

# Diet- and tissue-specific incorporation of isotopes in the shark *Scyliorhinus stellaris*, a North Sea mesopredator

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**ABSTRACT:** Elucidating predator–prey relationships is an important part of understanding and assessing the structure and function of ecosystems. Sharks are believed to play a significant role in marine ecosystems, although their specific trophic ecology is largely unexplored. Stable isotopes of nitrogen ( $\delta^{15}\text{N}$ ) and carbon ( $\delta^{13}\text{C}$ ) are a widely applied tool in food-web studies, but there is a need to quantify stable isotope dynamics in animals, particularly sharks. In this study, diet–tissue discrimination factors (DTDF = stable isotope in consumer tissue – stable isotope in diet) and turnover rates (time for the isotope to be assimilated into the consumer's tissue) of stable isotopes were estimated in blood, fin, and muscle tissue for the shark species *Scyliorhinus stellaris* fed 2 diets with different isotope values. Subsequently, these diet- and tissue-specific DTDFs were used in isotopic mixing models to quantify the diet of *Scyliorhinus canicula* caught in the North Sea and were compared with stomach content data. DTDFs for  $\delta^{15}\text{N}$  ( $\Delta^{15}\text{N}$ ) and  $\delta^{13}\text{C}$  ( $\Delta^{13}\text{C}$ ) ranged from  $-1.95$  to  $3.49\%$  and from  $0.52$  to  $5.14\%$ , respectively, and varied with tissue and diet type. Isotope turnover rates in plasma and red blood cells, expressed as half-lives, ranged from 39 to 135 d. Most of the variability in DTDFs reported in this and other studies with sharks can be explained by linear relationships between DTDF and dietary isotopic values. From these relationships, we propose a method for isotope mixing models using diet-specific DTDFs, which improves diet reconstruction estimates for animals, particularly mesopredator sharks that consume a large range of prey types.

**KEY WORDS:** Diet · Discrimination factor · Fractionation · Large-spotted dogfish · Nitrogen enrichment · SIAR · Turnover

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## INTRODUCTION

Many species of sharks are apex predators and are thought to play a significant role in marine ecosystems via regulation of community structure by top-down processes (Baum & Worm 2009, Ferretti et al. 2010). Increased fishing pressure has had direct and indirect negative effects on global shark populations, due in large part to their biological fragility (slow growth rate, low fecundity, and late age at maturity)

(Worm et al. 2003, Shepherd & Myers 2005, Ferretti et al. 2008, Hisano et al. 2011). Consequently, many shark species are now listed as threatened or endangered (IUCN 2011). Hence, knowledge of shark trophic ecology is crucial to understanding their ecological role in marine communities and in developing sound management plans for commercial stocks.

Several techniques can be used to study the diet of organisms, including direct observation of feeding behaviour, analysis of stomach contents, and exami-

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nation of chemical constituents, such as fatty acids or stable isotopes. Conventional methods (direct observations and stomach analyses) are useful for identifying specific prey taxa, but predation events are rarely observed or documented for sharks. Stomach content analyses generally require large sample sizes to accurately quantify long-term feeding patterns (see review by Cortés 1999, Wetherbee & Cortés 2004), which are difficult to obtain for most species of sharks, particularly those threatened or endangered. Moreover, stomach content analysis generally requires sacrificing the animal, and there are several sources of bias when estimating the proportions of dietary components based on stomach contents, including empty stomachs and the rapid digestion of soft-bodied prey. As a result, only the food items ingested at a specific point in time are considered, and not those that have been assimilated (Caut et al. 2008).

Analyses of the proportional abundance of stable isotopes of various elements in the different tissues of consumers and their potential prey have been used as an alternative approach to traditional dietary analyses (e.g. Hobson & Clark 1992a,b). This approach is based on the fact that stable isotopic ratios of nitrogen ( $^{15}\text{N}/^{14}\text{N}$ , expressed as  $\delta^{15}\text{N}$ ) and carbon ( $^{13}\text{C}/^{12}\text{C}$ , expressed as  $\delta^{13}\text{C}$ ) in consumer tissues reflect those of their prey in a predictable manner. Values of  $\delta^{13}\text{C}$  in organisms generally reflect the original source of carbon at the base of the food web (Kelly 2000). Values of  $\delta^{15}\text{N}$  increase with each trophic level, because organisms preferentially excrete the lighter nitrogen isotope. The values of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  provide a general and integrated estimate of the trophic level at which the species feeds; however, they usually do not provide the specific dietary information revealed by conventional diet analyses.

Despite the widespread use of stable isotopes, there are caveats and assumptions associated with employing them to study feeding ecology (Caut et al. 2008, Martínez del Rio et al. 2009). First, the change in isotopes between prey and consumer is not always consistent; this difference between the stable isotope composition of an animal's tissue and that of its diet is the diet–tissue discrimination factor (DTDF or  $\Delta^{15}\text{N}$  or  $\Delta^{13}\text{C}$ ). The DTDF can vary depending on a consumer's nutritional status, lipid content, quality of the diet consumed, size, age, dietary ontogeny, and the tissue and elemental/isotopic composition of both consumer and diet (reviews: Vander Zanden & Rasmussen 2001, Post 2002, McCutchan et al. 2003, Vanderklift & Ponsard 2003, Robbins et al. 2005, Caut et al. 2009). Accurate DTDFs are critical for most uses in ecology, for example, as input parameters in isotopic

mixing models used for diet reconstruction and trophic position estimates (Phillips 2001, Post 2002). Variability in these parameters has been shown to play a key role in the interpretation of results, especially due to the sensitivity of the models to these parameters (e.g. Caut et al. 2008, Hussey et al. 2010a). Second, when using stable isotopes for dietary analyses, it is important to understand the sampled tissue's turnover rate, or the time it takes for the isotope to be assimilated therein, to determine the time frame (i.e. days to years) that is represented by the isotopic signature of the tissue. This turnover time generally varies with tissue type and can provide different temporal estimates of diet or feeding ecology (MacNeil et al. 2006).

The uncertainty around DTDFs and turnover rates of stable isotopes, along with other factors, has resulted in numerous calls for laboratory experiments to determine DTDFs and turnover rates (Caut et al. 2008, Martínez del Rio et al. 2009). Although Fisk et al. (2002) pointed out the need for such research in sharks, only 5 controlled studies have been published (Hussey et al. 2010b, Logan & Lutcavage 2010a, Kim et al. 2012a,b, Malpica-Cruz et al. 2012). Due in large part to the difficulties of maintaining sharks in captivity for a significant length of time, these authors often used an opportunistic sampling methodology that relied on the tissue samples available, and thus their ability to calculate some of the required parameters is limited. Using 4 aquarium sharks that had been euthanized for medical reasons, Hussey et al. (2010b) modeled the average isotope value of the sharks' diet based on the different proportions of food given to them over the preceding year and the isotopic values of their prey. Logan & Lutcavage (2010a) collected juvenile sandbar sharks ( $n = 5$ ) and monitored blood and muscle isotopic values over a short period of time: during a pre-shift isotopic stabilization period of 2 wk and a feeding experiment of 46 to 55 d. Kim et al. (2012a) monitored isotopic values of the blood and muscle of 3 leopard sharks for >1000 d, but, unfortunately, did not report an estimation of tissue turnover. Finally, Malpica-Cruz et al. (2012) calculated isotopic incorporation in neonate to young-of-the-year leopard sharks consuming an artificial diet of commercial fish pellets. These studies reported isotopic incorporation rates that varied between tissues and with diet type. Clearly, there is a need for more controlled studies on isotope dynamics in sharks.

