

## Predicting trophic position in sharks of the north-west Atlantic Ocean using stable isotope analysis

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Trophic positions (TP) were estimated for the blue shark (*Prionace glauca*), shortfin mako (*Isurus oxyrinchus*), thresher shark (*Alopias vulpinus*), and basking shark (*Cetorhinus maximus*) using stable isotope ratios of carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ). The basking shark had the lowest TP (3.1) and  $\delta^{15}\text{N}$  value (10.4‰), whereas the thresher shark had the highest values (4.5, 15.2‰). Mako sharks showed considerable variation in TP and isotopic values, possibly due to foraging from both inshore and offshore waters. Thresher sharks were significantly more enriched in  $\delta^{15}\text{N}$  than blue sharks and mako sharks, suggesting a different prey base. The  $\delta^{13}\text{C}$  values of thresher sharks and mako sharks varied significantly, but neither was significantly different from that of blue sharks. No statistical differences were found between our TP estimations and those derived from published stomach contents analyses, indicating that stable isotope data may be used to estimate the trophic status of sharks.

As apex predators in the marine environment, the trophic ecology of sharks has been of interest in top-down models of feeding ecology where predation can affect community structure (Bowman et al., 2000; Link, 2002). Traditional studies have utilized stomach contents analysis to examine trophic ecology in sharks (reviewed by Cortés, 1999). Based on published stomach contents data, Cortés (1999) estimated the trophic positions (TP) of a number of shark species. In recent years, stable isotopes have been employed to calculate TP in a variety of species (e.g. Hobson & Welch, 1992; Cortés, 1999), but only a few studies have used stable isotopes to calculate TP in sharks (Rau et al., 1983; Fisk et al., 2002). The only study to compare the TP of sharks based on isotopic analysis was by Rau et al. (1983), but this study was limited to a single specimen from each species and only utilized  $^{13}\text{C}$  as a trophic indicator.

The stable isotope ratios of carbon ( $^{13}\text{C}/^{12}\text{C}$ ) and nitrogen ( $^{15}\text{N}/^{14}\text{N}$ ) present in predator tissues are directly related to those of their prey, and are transferred in a predictable manner (Peterson & Fry, 1987). Values for  $\delta^{13}\text{C}$  remain fairly constant from prey to predator, typically increasing by 0–1‰ per TP (Peterson & Fry, 1987; Hobson & Welch, 1992), whereas  $\delta^{15}\text{N}$  values increase by 3–4‰ (Peterson & Fry, 1987; Post, 2002). Thus, the former are often used as an indicator of a consumer's primary prey items, and the latter as a predictor of relative TP (Post, 2002). Stable isotopes provide distinct advantages over traditional diet and trophic analyses because: (1) dietary information represents assimilated, not just ingested prey; (2) isotopic compositions of consumer tissue represent long-term feeding behaviours (Peterson & Fry, 1987; Post, 2002); and (3)  $^{13}\text{C}$  ratios can be used to

distinguish between inshore and offshore feeding patterns (France, 1995).

While stable isotopic analysis (SIA) has become an increasingly popular technique in fish and trophic ecology, the assumptions involved in the analysis and the lack of known trophic fractionations for most species makes it crucial that isotopic data be compared to traditional diet analyses. This is of special importance with elasmobranchs where urea retention may affect nitrogen enrichment (Fisk et al., 2002). Knowledge of the food resources and trophic ecology of shark populations will provide a greater understanding of their role in marine food webs and the factors that may influence their seasonal distributions.

The purpose of this study was to calculate the TP of *Prionace glauca* L. (blue shark), *Isurus oxyrinchus* Rafinesque (shortfin mako), *Alopias vulpinus* Bonnaterre (thresher shark), and *Cetorhinus maximus* Gunnerus (basking shark) using SIA and to compare these results to those calculated from published stomach contents data. Common prey items from the north-west Atlantic Ocean were also analysed to examine our calculated TP for sharks in relation to other species within the food web, and to aid in identifying broad patterns in prey choice (i.e. fish, cephalopods, or planktonic prey). To our knowledge, this is the first isotopic study conducted on blue sharks and thresher sharks, and only the second on mako sharks (Rau et al., 1983).

