N$ and $\delta^{13}C$ range and regression line is that of a Bivariate linear model with 95% uncertainty intervals.

$\delta^{13}C$ values (ANOVA: $F_{12,269} = 59.53$, $p < 0.0001$ and $F_{12,269} = 45.20$, $p < 0.0001$, respectively) and cross-assemblage isotopic ranges of 5.5‰ for $\delta^{15}N$ and 4.9‰ for $\delta^{13}C$ (Fig. 2). A range of intraspecific variation in isotope values among species was observed with dusky (*Carcharhinus obscurus*: range/max. and min; 4.8‰: 11.4 to 16.2‰), white (*Carcharodon carcharias*: 3.2‰: 13.4 to 16.6‰), and scalloped hammerhead sharks (*Sphyrna lewini*: 3.1‰: 13.3 to 16.5‰) showing the largest range in $\delta^{15}N$ values, and tiger (*Galeocerdo cuvier*: 3.6‰: −14.3 to −17.9‰), white (range/max. and min; 2.8‰: −14.0 to −16.8‰), and scalloped hammerhead sharks (2.8‰: −14.3 to

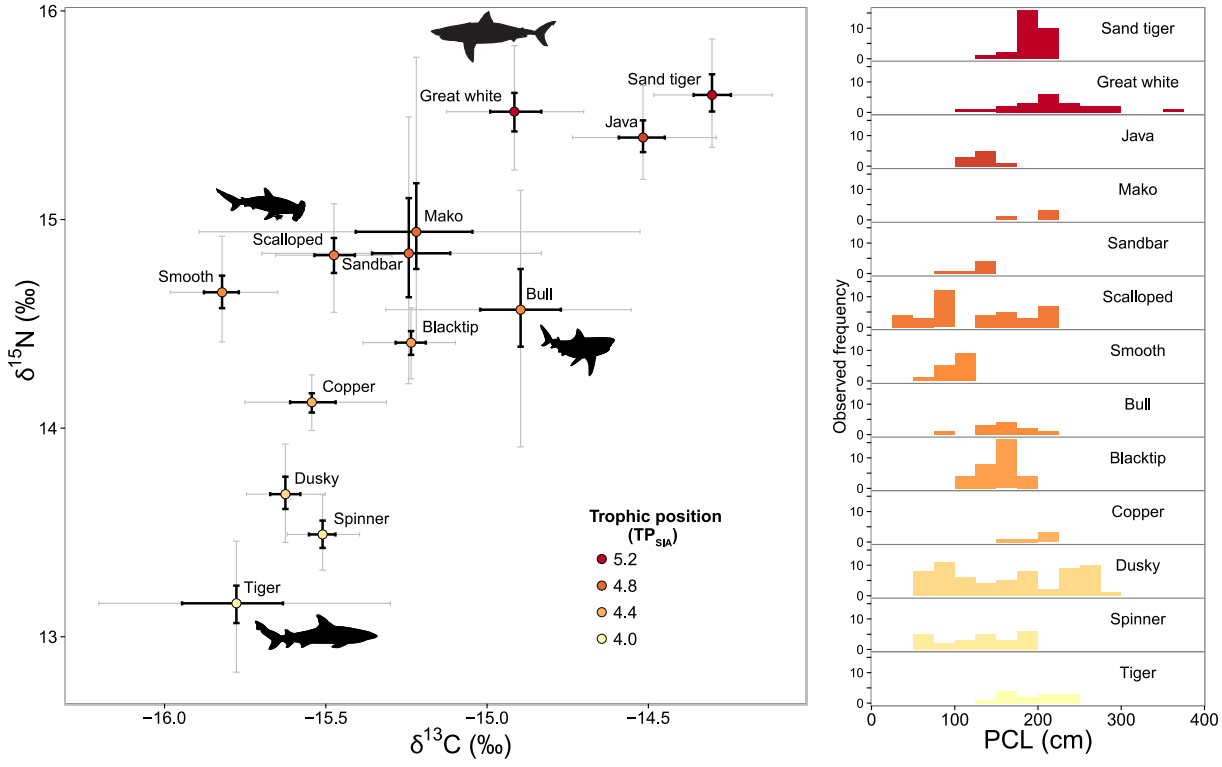

Fig. 3. Model mean $\delta^{15}N$ and $\delta^{13}C$ values for each shark species within the large shark assemblage. Black and grey error bars are 50% and 90% uncertainty intervals (UIs), respectively. Species are coloured by relative bulk isotope TP assignment (TP_{SIA}) and associated colour coded histograms detail the size distribution of animals included in the analyses.