Studies investigating the feeding ecology of sharks, especially those of species in decline or susceptible to the activities of commercial fisheries (Fer-

retti et al. 2008, Hisano et al. 2011), will probably continue to increase in the coming years. In this study, we first experimentally quantified  $\Delta^{15}\text{N}$  or  $\Delta^{13}\text{C}$  and isotope turnover rates in different tissues of the mesopredator shark *Scyliorhinus stellaris* fed 2 diets with different  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values (fish or mussel) for 240 d; recent evidence has shown strong relationships between  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  values in diet and the DTDF value (Overmyer et al. 2008, Dennis et al. 2010; see review by Caut et al. 2009). Second, these DTDFs were then used to interpret isotope data obtained for the small-spotted catshark *S. canicula* from the North Sea. The results of isotope mixing models were compared to stomach content data to assess the accuracy of the experimentally derived DTDFs.

## MATERIALS AND METHODS

### Laboratory experimental design

We first estimated the isotopic incorporation (discrimination factors and turnover) in different shark tissues to verify if there was a relationship between discrimination factors and diet isotopic values, as recently reviewed in Caut et al. (2009). This could have important effects on the isotopic model output and its interpretation. We held 26 male, 2 yr old large-spotted dogfish *Scyliorhinus stellaris* (mean  $\pm$  SD: length  $50.08 \pm 1.15$  cm, weight  $619.04 \pm 44.20$  g) for 12 mo on a constant diet prior to the experiments, at the Liege Aquarium-Museum (Belgium); all were born at the Aquarium. Dogfish were randomly divided into 2 dietary treatments with different isotopic values, fish (smelt *Osmerus eperlanus* [S]) or mussel (*Mytilus edulis* [M]) diet; individuals were each fed 30 g thrice weekly. The dogfish in each treatment were placed in a large aquarium separated by a transparent plastic window with an exchange of filtered water that maintained the same water conditions. After 120 d, 4 dogfish from both treatments ( $S_{120}$  and  $M_{120}$ ) were killed using a lethal dose of tricaine methanesulfonate (MS-222) and sampled for isotopic analysis, 6 dogfish were switched from the S to the M diet ( $S_{120}M_{120}$ ) and 6 were switched from the M to the S diet ( $M_{120}S_{120}$ ); they consumed the new diet for an additional 120 d. Three dogfish in each treatment continued on the same diet for 240 d ( $M_{240}$  and  $S_{240}$ ). Thus, we have used 2 long-term treatments with 2 different diet isotopic values ( $M_{240}$  and  $S_{240}$ ) to estimate precisely the isotopic incorporation. For the diet shift, we hypothesized that an

isotopic equilibrium was possible after 120 d. Thus, we aimed to compare the incorporation dynamics between different initial isotopic values. If the isotopic equilibrium was not achieved after 120 d, we could not calculate the DTDFs, but the diet switch provided insights into the turnover rates of the different diets.

Blood samples were taken and length and mass were measured at the start of the experiment and every 15 d for all individuals. Blood was obtained from the sinus vein (after anaesthesia with MS-222) using blood-collection kits (5 ml syringe +  $12.7 \times 31$  needle; WWR). The blood sample was immediately separated into red blood cells (RBC) and plasma components by centrifugation. At the end of the experiment (Day 240), 4 dogfish from both treatments were killed using a lethal dose of MS-222, and plasma, RBC, muscle, and fin were sampled. The isotope values of the diets were quantified for each treatment; samples were randomly taken from the stock throughout the experiment. All samples were kept at  $-20^\circ\text{C}$  until isotopic analysis.

### Field study procedures and stomach content analysis

Field samples of sharks and their potential diet items were collected in a restricted area in the southern half of the North Sea during the annual French International Bottom Trawl Survey (IBTS) in February 2008 (Fig. 1, see Heessen et al. 1997 for a complete description). The catch was categorized by species, and some individual whole fish were kept at  $-20^\circ\text{C}$  until isotopic analysis.

Blood from commercial shark species was collected from the sinus vein using blood-collection kits and then directly separated into RBC and plasma components by centrifuge. Dorsal muscle and stomach contents were also collected, and total length, mass, sex, and stomach fullness (i.e. contained food or empty) were recorded for each specimen.

Stomach contents were removed and preserved in alcohol (70%) for later identification to the lowest taxonomic level possible using a set of references for several taxonomic groups developed during the commercial trawl haul (including fish otoliths). The relative importance of each prey item was assessed in 2 ways: (1) the numerical index (NI), i.e. the percentage of each prey item relative to the total number of prey items (number of individuals in a prey category/total number of individuals among all prey categories  $\times 100$ ) and (2) the occurrence index (OI),

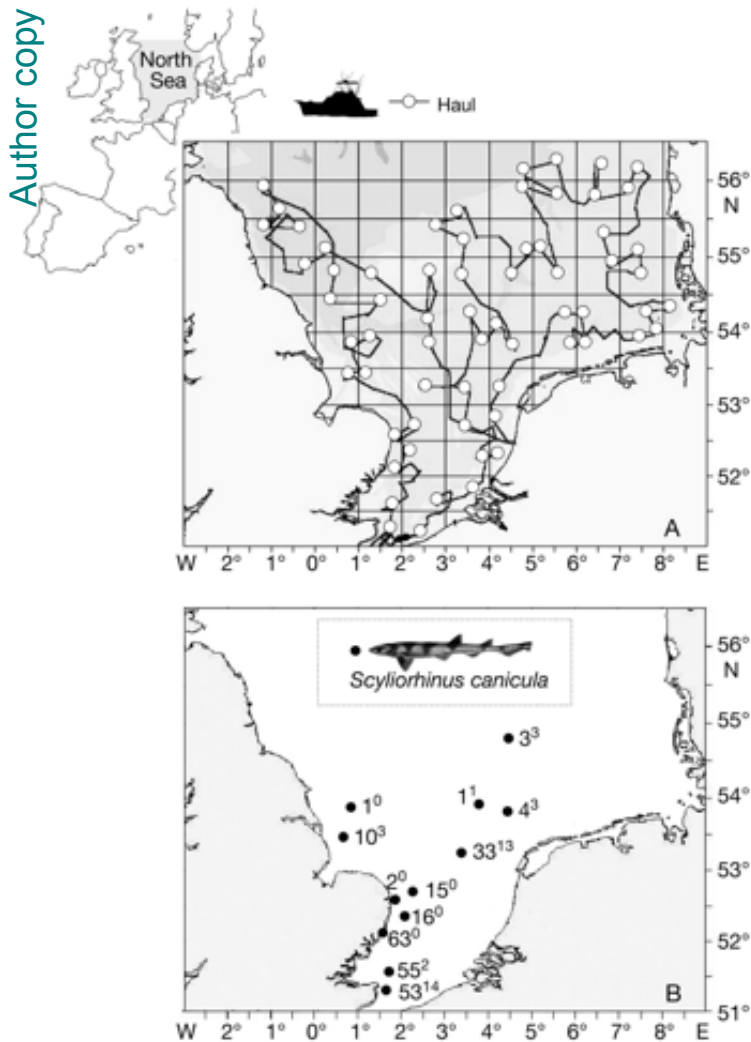


Fig. 1. (A) Map of the North Sea, where the annual French International Bottom Trawl Survey of 2008 (1 to 20 February) was conducted using randomized trawl hauls. One haul was randomly performed in each rectangle; the trawl hauls are represented by white circles. (B) Locations where *Scyliorhinus canicula* were collected, the values next to the black circles are the total numbers of individuals caught, and the superscripted values indicate the numbers of samples analysed ( $n = 39$ )

i.e. the percentage of each prey item in all non-empty stomachs (number of stomachs containing a prey category/total number of stomachs containing prey  $\times 100$ ). A cumulative prey curve was constructed to assess the adequacy of the number of stomachs sampled. The order of stomachs was randomized 10 times, and the mean ( $\pm$ SE) of unique prey items was plotted to minimize a possible bias resulting from the sampling order. The point at

which the prey curve achieved an asymptote identified the number of stomachs needed (Ferry et al. 1997). Identifiable prey items that were in good condition were kept at  $-20^{\circ}\text{C}$  until isotopic analysis, to increase the prey database.

