Diet- and tissue-specific isotopic incorporation in sharks: applications in a North Sea mesopredator

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14 **ABSTRACT**

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

33

34 **KEYWORDS :** diet; discrimination factor; fractionation; large-spotted dogfish; nitrogen
35 enrichment; SIAR; turnover.

INTRODUCTION

Many species of sharks are apex predators and are believed 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 examination 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 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 require sacrificing the animal and there are several sources of bias when estimating the proportions of dietary components based on stomach contents, including the rapid digestion of soft-bodied prey and empty stomachs. 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).

58 Analyses of the proportional abundance of stable isotopes of various elements in the
59 different tissues of consumers and their potential prey have been used as an alternative approach
60 to traditional dietary analyses (*e.g.* Hobson & Clark 1992*a*, 1992*b*). This approach is based on
61 the fact that stable isotopic ratios of nitrogen ($^{15}N/^{14}N$, expressed as $\delta^{15}N$) and carbon ($^{13}C/^{12}C$,
62 expressed as $\delta^{13}C$) in consumer tissues reflect those of their prey in a predictable manner. Values
63 of $\delta^{13}C$ in organisms generally reflect the original source of carbon at the base of the food web
64 (Kelly 2000). Values of $\delta^{15}N$ increase with each trophic level, because organisms preferentially
65 excrete the lighter nitrogen isotope. The values of $\delta^{15}N$ and $\delta^{13}C$ provide a general and integrated
66 estimate of the trophic level at which the species feeds; however, they usually do not provide the
67 specific dietary information revealed by conventional diet analyses.
68 Despite the widespread use of stable isotopes, there are caveats and assumptions
69 associated with employing them to study feeding ecology (Caut et al. 2008, Martínez del Rio et
70 al. 2009). First, the change in isotopes between prey and consumer is not always consistent; this
71 difference between the stable isotope composition of an animal's tissue and that of its diet is the
72 diet-tissue discrimination factor (DTDF or $\Delta^{15}N$ or $\Delta^{13}C$). The DTDF can vary depending on a
73 consumer's nutritional status, lipid content, quality of the diet consumed, size, age, dietary
74 ontogeny, and the tissue and elemental/isotopic composition of both consumer and diet (reviews:
75 Vander Zanden & Rasmussen 2001, Post 2002, McCutchan et al. 2003, Vanderklift & Ponsard
76 2003, Robbins et al. 2005, Caut et al. 2009). Accurate DTDFs are critical for most uses in
77 ecology, for example as input parameters in isotopic mixing models used for diet reconstruction
78 and trophic position estimates (Phillips 2001, Post 2002). Variability in these parameters has
79 been shown to play a key role in the interpretation of results, especially due to the sensitivity of
80 the models to these parameters (e.g. Caut et al. 2008, Husley et al. 2010*a*). Second, when using

81 stable isotopes for dietary analyses, it is important to understand the sampled tissue's turnover
82 rate, or the time it takes for the isotope to be assimilated therein, to determine the time frame (i.e.
83 days to years) that is represented by the isotopic signature of the tissue. This turnover time
84 generally varies with tissue type and can provide different temporal estimates of diet or feeding
85 ecology (MacNeil et al. 2006).

86 The uncertainty around DTDFs and turnover rates of stable isotopes, along with other
87 factors, has resulted in numerous calls for laboratory experiments to determine DTDFs and
88 turnover rates (Caut et al. 2008, Martínez del Rio et al. 2009). Although Fisk et al. (2002)
89 pointed out the need for such research in sharks, only five controlled studies have been published
90 (Hussey et al. 2010*b*, Logan & Lutcavage 2010*a*, Kim et al. 2012*ab*, Malpica-Cruz et al. 2012).
91 Due in large part to the difficulties of maintaining sharks in captivity for a significant length of
92 time, these authors often used an opportunistic sampling methodology that relied on the tissue
93 samples available, and thus their ability to calculate some of the required parameters is limited.
94 Using four aquarium sharks that had been euthanized for medical reasons, Hussey et al. (2010*b*)
95 modeled the average isotope value of the sharks' diet based on the different proportions of food
96 given to them over the preceding year and the isotopic values of their prey. Logan and Lutcavage
97 (2010*a*) collected juvenile sandbar sharks (n = 5) and monitored blood and muscle isotopic
98 values over a short period of time: during a pre-shift isotopic stabilization period of 2 weeks and
99 a feeding experiment of 46-55 days. Kim et al. (2012*a*) monitored isotopic values of the blood
100 and muscle of three leopard sharks for over 1000 days, but unfortunately lacked any estimation
101 of tissue turnover. Finally, Malpica-Cruz et al. (2012) calculated isotopic incorporation in
102 neonate to young-of-the-year leopard sharks consuming an artificial diet: commercial fish