−17.1‰) showing the largest $\delta^{13}C$ range. Neither bulk $\delta^{15}N$ nor $\delta^{13}C$ values showed a significant relationship with PCL across all species ($\beta_{1\delta^{15}N} = 4.7e^{-6}$ [95% UI = $-7.1e^{-6}$, $1.7e^{-6}$]; $\beta_{1\delta13C} = 7.4e^{-6}$ [95% UI = $-9.0e^{-6}$, $9.1e^{-6}$]; Fig. S2). The estimated slope of the bivariate regression between individual $\delta^{15}N$ and $\delta^{13}C$ data for all sharks was 1.7 [95% UI = 1.3, 2.3], suggesting $\delta^{13}C$ enrichment was <1‰ per TL (Fig. 2).

Having marginalized variability associated with animal length and variable sample sizes, sand tiger sharks (*Carcharias taurus*) had the highest $\delta^{15}N_{species}$ value of 15.6‰ and tiger shark the lowest $\delta^{15}N_{species}$ value at 13.2‰ (Fig. 3). While data at the individual level identified isotopic overlap within the large shark assemblage (Fig. 2), hierarchical model data ($\delta^{15}N_{species}$ and $\delta^{13}C_{species}$) identified a lesser degree of functional equivalence when considering all 13 large sharks sampled as a whole (Fig. 3).

Overall, differences in $\delta^{15}N$ values of pooled trophic and source amino acids confirmed the relative trophic structure for the subset of the sharks assigned through the bulk tissue isotope models, with one inconsistency being the ranking of scalloped hammerhead as the highest TP species of those analysed (Table 1). When considering absolute TP measured as the difference between glutamic acid and phenylalanine incorporated in to the dual TEF equation of Hoen et al. (2014), TP_{CSIA} of species were similar to TP_{SIA} with the exception of sand tiger shark (Fig. 4). This was a result of the unique phenylalanine $\delta^{15}N$ values of the sand tiger relative to other species examined. TP_{CSIA} of the scalloped hammerhead shark (TP = 5.4 ± 0.3) was higher than that of TP_{SIA} (4.7) as would be expected given the above TP ranking.

Although the arrangement of estimated bulk isotope and CSIA-AA TP among species was similar to that derived from conventional stomach content analyses, the magnitude and range of TP estimates was markedly different (Fig. 4). Bulk and CSIA-AA nitrogen isotopic compositions implied that large sharks feed across 1.2 and 1.3 trophic levels respectively, with species spanning primary to tertiary consumer roles (mean TP_{SIA} 4.0–5.2; TP_{SIA} range: 3.4–6.6, Figs. 1 and 4; and mean TP_{CSIA} 4.1–5.4, Fig. 4). This strongly contradicts current TP estimates from stomach content data (TP_{SCA}) that assume that large sharks are predominantly primary piscivores feeding across 0.4 of a trophic level (TP 4.1–4.5; Fig. 4). TP_{SIA} data validated expected trophic roles, with spinner sharks (*Carcharhinus brevipinna*), that consume zooplanktivores and primary piscivores feeding at a mean TP_{SIA} of 4.1 (range: 3.8–4.6), while species that feed on sharks and piscivorous fish, such as the java (*Carcharhinus amboinensis*) and sand tiger shark, (mean [range] TP_{SIA} 5.0 [4.8–5.3], 5.2 [4.6–6.1]), and marine mammals, such as the white shark, were tertiary consumers (5.2 [4.1–6.6]; Figs. 1, 3 and 4).