### Isotopic analyses

Shark tissues, food and prey items (including those collected from stomach contents) were freeze-dried and ground to a fine powder. For shark muscle, we compared isotopic values before and after lipid extraction. Lipid extraction was performed by rinsing samples with a 2:1 chloroform/methanol solvent and then drying them at  $60^{\circ}\text{C}$  for 24 h to remove any residual solvent. Extraction of lipids was not necessary for blood samples because the lipid component in blood is generally low (Caut et al. 2011). For all fish species, we mixed the whole body of the specimen and selected a homogenized subsample. For bivalves, gastropods, and hermit crabs, the shells were removed before analysis. Isotopic analyses were performed on 1 mg subsamples of homogenized materials loaded into tin cups.

Stable carbon and nitrogen isotope measurements were carried out using a continuous flow isotope ratio mass spectrometer (Optima, Micromass) coupled to a C-N-S elemental analyser (Carlo Erba). Stable C and N isotope ratios are expressed as:  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$ , where  $R$  is  $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$  for  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$ , respectively.  $R_{\text{standard}}$  is the ratio of the international references PDB for carbon and AIR for nitrogen. One-hundred replicate assays of internal laboratory standards indicate maximum measurement errors (SD) of  $\pm 0.20\text{‰}$  and  $\pm 0.15\text{‰}$  for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  measurements, respectively.

### Isotopic turnover and DTDf

For the 2 treatments continued on the same diet for 240 d ( $M_{240}$  and  $S_{240}$ ), following the diet switch at  $t_0$ , turnover rates of isotopes were quantified by fitting the data using a Marquardt non-linear fitting routine (NLIN, SAS) using the following equations:

$$y = a + be^{ct} \quad (4)$$

where  $y$  is  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$ ,  $a$  is the isotope value approached asymptotically ( $\delta X_{(\infty)}$ ),  $b$  is the total change in values after the diets were switched at  $t_0$  ( $\delta X_{(\infty)} - \delta X_{(t_0)}$ ),  $c$  is the turnover rate, and  $t$  is the time in days since the switch. In order to find the length of time

required for  $\alpha$  percent turnover, we solved the equation (Tieszen et al. 1983):

$$T = \ln[(1 - \alpha / 100) / c] \quad (2)$$

where  $T$  is the time in days,  $\alpha$  is percent turnover, and  $c$  is the turnover rate of the tissue. To calculate turnover rate half-lives (50% turnover) and near-complete turnover (95% turnover), the equation is solved for  $\alpha = 50$  and  $\alpha = 95$ , respectively.

DTDFs between a food resource (food) and a consumer (shark) are described in terms of the difference in delta ( $\delta$ ) values using  $\Delta$  notation, where DTDF ( $\Delta$ ) =  $X_{(\infty)\text{shark}}$  (obtained by the fitted model) –  $X_{\text{food}}$ , where  $X$  is  $\delta^{13}\text{C}$  or  $\delta^{15}\text{N}$  and values were only calculated for sharks held on the same diet for 240 d ( $M_{240}$  and  $S_{240}$ ).

### Isotopic model

The relative isotopic contribution of prey to the diet of sharks in the North Sea was calculated using the SIAR package (Parnell et al. 2010). This model uses Bayesian inference to solve for the most likely set of proportional dietary contributions given the isotopic ratios of a set of possible food sources and a set of consumers. The model assumes that each target value comes from a Gaussian distribution with an unknown mean and standard deviation. The structure of the mean is a weighted combination of the isotopic values of the food sources. The weights are made up of dietary proportions (which are given a Dirichlet prior distribution) and the concentration dependencies given for the different food sources. The standard deviation is divided between the uncertainty around the discrimination corrections and the natural variability between target individuals (for more information see Jackson et al. 2009, Moore & Semmens 2008, Parnell et al. 2010). Throughout this paper, the mean dietary proportions from isotope analyses will be followed by their 95% confidence interval (CI). To represent the sharks, we used plasma and muscle tissues because the turnover rates of stable isotopes are different for each, reflecting a short and longer assimilation time, respectively (MacNeil et al. 2006). Isotopic models typically use the mean  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values for each type of diet, corrected by the DTDF. To build our set of different potential prey species, we used isotope values for prey species found in the stomach contents and added values for other species from the literature (Kaiser & Spencer 1994, Olaso et al. 1998, 2005, Valls et al. 2011, Filipe et al. 2012) to limit the bias due to the sampling size

of the stomach analysis. We grouped the different prey species according to taxa and type of consumer (e.g. detritivores) for isotopic model analysis. Because lipids were not extracted from the prey species, we used the general correction for lipid content for aquatic species when the C/N ratio of the tissue being analyzed was  $>3.5$  (following the equation by Post et al. [2007]:  $\delta^{13}\text{C}_{\text{normalized}} = \delta^{13}\text{C}_{\text{untreated}} - 3.32 + 0.99 \text{ C/N}$ ).

DTDFs depend on several sources of variation (e.g. taxon, environment, and tissue). Previous laboratory work had shown significant relationships between the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of diets and the corresponding  $\Delta^{15}\text{N}$  and  $\Delta^{13}\text{C}$  of the different tissues of consumers fed on those diets (e.g. reviewed in Caut et al. 2009). Thus, the  $\Delta^{13}\text{C}$  and  $\Delta^{15}\text{N}$  of plasma and muscle were calculated for each dietary item using regressions between shark  $\Delta^{13}\text{C}$  and  $\Delta^{15}\text{N}$  and the corresponding dietary isotopic ratios; these regressions utilized experimental data from our and 3 other studies on sharks fed a known natural diet (Hussey et al. 2010b, Kim et al. 2012a,b following Caut et al. 2008). Moreover, we ran a SIAR mixing model using the common fish fixed discrimination factors (FDFs) of 1‰ for  $\delta^{13}\text{C}$  and 3.2‰ for  $\delta^{15}\text{N}$  (Post et al. 2007) and compared the outputs with the run of the model using the DTDFs estimated with our regressions.

### Statistical analyses

We performed generalized linear models to test (1) the effect of lipid extraction on the isotopic ratios of shark muscle (captive [*Scyliorhinus stellaris*] and wild individuals [*S. canicula*]) and the 2 diets (M and S)—values resulting from lipid extraction are noted hereafter by the subscript <sub>DEL</sub>; (2) the isotopic difference between the 2 control diets; (3) the effect of the 2 control diets on body mass growth; (4) the effect of sex and body mass on the isotopic values of *S. canicula*; and (5) the difference in isotope values between tissues (plasma and muscle) in both captive and wild individuals.

To compare the isotopic ratios of each tissue (muscle and fin) among the 2 groups having consumed the same diet ( $M_{120}$  vs.  $M_{240}$  and  $S_{120}$  vs.  $S_{240}$ ), we performed pairwise comparisons using Kruskal-Wallis non-parametric tests (hereafter KW).

Computations were performed with STATISTICA 6.0 (StatSoft) and isotopic incorporation data were fitted using a Marquardt non-linear fitting routine (NLIN, SAS). The level of significance for statistical analysis was set at  $\alpha = 0.05$ .