### MATERIALS AND METHODS

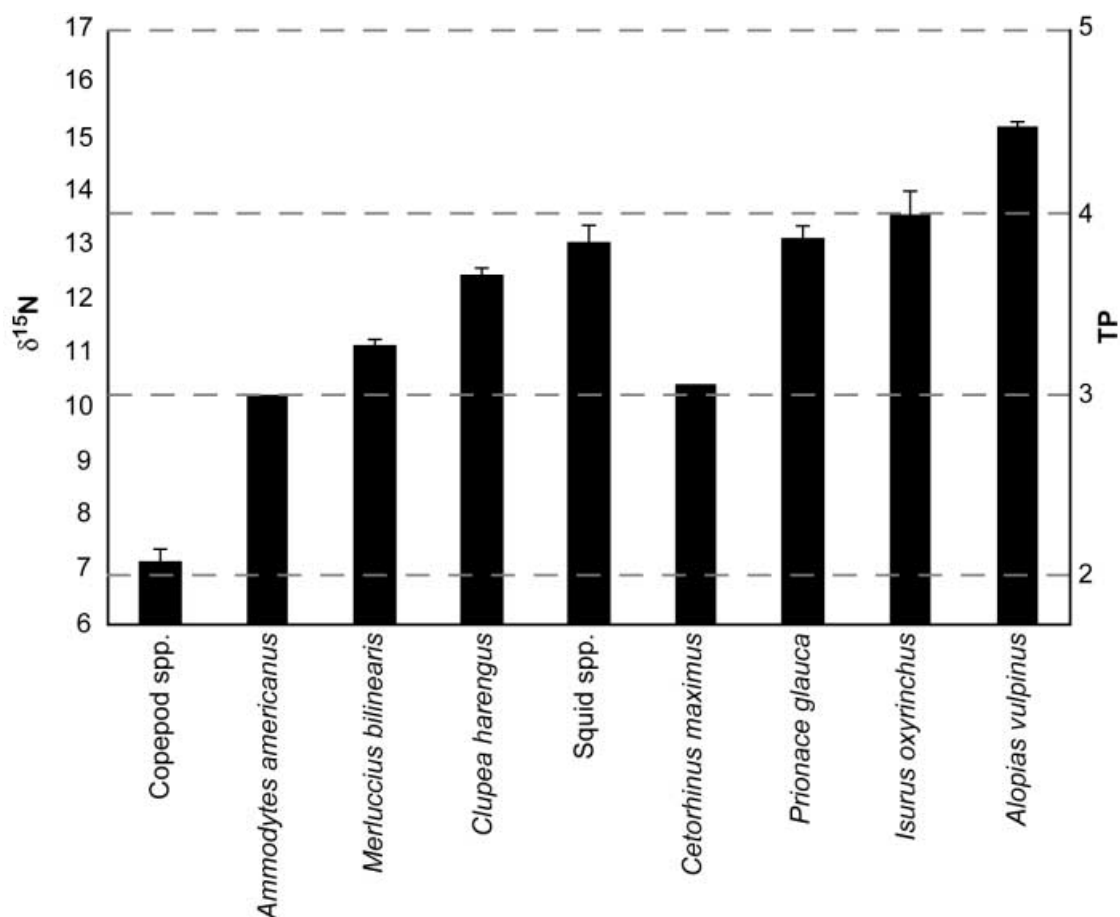
#### Sample collection

Tissue samples from *Prionace glauca* (N=5), *Isurus oxyrinchus* (N=5), and *Alopias vulpinus* (N=4) were

**Table 1.** Mean (SD)  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotopic values for selected species.

Sample	N	$\delta^{13}\text{C}$ (‰)		$\delta^{15}\text{N}$ (‰)	
		Mean (SD)	Range	Mean (SD)	Range
Sharks					
<i>Cetorhinus maximus</i>	1	-22.5		10.4	
<i>Prionace glauca</i>	5	-16.9 (0.10)	-17.1 to -16.5	13.1 (0.25)	12.5 to 13.7
<i>Isurus oxyrinchus</i>	5	-16.6 (0.23)	-17.1 to -15.9	13.6 (0.48)	12.2 to 15.2
<i>Alopias vulpinus</i>	4	-17.5 (0.08)	-17.7 to -17.3	15.2 (0.14)	14.8 to 15.5
Other					
Copepod spp.	*	-21.8	-22.2 to -21.4	7.1	7.0 to 7.3
<i>Ammodytes americanus</i>	6	-21.0 (0.24)	-21.9 to -20.2	10.2 (0.09)	9.9 to 10.5
<i>Merluccius bilinearis</i>	5	-18.7 (0.14)	-19.1 to -18.4	11.1 (0.14)	10.8 to 11.5
<i>Clupea harengus</i>	2	-21.1	-21.1 to -21.1	11.3	10.7 to 11.9
Squid spp.	9	-17.8 (0.39)	-19.8 to -17.0	12.7 (0.20)	11.9 to 13.7

\*, Copepods were analysed in two groups of 15–20 individuals. N, number; SD, standard deviation.