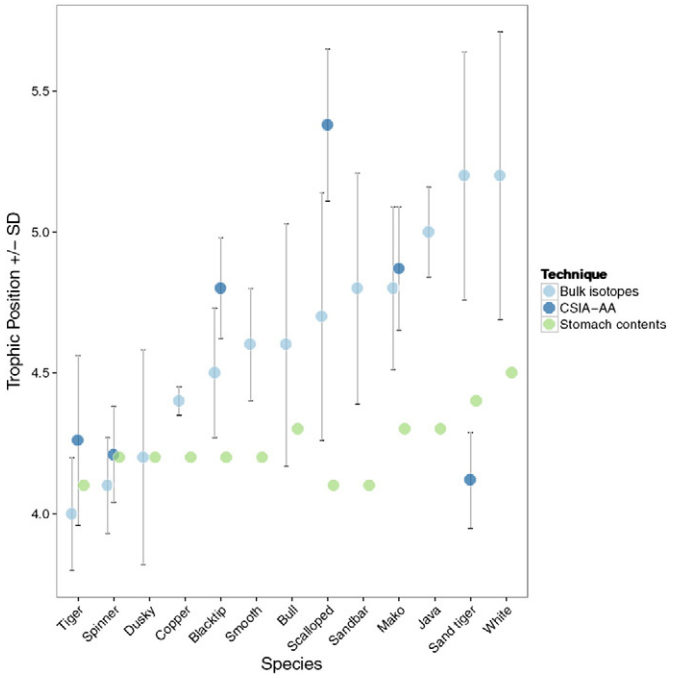

Fig. 4. Absolute trophic positions (TPs) of thirteen species that form the "large shark assemblage" calculated from (a) stomach content analysis according to Cortés (1999; TP_{SCA}; green circles) (b) a dietary $\delta^{15}N$-dependent $\Delta^{15}N$ enrichment model to quantify species TP based on whale shark/devil rays occupying a TP of 3 (TP_{SIA}; light blue circles) and (c) the difference in weighted mean $\delta^{15}N$ values between trophic and source amino acids (AAs) following the equation of Hoen et al. (2014) to provide an "internally normalized" estimate of TP (TP_{CSIA}; dark blue circles). Species are ordered by increasing TP calculated according to method (b).

4. Discussion

The application of bulk nitrogen isotopic data and CSIA-AA confirms that the estimated TP of large sharks and the trophic structure at the top of the southern Africa marine food webs is both higher and more complex than previously thought, with important consequences for understanding predator dynamics and potential trophic cascades. Contrary to mainstream food web theory, recent work suggests that functional equivalence and functional redundancy at high trophic levels are likely to be rare (Chalcraft and Resetarits, 2003a,b; Loreau, 2004; Resetarits and Chalcraft, 2007; Heithaus et al., 2013). In light of our data, this suggests that ecosystem processes arising from top predator interactions, such as food web stability, may be dependent on diverse functional effects rather than compensatory ones. Given high species-specific trophic diversity, declines among large sharks may have had weaker but more pervasive effects on global ecosystems than those previously reported for the removal of an entire functional group (Myers et al., 2007).

Large shark assemblages are known to exhibit high variation in spatial movements and habitat use (Bonfil et al., 2005; Dicken et al., 2007; Hussey et al., 2009; Diemer et al., 2011), morphological design and feeding behaviour (Motta and Huber, 2012), life-history characteristics (e.g. nursery area use, growth rate, age at maturity, fecundity; Cortés, 2000; Garcia et al., 2008), and diet (Wetherbee and Cortes, 2004). Several top predators, including sharks, have shown high inter-species variability in $\delta^{13}C$ and $\delta^{15}N$, with discrete feeding modes across trophic levels four and five (Hussey et al., 2012a; Madigan et al., 2012). In

Table 1
Relative trophic structure/rank of the large shark assemblage off southern Africa according to bulk $\delta^{15}N$ values incorporated into a dietary $\delta^{15}N$-dependent $\Delta^{15}N$ enrichment model (TP_{SIA}), compound specific nitrogen isotope analysis of individual amino acids (CSIA-AAs: TP $structure_{CSIA}$) using the difference in the weighted mean $\delta^{15}N$ values of 'trophic' and 'source' amino acids and stomach content data (TP_{SCA}).

Ranking (from high to low TP)	Bulk isotopes TP_{SIA}	TP $structure_{CSIA}$ (trophic–source; difference in weighted mean)[a]	Stomach contents TP_{SCA}[b]
1	Sand tiger (5.2 ± 0.44)	Scalloped (28.4 ± 0.08)	Sand tiger (4.4)
2	Mako (4.8 ± 0.29)	Sand tiger (25.0 ± 0.14)	Mako (4.3)
3	Scalloped (4.7 ± 0.44)	Mako (21.2 ± 0.06)	Blacktip (4.2)
4	Blacktip (4.5 ± 0.23)	Blacktip (21.0 ± 0.08)	Spinner (4.2)
5	Spinner (4.1 ± 0.17)	Tiger (20.7 ± 0.03)	Scalloped (4.1)
6	Tiger (4.0 ± 0.20)	Spinner (18.6 ± 0.05)	Tiger (4.1)
7	Whale (3.0)	Whale (10.1 ± 0.05)	Whale (3.5)

[a] Trophic amino acids are valine, alanine, leucine, proline, aspartic acid, and glutamic acid. Source AAs are glycine and phenylalanine.
[b] Trophic position values calculated from standardised stomach contents according to Cortés (1999).

South Africa, elasmobranchs represent an important component of the overall diet of a number of large predatory sharks including mako (*Isurus oxyrinchus*), java, sand tiger and white, with common prey including dusky and scalloped hammerhead sharks (Cliff et al., 1990; Cliff and Dudley, 1991a, 1991b; Hussey et al., 2011). Our assigned mean TP_{SIA} for these large sharks of 4.8, 5.0, 5.2 and 5.2, respectively, position them between secondary and tertiary piscivores feeding in excess of TP_{SCA} 4 (Fig. 1).