**Table 1. *Scyliorhinus stellaris*. (A) Effect of lipid extraction on the nitrogen ( $\delta^{15}\text{N}$ , ‰) and carbon ( $\delta^{13}\text{C}$ , ‰) isotopic values of the 2 diet treatments (mussels [M] and smelt [S]), values resulting from lipid extraction are denoted by the subscript DEL. (B) Exponential equations (with  $R^2$ ) and statistics of convergent equations of stable isotope incorporation in plasma and red blood cells (RBC) of dogfish under controlled conditions. Nitrogen and carbon diet–tissue discrimination factors ( $\Delta$ , ‰) and turnover rates ( $t_{50\%}$  and  $t_{95\%}$ ) in days in different dogfish tissues (calculated from lipid-extracted control diet samples for  $\delta^{13}\text{C}$  and non-lipid-extracted samples for  $\delta^{15}\text{N}$ ) are listed. (C) Difference between the isotopic ratios of each tissue (muscle and fin) among the 2 groups having consumed the same diet for 120 and 240 d ( $M_{120}$  vs.  $M_{240}$  and  $S_{120}$  vs.  $S_{240}$ ; Kruskal-Wallis test). Nitrogen and carbon discrimination factors ( $\Delta$ , ‰) were calculated at time 240 d**

(A)	Diet	Nitrogen			Carbon											
		$\delta^{15}\text{N} \pm \text{SD}$	df	F	p	$\delta^{13}\text{C} \pm \text{SD}$	df	F	p							
Mussel	M	9.72 ± 0.24	1,18	0.09	0.742	17.42 ± 0.15	1,18	14.59	<0.001							
	M <sub>DEL</sub>	9.82 ± 0.25				-16.49 ± 0.19										
Smelt	S	17.45 ± 0.27	1,14	0.14	0.726	-24.03 ± 0.51	1,14	8.56	0.020							
	S <sub>DEL</sub>	17.59 ± 0.27				-22.26 ± 0.33										
(B)	Diet	Equation ( $R^2$ )	df	F	p	$\Delta$	$t_{50\%}$	$t_{95\%}$								
Plasma	M <sub>240</sub>	$Y = 12.77 + 2.12e^{-0.0063x}$ (0.84)	2,58	148.96	<0.001	3.05	110	476	$Y = -13.70 - 3.48e^{-0.0114x}$ (0.87)	2,58	188.91	<0.001	2.79	61	263	
	S <sub>240</sub>	$Y = 17.87 - 3.24e^{-0.0176x}$ (0.85)	2,59	158.32	<0.001	0.42	39	170	$Y = -19.05 + 1.86e^{-0.0119x}$ (0.57)	2,59	37.13	<0.001	3.21	58	252	
RBC	M <sub>240</sub>	$Y = 12.91 + 1.03e^{-0.0116x}$ (0.70)	2,58	66.73	<0.001	3.19	60	258	$Y = -15.27 - 2.45e^{-0.0074x}$ (0.76)	2,58	87.69	<0.001	1.22	94	405	
	S <sub>240</sub>	$Y = 18.15 - 4.52e^{-0.0052x}$ (0.87)	2,58	188.66	<0.001	0.7	135	582	$Y = -20.25 + 2.59e^{-0.0053x}$ (0.76)	2,58	90.90	<0.001	2.01	130	561	
(C)	Effect		df	H	p	$\Delta$				df	H	p	$\Delta$			
Muscle	M <sub>120</sub> vs. M <sub>240</sub>		1,7	1.13	0.289	3.49	M diet			1,7	4.58	0.032	0.52	M diet		
	S <sub>120</sub> vs. S <sub>240</sub>		1,7	2.00	0.157	-1.81	S diet			1,7	0.00	1	4.28	S diet		
Fin	M <sub>120</sub> vs. M <sub>240</sub>		1,6	0.86	0.335	0.49	M diet			1,6	3.43	0.064	1.06	M diet		
	S <sub>120</sub> vs. S <sub>240</sub>		1,7	4.50	0.034	-1.95	S diet			1,7	4.50	0.034	5.14	S diet		

**RESULTS**

**Experimental study**

Stable isotopes of the control diets (S and M)

Lipid extraction had a significant effect on the  $\delta^{13}\text{C}$  of the 2 control diets, but not on the  $\delta^{15}\text{N}$  (Table 1A). Thus, lipid-extracted  $\delta^{13}\text{C}_{\text{DEL}}$  and non-lipid-extracted  $\delta^{15}\text{N}$  values were used to estimate DTFD and mixing models, and these values were significantly different between the 2 control diets ( $\delta^{13}\text{C}$ :  $F_{1,16} = 249.55$ ,  $p < 0.001$ ;  $\delta^{15}\text{N}$ :  $F_{1,16} = 453.81$ ,  $p < 0.001$ ). Moreover, the 2 control diets had no significant effect on body mass growth during the experiment ( $F_{1,23} = 3.83$ ,  $p < 0.063$ ).

Blood isotopic incorporation

The blood C/N ratio in *Scyliorhinus stellaris* was low ( $C/N < 3.5$ ; Post et al. 2007), confirming that it was unnecessary to perform lipid extraction on these tissues (mean ± SD: plasma  $C/N = 1.93 \pm 0.03$ ; RBC  $C/N = 2.26 \pm 0.03$ ,  $n = 380$ ). An exponential model significantly fit values of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  for plasma and RBC for M<sub>240</sub> and S<sub>240</sub> treatments (Fig. 2, Table 1B). Half-life estimates for isotopic incorporation rates of  $\delta^{15}\text{N}$  (39 to 110 d) and  $\delta^{13}\text{C}$  (58 to 61 d) in plasma were lower than those in RBC ( $\delta^{15}\text{N}$ : 60 to 135 d and  $\delta^{13}\text{C}$ : 94 to 130 d), but the range in values did overlap.

In all diet treatments, plasma and RBC were enriched in  $^{15}\text{N}$  and  $^{13}\text{C}$  relative to dietary values (Table 1B). The  $\Delta^{15}\text{N}$  ranged from 0.42 to 3.05 for plasma and 0.70 to 3.19 for RBC, and the  $\Delta^{13}\text{C}$  ranged from 2.79 to 3.21 for plasma and 1.22 to 2.01 for RBC. The value of  $\Delta^{15}\text{N}$  was greater for the M than for the S diet, but the inverse was true for  $\Delta^{13}\text{C}$ . It seemed to be more appropriate to use parameters estimated from the group fed the same diet over the longest period (S<sub>240</sub> and

$M_{240}$ ) for models. Indeed, the fitted equations were better adjusted when the data set approached an asymptote (i.e. equilibrium) (data for 120 d treatment not shown) and plasma and RBC isotope values did not reach an asymptote for treatments with a diet shift ( $S_{120}M_{120}$  or  $M_{120}S_{120}$ ; Fig. 2).

### Muscle and fin isotopic incorporation

Lipid extraction had no significant effect on the  $\delta^{13}C$  and  $\delta^{15}N$  values of muscle ( $\delta^{13}C$ :  $F_{1,50} = 0.10$ ,  $p = 0.748$ ;  $\delta^{15}N$ :  $F_{1,50} = 0.23$ ,  $p = 0.631$ ), which was consistent with the tissue's low C/N ratio (mean  $\pm$  SD =  $2.81 \pm 0.01$ ,  $n = 26$ ). We did not perform lipid extraction on fin samples because their C/N ratio was also very low ( $2.53 \pm 0.01$ ).

A comparison of  $\delta^{15}N$  and  $\delta^{13}C$  in the 3 tissues (muscle, fin, and whole blood) at 120 and 240 d for individuals fed the same diet revealed a different trend for the M and S diets. In the S diet treatment, there were significant differences between the  $S_{120}$  and  $S_{240}$  groups in  $\delta^{15}N$  and  $\delta^{13}C$  for fin, but no difference was found for muscle (Table 1C). In contrast, in the M diet treatment, there were no significant differences between the  $M_{120}$  and  $M_{240}$  groups in  $\delta^{15}N$  or  $\delta^{13}C$  for any of the tissues, except for muscle  $\delta^{13}C$  (Table 1C).

Finally, for samples from individuals consuming the same diet over the entire 240 d of the study, there were significant differences in  $\delta^{13}C$  and  $\delta^{15}N$  between the treatments (M and S) for muscle (KW test— $\delta^{13}C$ :  $H_{1,6} = 3.97$ ,  $p = 0.046$ ;  $\delta^{15}N$ :  $H_{1,6} = 3.86$ ,  $p = 0.049$ ), but not for fin tissues ( $\delta^{13}C$ :  $H_{1,5} = 3.00$ ,  $p = 0.083$ ;  $\delta^{15}N$ :  $H_{1,5} = 3.00$ ,  $p = 0.083$ ). In addition, although we did not have the possibility of verifying and measuring isotopic equilibrium for muscle and fin tissues, we calculated the DTDF after 240 d on the control diet for the sake of comparison. We found the same trend: a higher degree of differentiation between diets (M vs. S) than between tissues, consistent with results from plasma and RBC;  $\Delta^{15}N$  was greater in the M diet than in the S diet, and the inverse was true for  $\Delta^{13}C$  (Table 1C).