**Figure 1.**  $\delta^{15}\text{N}$  values and estimated trophic position (TP) for all species in the study (mean  $\pm$  SE). Dashed lines represent estimated trophic levels based on a 3.4‰  $^{15}\text{N}$  fractionation per TP. Sandlance (*Ammodytes americanus*) was used as an estimator for  $^{15}\text{N}$  base and assigned an TP of 3.0.

collected at recreational fishing tournaments on Cape Cod and Martha's Vineyard, MA in July and August, 2001. Tournament participants caught the sharks from shelf waters in an area extending approximately 50 to 160 km south to south-east of Martha's Vineyard. The *Cetorhinus maximus* sample was obtained from a specimen that stranded on a Cape Cod beach in September 2001.

Tissue samples were removed from the caudal region of each shark and frozen. Prior to analysis, the tissues were

thawed in flowing seawater and a small section of white muscle was excised from just below the skin and connective tissue. Muscle samples were rinsed with distilled water to remove any excess superficial debris and dried in a drying oven until a constant weight had been reached.

Representative prey items included *Merluccius bilinearis* Mitchell (silver hake), *Clupea harengus* L. (Atlantic herring), squid spp., and copepod spp. These specimens were obtained from National Marine Fisheries Service

(NMFS) trawls conducted along the Massachusetts coast and Stellwagen Bank during the summers of 2000 and 2001. Muscle tissue collection and preparation for isotopic analysis were identical to those of shark samples. Copepods were dried whole in two groups of approximately 15 to 20 individuals.

#### Stable isotope analysis

Approximately 1 to 2 mg of ground tissue was used to determine the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values. Samples were combusted using a Carlo Erba 2100 Elemental Analyzer interfaced via continuous flow to a Finnigan Mat Delta Plus isotope-ratio mass spectrometer. Stable isotope abundances were measured by comparing the ratio of the two most abundant isotopes (e.g.  $^{13}\text{C}/^{12}\text{C}$  and  $^{15}\text{N}/^{14}\text{N}$ ) in the sample to the international standard. Results are expressed in terms of parts per thousand (‰) deviation from the standard using the equation:

$$\delta X = \left[ \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right] \times 1000\text{‰} \quad (1)$$

where X is  $^{13}\text{C}$  or  $^{15}\text{N}$  and R is the isotopic ratio  $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$  (Peterson & Fry, 1987). Standards used for carbon and nitrogen were Pee Dee Belemnite and atmospheric nitrogen (air), respectively. The Colorado Plateau Stable Isotopes Laboratory at Northern Arizona University performed the analysis of  $^{13}\text{C}$  and  $^{15}\text{N}$ .

#### Trophic position analysis

Relative TP were estimated using the equation:

$$\text{trophic position} = \lambda + \frac{(\delta^{15}\text{N}_{\text{consumer}} - \delta^{15}\text{N}_{\text{base}})}{\Delta_n} \quad (2)$$

where  $\lambda$  is the TP of the organism used to estimate  $\delta^{15}\text{N}_{\text{base}}$ ,  $\Delta_n$  is the enrichment in  $^{15}\text{N}$  per trophic level, and  $\delta^{15}\text{N}_{\text{consumer}}$  is the direct measurement of  $\delta^{15}\text{N}$  for the target species (Post, 2002). The species used as an estimate for  $\delta^{15}\text{N}_{\text{base}}$  should share the same habitat as the target species and should integrate the isotopic signature of the food web at a time scale large enough to minimize the effects of short-term variation (Post, 2002). Given its abundance throughout the north-west Atlantic, *Ammodytes americanus* DeKay (American sandlance) was used as the estimate for  $^{15}\text{N}$  base. As a secondary consumer, it was assigned a trophic level of 3.0. Since no fractionation data exist for elasmobranchs, the mean terrestrial and aquatic enrichment of  $\delta^{15}\text{N}=3.4$  was assumed for all trophic estimations (Post, 2002).