Observed differences between TP_{SIA} estimates and conventional stomach contents (TP_{SCA}) have previously been reported (Hussey et al., 2012a). TP_{SIA} for Atlantic cod (*Gadus morhua*) and horse mackerel (*Trachurus trachurus*), for example, were 5.2 and 5.1, respectively (Jennings et al., 2002; Huckstadt et al., 2007), compared to 4.4 and 3.6 reported in Fishbase. The major source of this discrepancy is thought to be the broad prey functional groups used to calculate TP_{SCA} (Hussey et al., 2012a) that often includes a single group of 'teleosts' to represent a wide range of trophic roles, from zooplanktivores to tertiary piscivores. Similar to the assumed trophic structure of larger sharks, this simple functional grouping compresses less-abundant upper trophic level teleosts into a lower, narrower trophic range, resulting in de facto truncation at the top of the food web.

Estimating trophic position using bulk stable isotopes is contingent on the selection of an appropriate diet-tissue discrimination factor that scales TP_{SIA} to observed levels of isotope enrichment (Hussey et al., 2012b, 2014a,b; Madigan et al., 2012). We overcame this with a recently developed dietary $\delta^{15}N$-dependent $\Delta^{15}N$ enrichment model that accounts for known effects of dietary $\delta^{15}N$ values on discrimination (Hussey et al., 2010; Kim et al., 2012; Caut et al., 2013; Madigan et al., 2012; Olin et al., 2013). Estimation of TP_{SIA} is also dependent however, on selecting an accurate baseline organism for the study system (Cabana and Rasmussen, 1996). As CSIA-AA does not require baseline species, the general agreement between CSIA-AA and model $\delta^{15}N$ trophic structure, along with the minimal bias between the regression of bulk $\delta^{15}N$ and $\delta^{13}C$ values, indicated that baseline effects were adequately represented in this study.

Determining the effects of shark removals on marine ecosystems has proven exceptionally difficult to date, in part due to limited understanding of their trophic roles. Supposed trophic cascades as a result of shark removal have been reported (Stevens et al., 2000; Myers et al., 2007; Ferretti et al., 2010; Rupert et al., 2013), while other large-scale ecosystem models (Kitchell et al., 2002) and monitoring of variations in abundance, size structure and biomass of trophic guilds across fished to protected systems have failed to detect effects (Rizzari et al., 2014). Ironically, arguments both for and against trophic cascades have suggested that results were due to the assumption that large sharks comprise a single functional group (Kitchell et al., 2002; Myers et al., 2007). Such functional similarity is thought to be responsible for the relationship between biodiversity and ecosystem stability and function (Naem, 1998; Walker, 1991) where, with increasing biodiversity, the number of functionally equivalent species increases to provide compensatory effects that safeguard food web function against the loss or breakdown of species-level interactions. Yet experimental manipulation of multiple predators, considered functionally similar, suggests that functional equivalence is limited among higher trophic levels (Chalcraft and Resetarits, 2003a,b; Loreau, 2004; Resetarits and Chalcraft, 2007). While we do not directly determine the functional roles of large sharks, the expanded trophic range of the large shark assemblage indicates limited functional equivalence (sensu Chesson, 2000). Consequently the loss of individual shark species will likely have weaker but more pervasive effects on food web structure and stability than currently assumed.

5. Summary

Unravelling the consequences of shark exploitation for food web structure and stability is an important factor if we are to understand what potentially critical ecosystem function has been lost through widespread exploitation throughout the 20th century. Species-specific variation in shark-food web interaction strengths could generate a range of effects dependent on the species or combination of species removed, including possible trophic cascades within the shark assemblage itself. To date, the study of trophic cascades resulting from large shark removal has only examined food web effects related to reduction in numbers of the entire assemblage (Myers et al., 2007) and has been highly controversial. Individual species depletion effects will be still more difficult to tease apart but will better reflect the complexity of top down control exerted by large marine predators. Our reassignment of shark TPs within the large shark assemblage, verified using both bulk and CSIA-AA methods, will impact model predictions from mass-balance ecosystem approaches and directly alter the estimates of pristine biomass for these large sharks Hussey et al. (2014a). Given that other upper trophic level guilds likely exhibit similar trophic complexity that has previously gone unrecognized (e.g. Madigan et al., 2012), these results may have widespread implications for food webs generally.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.fooweb.2015.04.002.

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