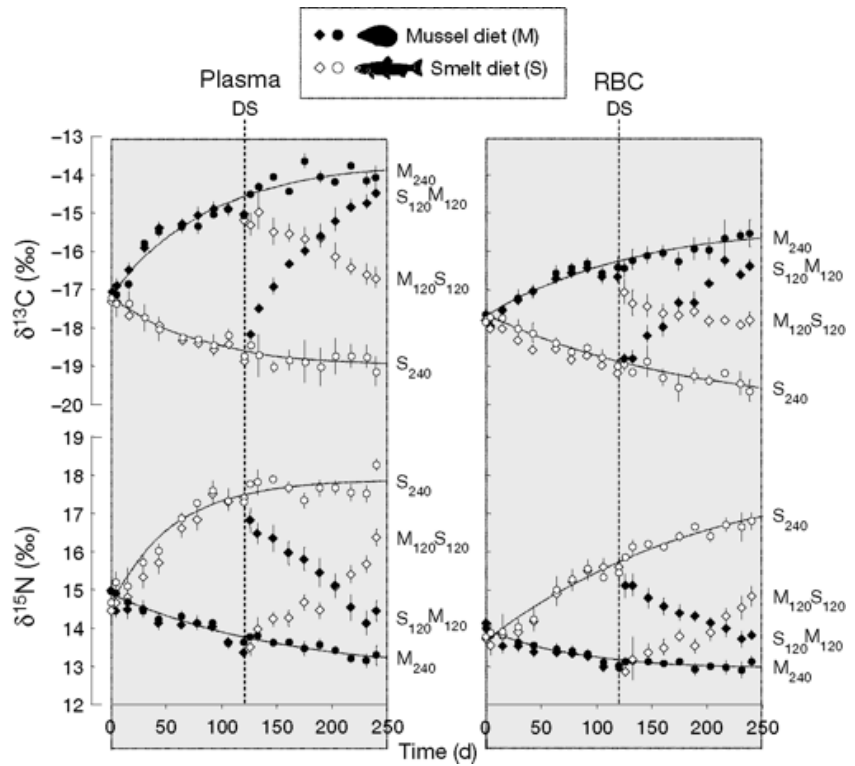


Fig. 2. *Scyliorhinus stellaris*. Nitrogen and carbon isotopic values (mean  $\pm$  SD) of plasma and red blood cells (RBC) for the different diet treatments: (1)  $S_{120}M_{120}$  = switched from smelt (S) to mussel (M) diet at 120 d (DS: diet shift); (2)  $M_{120}S_{120}$  = switched from M to S diet at 120 d; and (3)  $M_{240}$  and  $S_{240}$  = remained on the same diet (M or S) for 240 d. The diet treatments  $M_{120}$  and  $S_{120}$  are not separately plotted as they represent the first part of the DS experiments (0 to 120 d), before the DS occurred

### Field study

#### Wild shark isotopic values

Over the 67 total hauls, 255 small-spotted catsharks *Scyliorhinus canicula* were caught (Fig. 1). In total, 39 individuals of *S. canicula* (10 $\sigma$  and 29 $\varphi$ ) were sampled for isotopes and stomach contents, with a mean ( $\pm$  SD) total length and mass of ( $\sigma$ )  $505 \pm 14$  mm and ( $\varphi$ )  $545 \pm 41$  g. Among them, 20.5% of the sharks sampled had empty stomachs.

Lipid extraction had no effect on  $\delta^{15}N$  and  $\delta^{13}C$  in muscle samples ( $\delta^{13}C$ ,  $F_{1,76} = 0.54$ ,  $p = 0.464$  and  $\delta^{15}N$ ,  $F_{1,76} = 1.14$ ,  $p = 0.289$ ), a result that is consistent with this tissue's lower C/N ratio (mean  $\pm$  SD =  $2.74 \pm 0.02$ ). Similarly, the C/N ratio of plasma ( $1.48 \pm 0.06$ ) was lower than that of muscle, which meant that no lipid extraction of plasma was necessary. There were no significant effects of mass or sex on  $\delta^{13}C$  or  $\delta^{15}N$  for *S. canicula* ( $\delta^{13}C_{\text{Muscle}}$ : mass

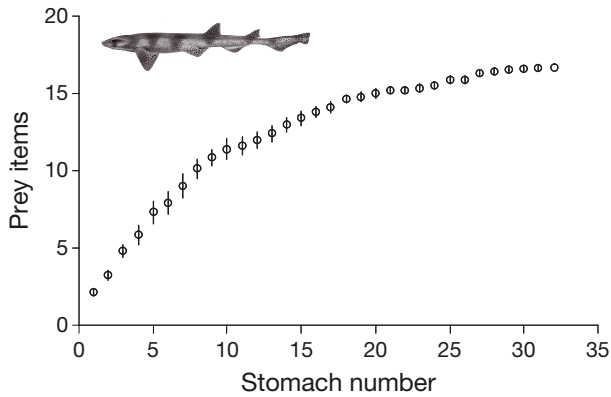


Fig. 3. *Scyliorhinus canicula*. Randomized cumulative prey curve for *S. canicula*. Mean values of 10 randomizations are presented ( $\pm$ SE)

$F_{1,36} = 1.35$ ,  $p = 0.253$  and sex  $F_{1,36} = 1.23$ ,  $p = 0.274$ ;  $\delta^{15}\text{N}_{\text{Muscle}}$ : mass  $F_{1,36} = 2.78$ ,  $p = 0.104$  and sex  $F_{1,36} = 3.06$ ,  $p = 0.089$ ;  $\delta^{13}\text{C}_{\text{Plasma}}$ : mass  $F_{1,36} = 0.82$ ,  $p = 0.371$  and sex  $F_{1,36} = 0.86$ ,  $p = 0.361$ ;  $\delta^{15}\text{N}_{\text{Plasma}}$ : mass  $F_{1,36} = 2.30$ ,  $p = 0.138$  and sex  $F_{1,36} = 0.77$ ,  $p = 0.386$ ). However, there was a significant difference between muscle and plasma isotope values ( $\delta^{13}\text{C}$ :  $F_{1,76} = 21.24$ ,  $p < 0.001$ ;  $\delta^{15}\text{N}$ :  $F_{1,76} = 43.01$ ,  $p < 0.001$ ), with muscle having higher  $\delta^{15}\text{N}$  but lower  $\delta^{13}\text{C}$  (Table 2).

#### Conventional diet analysis

The cumulative prey curve for *Scyliorhinus canicula* reached a well-defined asymptote, indicating that sample size was sufficient to adequately describe the diet (Fig. 3). *S. canicula* had a varied diet based on stomach contents, which was composed of 17 different taxa belonging to 5 taxonomic groups: Annelida, Decapoda, Mollusca, Echinodermata, and Teleostei. Decapods were by far the most abundant, according to the numerical (NI) and occurrence indices (OI), with values between 45 and 63%, respectively (Fig. 4, see also Table S1 in the Supplement at [www.int-res.com/articles/suppl/m492p185\\_supp.pdf](http://www.int-res.com/articles/suppl/m492p185_supp.pdf)). Teleostei was predominantly represented by 2 species: *Ammodytes tobianus* and *Buglossidium luteum*. The remaining prey groups, Mollusca and Echinodermata, represented less than ~25% of the diet in both indices. However, Mollusca was represented by only 1 species, *Buccinum undatum*, which was the second most important prey species after *Liocarcinus depurator* (Table S1).