## RESULTS

The isotopic values for all species in the study are summarized in Table 1. We found significant differences between *Prionace glauca*, *Isurus oxyrinchus*, and *Alopias vulpinus*  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values (analysis of variance,  $F=7.91$ ,  $P<0.01$ , and  $F=9.40$ ,  $P<0.01$ , respectively). A posteriori Tukey's multiple comparisons tests showed that mean  $\delta^{15}\text{N}$  values did not differ significantly between *I. oxyrinchus* and *P. glauca* ( $P=0.51$ ), and both species were significantly more depleted in  $\delta^{15}\text{N}$  than *A. vulpinus*

**Table 2.** Trophic position calculations for shark species in the present study.  $TP_{\text{diet}}$  is the mean estimated trophic position of shark species from Cortés (1999), the range of values is indicated in parentheses. A posteriori chi-squared analysis on species with  $N>1$  showed no significant differences ( $P>0.05$ ) between TP calculations using  $\delta^{15}\text{N}$  and those from diet analysis.

Species	N	$TP_{\delta^{15}\text{N}}$ (Range)	$TP_{\text{Diet}}$
<i>Cetorhinus maximus</i>	1	3.1	3.2
<i>Prionace glauca</i>	5	3.8 (3.7 to 4.0)	4.1
<i>Isurus oxyrinchus</i>	5	4.0 (3.6 to 4.5)	4.3
<i>Alopias vulpinus</i>	4	4.5 (4.3 to 4.6)	4.2

N, number;  $TP_{\delta^{15}\text{N}}$ , trophic position based on N stable isotopes;  $TP_{\text{diet}}$ , trophic position based on diet.

( $P=0.02$  and  $P=0.003$ , respectively). The mean  $\delta^{13}\text{C}$  value of *I. oxyrinchus* was significantly more enriched than that of *A. vulpinus* ( $P=0.005$ ), but neither *A. vulpinus* ( $P=0.07$ ) nor *I. oxyrinchus* ( $P=0.28$ ) differed significantly from *P. glauca*. *Isurus oxyrinchus* isotopic values fluctuated substantially, ranging from  $-17.08$  to  $-15.89\text{‰}$  for  $\delta^{13}\text{C}$  and from  $12.22$  to  $15.20\text{‰}$  for  $\delta^{15}\text{N}$ , whereas *P. glauca* and *A. vulpinus* values were less variable (Table 1). *Cetorhinus maximus* had the lowest values of both  $\delta^{15}\text{N}$  ( $10.4\text{‰}$ ) and  $\delta^{13}\text{C}$  ( $-22.49\text{‰}$ ) (Table 1).

An a posteriori chi-squared analysis on the three species with  $N>1$  showed no statistical differences between the observed TP calculated by stable isotope analysis and the expected TP derived from diet data by Cortés (1999): *I. oxyrinchus*— $\chi^2=0.107$ ,  $df=4$ ,  $P>0.05$ ; *P. glauca*— $\chi^2=0.196$ ,  $df=4$ ,  $P>0.05$ ; *A. vulpinus*— $\chi^2=0.072$ ,  $df=3$ ,  $P>0.05$  (Table 2) (StatView 5.0.1, SAS Systems, Cary, NC). The TP of *C. maximus* (3.1) was also very similar to the estimation by Cortés (1999), and was exactly one full TP above that for copepods (Figure 1). *Alopias vulpinus* was estimated to be feeding at the highest TP (4.5), approximately a full trophic level above fish prey and squid (Figure 1).

## DISCUSSION

Calculated TP for *Prionace glauca*, *Isurus oxyrinchus*, and *Alopias vulpinus* based on SIA did not differ significantly from those determined by Cortés (1999) using stomach contents analysis. However, it is important to note that our data rely on the choice of 3.4 as the trophic enrichment for  $^{15}\text{N}$ . While this value is currently the best estimate available (see Post, 2002), the actual fractionation values for the species in this study have not been determined. However, in support of the TP determination, *Cetorhinus maximus*, known to feed solely on zooplankton (reviewed in Cortés, 1999), was found to be one full TP above copepod samples.