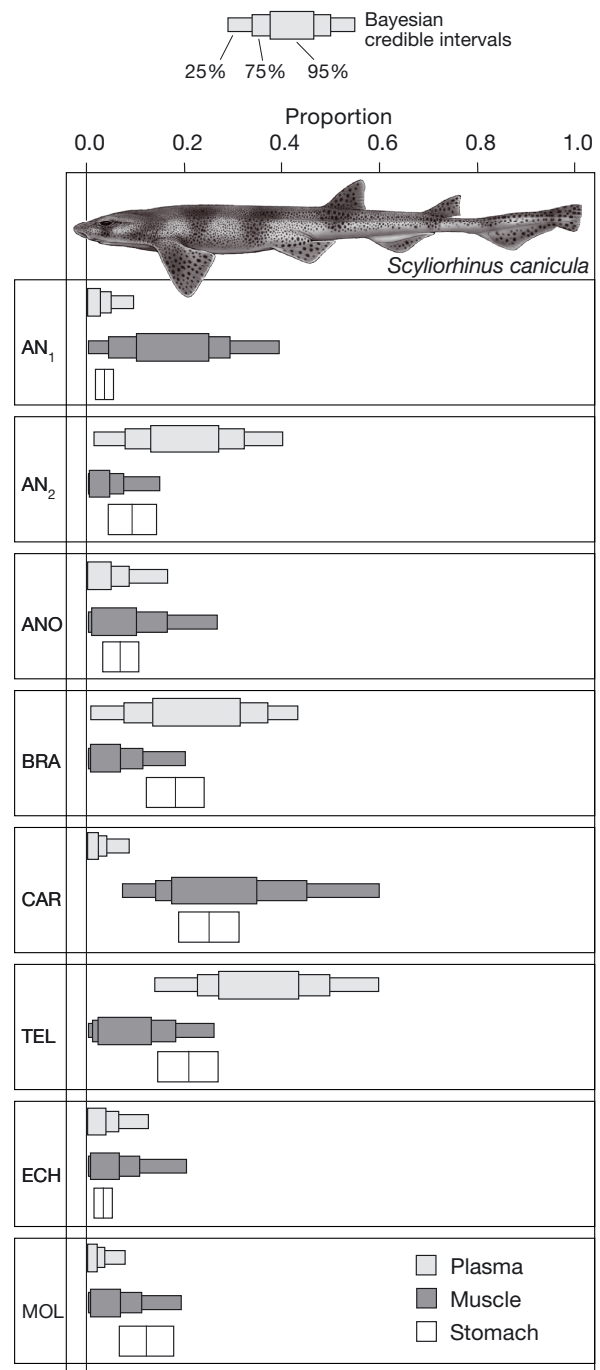


Fig. 4. *Scyliorhinus canicula*. Proportional contribution of different potential prey to the diets of *S. canicula* based on plasma and muscle isotopes (SIAR model) and stomach contents (numerical index: mean  $\pm$  SD). Boxplots (x-axis) show the distribution of possible contributions from each prey source to the diet of *S. canicula* that result from the application of the SIAR isotopic model. Values shown are the 25, 75, and 95% credibility internals, respectively, for these distributions. Abbreviations for *S. canicula* prey groups are as follows — AN<sub>1</sub>: Annelida Group 1; AN<sub>2</sub>: Annelida Group 2; ANO: Anomura; BRA: Brachyura; CAR: Caridae; TEL: Teleostei; ECH: Echinodermata; MOL: Mollusca



## Isotopic diet analysis

Eighty-two different prey items of 6 different Orders were caught over a total of 63 hauls (Fig. 1, see Table S2 in the Supplement at [www.int-res.com/articles/suppl/m492p185\\_supp.pdf](http://www.int-res.com/articles/suppl/m492p185_supp.pdf)). We used previous studies (see 'Materials and methods') and stomach content data from collected sharks to choose likely prey items for isotope analysis and inclusion in the isotope mixing models (see Table 2 and Table S1 in the Supplement for list of species). Most of these were collected from trawls, but some were from stomach contents (e.g. 2 different groups of Annelida denoted as Annelida<sub>1</sub> and Annelida<sub>2</sub>).

Strong significant regressions were found relating shark tissue (plasma and muscle)  $\Delta^{13}\text{C}$  and  $\Delta^{15}\text{N}$  to the corresponding dietary isotopic values from controlled natural diet experiments with sharks (Fig. 5). These regression equations allowed for the estimation of  $\Delta^{13}\text{C}$  and  $\Delta^{15}\text{N}$  for sharks based on the isotope values of the individual diet types collected from the ecosystem (Table 2), which were used in the isotopic model SIAR.

Depending on whether plasma or muscle was used, different potential prey contributions for *Scyliorhinus canicula* were found (Fig. 4). Using plasma, the model suggested 3 principal resources (mean percent): Teleostei (36%), Brachyura (23%), and Annelida<sub>2</sub> (21%). In contrast, when muscle was used, Caridae (31%), Annelida<sub>1</sub> (19%), and Teleostei (12%)

constituted the bulk of the diet based on the mixing model. Compared with the stomach contents, the mixing model underestimated the contribution of Caridae and Teleostei for muscle and plasma, respectively, and overestimated the importance of Annelids for both tissues (Fig. 4). Moreover, when we ran the SIAR mixing model using the common fish FDFs and compared it to the results from the model run using the DTDFs estimated with our regressions, we observed from the muscle tissue an overestimation of the importance of Brachyura (21%), Teleostei (19%), and Annelida<sub>2</sub> (19%), and an underestimation of the contribution of Caridae (5%) and Annelida<sub>1</sub> (6%). In contrast, when muscle was used, the FDF model strongly overestimated Annelida<sub>2</sub> (42%) and underestimated Brachyura (9%) and Teleostei (3%) (Fig. 6).

## DISCUSSION

## Isotopic incorporation

Although stable isotope analysis has become an increasingly popular technique in animal trophic ecology, the assumptions involved in the analyses and the lack of information for most taxa make experimental studies that quantify accurate DTDFs and turnover rates of tissues imperative. The application of an accurate DTDF is highly important, as it has

Table 2. *Scyliorhinus canicula*. Mean isotopic values ( $\pm$ SD, in parentheses) of carbon ( $\delta^{13}\text{C}_{\text{DEL}}$ , lipid-extracted) and nitrogen ( $\delta^{15}\text{N}$ ) in the muscle and plasma of *S. canicula* and their prey items from the North Sea, and estimated diet-item specific diet tissue discrimination factors (DTDFs) for the isotopic model. Prey items were chosen based on their presence in collected stomach contents or identified from the literature for this species. Species-specific DTDFs ( $\Delta$ : P = plasma and M = muscle) were generated from  $\Delta$ -diet isotope relationships generated from experimental data (see Fig. 3) and were used in the isotopic mixing model SIAR

	n	Isotopic values		Estimated DTDFs			
		$\delta^{13}\text{C}_{\text{DEL}}$	$\delta^{15}\text{N}$	$\Delta^{13}\text{C}_{\text{P}}$	$\Delta^{15}\text{N}_{\text{P}}$	$\Delta^{13}\text{C}_{\text{M}}$	$\Delta^{15}\text{N}_{\text{M}}$
<b><i>S. canicula</i></b>							
Muscle	39	-16.15 (0.09)	16.11 (0.14)				
Plasma	39	-15.47 (0.18)	14.87 (0.15)				
<b>Prey items</b>							
<b>Annelida</b>							
Annelida Group 1	5	-16.47 (0.20)	14.99 (0.62)	2.74 (0.02)	1.38 (0.21)	0.36 (0.11)	0.97 (0.43)
Annelida Group 2	1	-17.43	11.61	2.81	2.53	0.91	3.27
<b>Arthropoda (Decapoda)</b>							
Anomura	7	-16.67 (0.44)	13.14 (0.82)	2.75 (0.03)	2.01 (0.28)	0.47 (0.25)	2.26 (0.57)
Brachyura	17	-17.67 (0.12)	12.39 (0.48)	2.83 (0.01)	2.27 (0.16)	1.05 (0.07)	2.77 (0.33)
Caridae	14	-16.62 (0.21)	16.07 (0.24)	2.75 (0.02)	1.01 (0.08)	0.44 (0.12)	0.23 (0.16)
Chordata (Teleostei)	38	-18.44 (0.19)	13.79 (0.21)	2.89 (0.01)	1.79 (0.07)	1.49 (0.11)	1.81 (0.14)
Echinodermata	3	-16.04 (0.40)	12.47 (0.64)	2.70 (0.03)	2.24 (0.22)	0.11 (0.23)	2.72 (0.44)
Mollusca	5	-15.04 (0.46)	12.80 (0.38)	2.62 (0.04)	2.12 (0.13)	-0.46 (0.26)	2.49 (0.26)

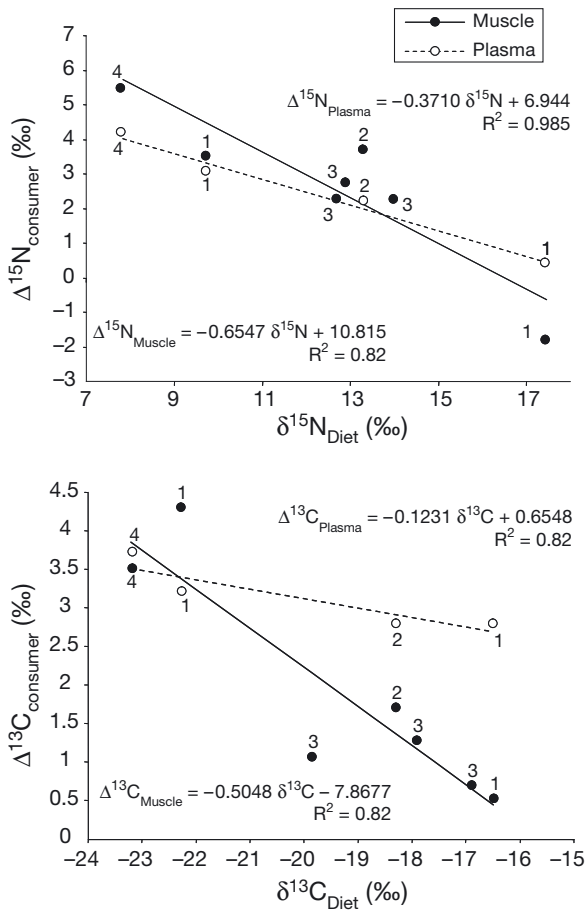


Fig. 5. *Scyliorhinus canicula*. Relationship between the mean values of (A) nitrogen isotopic ratios ( $\delta^{15}\text{N}$ ) and diet–tissue discrimination factors (DTDFs,  $\Delta^{15}\text{N}$ ) and (B) carbon isotopic ratios ( $\delta^{13}\text{C}$ ) and DTDFs ( $\Delta^{13}\text{C}$ ) for the different tissues sampled (black: muscle; white: plasma) for laboratory-derived DTDFs. The number next to each point identifies the shark study (1: present study; 2: Kim et al. 2012a; 3: Hussey et al. 2010b; 4: Kim et al. 2012b). Equations, regression coefficients, and fits are shown for the significant models

been shown to be variable across tissues, species, and dietary isotopic values (Caut et al. 2009, Martínez del Rio et al. 2009). A recent debate about the effect of an inadequate DTDF obtained from teleost fish that was applied to elasmobranchs has shown the importance of this parameter in the interpretation of trophic ecology in sharks (Hussey et al. 2010a, Logan & Lutcavage 2010a,b). Because of the unique physiology of sharks, in particular urea retention in tissues for osmoregulation, the estimation of shark-specific DTDFs is even more imperative (Fisk et al. 2002, Hussey et al. 2012).

Only 3 studies have estimated DTDFs for various tissues of sharks consuming a natural diet, and they include a wide range of estimates for  $\Delta^{15}\text{N}$  (2.3 to 5.5‰) and  $\Delta^{13}\text{C}$  (0.9 to 3.5‰) (Hussey et al. 2010b, Kim et al. 2012a,b; see values in Fig. 3). In our study, we also found a range of DTDFs depending on the type of diet and tissue ( $\Delta^{15}\text{N}_{\text{Mussel}} = 3.49\text{‰}$  or  $\Delta^{15}\text{N}_{\text{Smelt}} = -1.81\text{‰}$  and  $\Delta^{13}\text{C}_{\text{Mussel}} = 0.52\text{‰}$  or  $\Delta^{13}\text{C}_{\text{Smelt}} = 4.28\text{‰}$ ). This variability in DTDFs across these studies was largely explained by dietary isotopic values ( $R^2 = 0.82$  to  $0.98$ ; Fig. 3), which produced a negative linear  $\Delta$ -diet isotope value relationship that has been reported for other taxa under controlled-diet experiments (Overmyer et al. 2008, Dennis et al. 2010) and in compilations of published literature values (Caut et al. 2009). We also found good agreement between DTDFs for tissues across both diets ( $\Delta^{15}\text{N}_{\text{Mussel}} > \Delta^{15}\text{N}_{\text{Smelt}}$  and inversely  $\Delta^{13}\text{C}_{\text{Mussel}} < \Delta^{13}\text{C}_{\text{Smelt}}$ ). However, different amino acids in a single tissue can vary in their isotopic values by  $>15\%$  (e.g. Hare et al. 1991), due to variation in the amino acid proportions within different proteins. Thus, our dissimilarity in DTDFs among tissue types could be interpreted as a consequence of this amino acids composition.

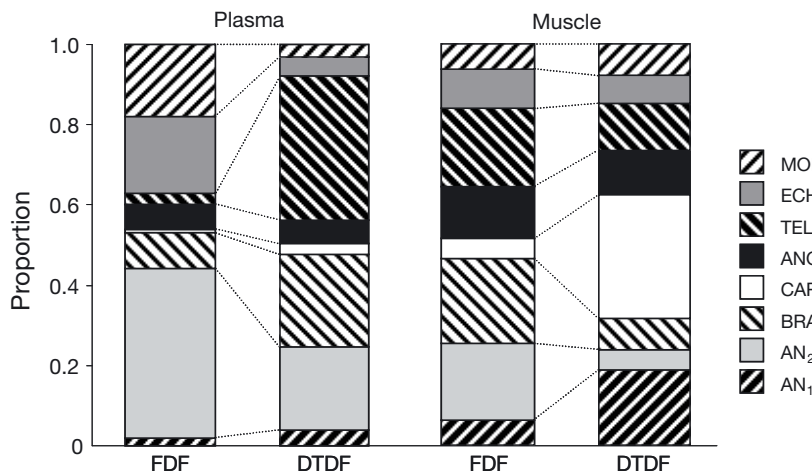


Fig. 6. *Scyliorhinus canicula*. Mean proportional contribution of different potential prey types to the diets of *S. canicula* based on plasma and muscle isotopes (SIAR model) with fixed discrimination factors (FDFs,  $\Delta^{13}\text{C} = 1\text{‰}$  and  $\Delta^{15}\text{N} = 3.2\text{‰}$ ) and diet–tissue discrimination factors (DTDFs) estimated by regressions (see Fig. 4). Abbreviations for *S. canicula* prey groups are as follows — AN<sub>1</sub>: Annelida Group 1; AN<sub>2</sub>: Annelida Group 2; BRA: Brachyura; CAR: Caridae; ANO: Anomura; TEL: Teleostei; ECH: Echinodermata; MOL: Mollusca

Previous studies have also found that DTDFs directly increase with protein content (Pearson et al. 2003) and directly decrease with protein quality (Florin et al. 2011; see quality or quantity hypothesis, Caut et al. 2010). The variation in DTDFs in our study may also be explained by differences in the protein quantity and quality between the invertebrate (M diet) and fish (S diet) used (%N = 8 for Mollusca versus 12 for fish in wild caught samples; see Table S2 in the Supplement). Given the strength of DTDF–diet isotope value relationships found across studies that included invertebrate and fish diet items, we think this relationship is more important. Regardless, these relationships are based on animals that are the potential prey consumed by elasmobranch mesopredators, in the natural environment.