Our data do not support the hypothesis that the retention of urea in sharks may alter the calculated TP based on  $\delta^{15}\text{N}$  values. Fisk et al. (2002) hypothesized that the retention of urea for osmotic balance in elasmobranchs might cause the nitrogen isotope ratio to be more depleted, resulting in the underestimation of elasmobranch TP. However, the degree to which urea affects  $^{15}\text{N}$  fractionation might be a function of differing urea concentrations

within various tissues or between various species of elasmobranchs. Controlled laboratory experiments on elasmobranchs to explore differing urea concentrations within tissues or on elasmobranchs with known differing urea concentrations may aid in elucidating the effects of urea concentration on  $^{15}\text{N}$  fractionation.

High variability in *I. oxyrinchus*  $\delta^{15}\text{N}$  values warrants caution when making any conclusions regarding the exact TP of this species. Body size did not appear to be a major source of variability in the  $\delta^{15}\text{N}$  values of *I. oxyrinchus*. The largest of our specimens (555 kg), a current world record, showed only an intermediate nitrogen value (13.7‰), whereas an individual weighing less than 135 kg produced the highest value (15.2‰). Substantial differences in the diet of *I. oxyrinchus* between inshore ( $\leq 91$  m depth) and offshore waters ( $> 91$  m depth) may be a more likely explanation. Stillwell & Kohler (1982) noted that fish were the predominant prey items for inshore shortfin mako (85% total prey volume), while cephalopods became more important offshore. Although many of their offshore samples were from outside our study area (beyond the continental shelf), *I. oxyrinchus* are known to migrate between inshore and offshore environments (Stillwell & Kohler, 1982). Thus, large variation in  $\delta^{15}\text{N}$  values would be expected since inshore food webs have more trophic levels (Link, 2002), allowing for additional  $\delta^{15}\text{N}$  fractionations and more enriched  $\delta^{15}\text{N}$  values. This explanation is also supported by the  $\delta^{13}\text{C}$  data. The  $\delta^{13}\text{C}$  values from inshore food webs tend to be more  $\delta^{13}\text{C}$  enriched than those from offshore environments (France, 1995). In our study, *I. oxyrinchus* with the most enriched  $\delta^{15}\text{N}$  (15.2‰) also had the most enriched  $\delta^{13}\text{C}$  ( $-15.9$ ‰), whereas the specimen with the most depleted  $\delta^{15}\text{N}$  (12.2‰) produced the most depleted  $\delta^{13}\text{C}$  value ( $-17.1$ ‰). A larger study sampling *I. oxyrinchus* from inshore and offshore habitats is needed to further evaluate the use of SIA on this species.

Unlike *I. oxyrinchus*, variability in  $\delta^{15}\text{N}$  values for *P. glauca* and *A. vulpinus* was minimal. Trophic position calculations for *P. glauca* and *A. vulpinus* using  $\delta^{15}\text{N}$  values did not differ significantly from those found by Cortés (1999). Since *A. vulpinus* were found to differ significantly in  $\delta^{15}\text{N}$  values from the other sharks, it is possible that they were exploiting a different prey base. Comparisons with isotopic values of prey species suggest that *P. glauca* and *I. oxyrinchus* forage primarily on fish prey, which is supported by findings in Stillwell & Kohler (1982), Cortés (1999), and Bowman et al. (2000). However, dietary information on *A. vulpinus* is less complete and often contradictory. Bowman et al. (2000) indicated *A. vulpinus* feed almost solely on fish (97.1%), whereas Cortés (1999) found that they rely heavily on cephalopods (71.8%). This emphasizes the problems with stomach contents-based trophic analysis because ingested prey items can vary both spatially and temporally. Our data agree more closely with those of Cortés (1999) since squid had higher  $\delta^{15}\text{N}$  values and less depleted  $\delta^{13}\text{C}$  values than fish prey.

The similarity between TP estimations produced using stable isotope data and stomach contents data suggest that SIA may be successfully employed to investigate the trophic ecology of elasmobranchs. The SIA provides several advantages over conventional stomach contents analysis, including non-lethal or less intrusive sampling, and the dampening of temporal and spatial biases. However, the noted lack of isotopic studies on elasmobranchs, the relatively small sample sizes in this study, and the potential effects of urea retention in some species warrant a more detailed analysis and further exploration of this application to elasmobranch feeding biology.

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