In addition to using appropriate DTDFs, it is important to consider the turnover rate of isotopes in different tissues so that the time scale can be considered when interpreting the trophic ecology of the predator. Previous studies on elasmobranch turnover rates estimated that complete nitrogen and carbon turnover differed among tissues, ranging from a minimum of approximately 6 mo for plasma, 8 mo for whole blood, to >2 yr for muscle (MacNeil et al. 2006, Logan & Lutcavage 2010a, Kim et al. 2012b, Malpica-Cruz et al. 2012). Although the physiology of the species and experimental conditions (e.g. temperature) used in the present study could be different (e.g. metabolism or size), the turnover rates were in the same range as found in those previous studies and followed the classical tissue gradient of plasma < RBC < muscle. Moreover, the difference in turnover rate between diets depends probably on the direction and isotopic amplitude of the diet shift (moving to a lower or higher isotope value), as observed in other studies (e.g. MacNeil et al. 2006, Caut et al. 2011).

The reliability of the DTDF value is dependent on the assumption that isotope values in the tissue have achieved equilibrium with the diet, to calculate DTDF. Thus the duration of the experiment plays an important role in the accurate estimation of the DTDF. Although earlier studies found the same range of isotopic turnover rates as this study (modeled by exponential equations), the duration of the previous experiment was generally much shorter than the time-to-equilibrium (entire turnover) for the tissues examined: 29 and 34 d in MacNeil et al. (2006), 60 d in Logan & Lutcavage (2010a), 192 d in Malpica-Cruz et al. (2012), and >300 d in Kim et al. (2012b). In our study, we estimated the DTDFs from animals maintained on the same diet for 240 d (longest time), because the exponential models fit-

ting isotopic incorporation in tissues are extremely sensitive to the duration of the experiment. Indeed, we observed differences between the exponential fit results at 120 d and at 240 d ( $S_{120}$  vs.  $S_{240}$  or  $M_{120}$  vs.  $M_{240}$ ).

### Application of diet and tissue-specific DTDFs in mesopredators

Although mesopredators play a key role in marine ecosystems, many isotopic studies focus on top predators, probably because such species are more appealing and challenging to study with traditional methods. Mesopredators link different food webs and trophic levels in marine ecosystems, contributing to system dynamics and stability (Matich et al. 2011). *Scyliorhinus canicula* was caught mainly near the coast and in shallow water (~40 m), and thus fed on a variety of bottom invertebrates (including polychaetes, crustaceans, and molluscs) and fishes. The prey diversity observed in the shark stomachs in our study was lower than that found in previous studies of stomach contents in this species (Olaso et al. 1998, 2005, Rodriguez-Cabello et al. 2007, Valls et al. 2011, Filipe et al. 2012), which could be due in part to our low sample size. However, this species appears to have low variability in its diet with the same principal prey taxa. As well, Filipe et al. (2012) found a stable cumulative trophic diversity from 30 to 40 stomachs sampled, which is both in the range of stomachs sampled and consistent with our cumulative prey curve. None of these studies were carried out in the North Sea, but we found the same principal types of prey (fish, Decapoda crustaceans, and molluscs).

Using our  $\Delta$ -diet isotope value relationships for plasma and RBC, specific DTDFs were generated for each potential prey of the wild-caught *Scyliorhinus canicula* and used to generate isotope values for incorporation in mixing models (Table 2). These models confirmed our and previous stomach content results, indicating high levels of invertebrate consumption, especially of crustaceans (Decapoda). However, we found differences in the prey proportions that were estimated from muscle versus plasma isotopes. Plasma results, which represent a shorter time scale (170 to 476 d based on  $t_{95\%}$ ), showed a higher proportion of fish in the diet than results from muscle. We do not have a turnover estimate for muscle, but estimates from other studies have suggested a higher turnover rate (>400 d; MacNeil et al. 2006, Logan & Lutcavage 2010a, Kim et al. 2012b). This recent trophic shift could confirm the size-related

dietary variability observed in this species (Olaso et al. 1998, 2005, Rodriguez-Cabello et al. 2007); when *S. canicula* is growing, it decreases consumption of crustaceans and increases that of fish. Individuals caught in our study were in the range of NE Atlantic maturity size (52 to 65 cm and 49 to 55 cm for females and males, respectively; Ellis & Shackley 1997) and our sampling was outside the egg-laying period established during the summer (Capapé et al. 1991, Ellis & Shackley 1997), which would suggest the animals sampled were mature. Thus, the isotopic model results show that the sharks had probably recently undergone a diet shift.

#### **Caveats in applying stable isotopes in the study of sharks**

Although stable isotope analysis is a powerful tool when used to understand trophic levels, it is not without limitations and potential problems. First, currently this technique should be associated with traditional diet analysis (of stomach contents) if the goal is to identify specific prey. The uncertainty around appropriate DTDFs could lead to false conclusions, and the use of different DTDFs will result in very different results (e.g. Caut et al. 2008, Hussey et al. 2010a; Fig. 6). Second, if the shark species studied move between areas with different baseline  $\delta^{15}\text{N}$  or available prey, their tissues will never reach isotopic equilibrium with each habitat's local prey (based on our and other turnover rate estimates which suggest that it takes approximately 0.5 to 1.5 yr to approach equilibrium); instead, their tissues will reflect their average diet over the time of turnover. Thus, turnover makes interpreting resource choices at a given point in time challenging, but can provide a broad-scale perspective to the feeding ecology of the species. Indeed, it represents the diet over the period of tissue turnover and not only that during the sampling period (e.g. stomachs). Third, as we have done in this study, it is important to focus on the most important potential prey species, because it is difficult or impossible to make conclusions regarding the consumption of specific prey items when a large number of prey with similar stable isotope values are present (Caut et al. 2008).

Stable isotopes in sharks should be assessed with caution, especially if dietary shifts occur over short time scales. Thus, the type of predator tissue used defines the time scale of the phenomenon studied. Plasma tissue could be used to interpret dietary shifts over the scale of a year, while muscle tissue reflects

shifts over many years. However, exceptions may be made if the isotopic amplitude of the phenomenon observed is high and reaching equilibrium is unnecessary to the interpretation of isotopic data (e.g. a trophic shift between prey with clearly different isotopic values). Although stable isotopes have been successfully used in shark species to examine animal origin and movement (e.g. Abrantes & Barnett 2011, Hussey et al. 2011, 2012), it is very difficult to work at a scale of <6 mo (minimum turnover time for the plasma), especially if the difference in isotopic values related to trophic shift is small.

In conclusion, baseline information on the biology of sharks and other heavily exploited species has recently increased. Information on diet and trophic position can contribute to our understanding of species ecology, management plans for commercial stocks, and conservation plans for endangered species (Shiffman et al. 2012). Published data are too often limited to the qualitative determination of stomach contents over a short time period and provide no sense of the relative contribution of each prey species over the integrated assimilation period. Given the opportunistic feeding behaviour of many sharks, stomach content data are usually insufficient to adequately characterize the trophic position of the various species studied, except in rare instances where regular and longer term stomach content data sets are available. Conventional methods are, however, complementary to isotopic analysis, because they provide a taxonomic resolution of diet that is necessary before choosing the diet composition of the consumer for isotope mixing models (Caut et al. 2008). Thus, the optimal approach is to combine isotopic analysis with conventional methods. However, the shark-specific patterns of isotopic incorporation (higher turnover and variable discrimination factors) may represent an obstacle in trophic interpretations. For example, the use of multi-tissue analyses, which are generally recommended (e.g. Fisk et al. 2002, Kinney et al. 2011), requires information on the turnover rate of each tissue analyzed, the prey consumed during the given time scale, as well as the DTDFs. Moreover, the accurate interpretation of inter-tissue isotopic differences due to amino acid composition, requires careful consideration of which DTDF value to use, and can help to more accurately elucidate the trophic ecology of the study animals. The use of our DTDFs that are scaled to the diet isotope values could be the first step towards more accurate mixing models, especially those utilized for mesopredators, which are known to consume a variety of potential prey with a wide range of isotopic values.

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