103 pellets. These studies reported isotopic incorporation rates that varied between tissues and with
104 diet type. Clearly, there is a need for more controlled studies on isotope dynamics in sharks.
105 Studies investigating the feeding ecology of sharks, especially those of species in decline
106 or susceptible to the activities of commercial fisheries (Ferretti et al. 2008, Hisano et al. 2011),
107 will probably continue to increase in the coming years. In this paper, we first experimentally
108 quantify $\Delta^{15}N$ or $\Delta^{13}C$ and isotope turnover rates in different tissues of a mesopredator shark
109 *Scyliorhinus stellaris*, fed two diets with different $\delta^{15}N$ and $\delta^{13}C$ values (fish or mussel) for 240
110 days; recent evidence has shown strong relationships between $\delta^{15}N$ and $\delta^{13}C$ values in diet and
111 the DTDF value (Overmyer et al. 2008, Dennis et al. 2010; see review Caut et al. 2009). These
112 DTDFs where then used to interpret isotope data obtained for the small-spotted catshark
113 (*Scyliorhinus canicula*) from the North Sea. The results of isotope mixing models were
114 compared to stomach content data to assess the accuracy of the experimentally derived DTDFs,
115

116 # MATERIALS AND METHODS

117 **Laboratory experimental design**

118 Firstly, we aimed to estimate the isotopic incorporation (discrimination factors and turnover) in
119 different shark tissues to verify if there was a relationship between discrimination factors and
120 diet isotopic values, as recently reviewer in Caut et al. 2009. This could have important effects in
121 the isotopic model output and interpretation of such. For that, twenty-six male, 2 year old large-
122 spotted dogfish (*Scyliorhinus stellaris*, length 50.08±1.15 cm (mean ± SD) and weight
123 619.04±44.20 gr) were held for 12 months on a constant diet prior to the experiments, at the
124 Liege Aquarium-Museum (Belgium); all were born at the Aquarium. Dogfish were randomly
125 divided into two dietary treatments with different isotopic values, fish (smelt *Osmerus eperlanus*

126 (S)) or mussel (*Mytilus edulis* (M)) diet; individuals were each fed 30 grams three times per
127 week. The dogfish in each treatment were placed in a large aquarium separated by a transparent
128 plastic window with an exchange of filtered water that maintained the same water conditions.
129 After 120 days, four dogfish from both treatments were sacrificed using a lethal dose of tricaine
130 methanesulfonate and sampled as above (S_{120} and M_{120}), six dogfish were switched from the S to
131 M diet ($S_{120}M_{120}$) and six were switched from the M to S diet ($M_{120}S_{120}$); they consumed the new
132 diet for an additional 120 days. Three dogfish in each treatment continued on the same diet for
133 240 days (M_{240} and S_{240}). Thus, we have used two long-term treatments with two different diet
134 isotopic values (M_{240} and S_{240}) to estimate precisely the isotopic incorporation. For the diet shift,
135 we hypothesized that an isotopic equilibrium was possible after 120 days. Thus we aimed to
136 compare the incorporation dynamics between different initial isotopic values. If the isotopic
137 equilibrium was not achieved after 120 days, we could not calculate the DTDFs, but the diet
138 switch provided insights into the turnover rates of the different diets.

139 Blood samples were taken and length and mass were measured at the start of the
140 experiment and every 15 days for all individuals. Blood was obtained from the sinus vein (after
141 anaesthesia with tricaine MS-222) using blood-collection kits (syringe 5ml + needle 12.7 x 31;
142 WWR, France). The blood sample was immediately separated into red blood cells (RBC) and
143 plasma components by centrifugation. At the end of the experiment (day 240), four dogfish from
144 both treatments were sacrificed using a lethal dose of tricaine methanesulfonate (MS-222) and
145 plasma, RBC, muscle, and fin were sampled. The isotope values of the diets were quantified for
146 each treatment; samples were randomly taken from the stock throughout the experiment. All
147 samples were kept at -20°C until isotopic analysis.
148

149 **Field study procedures and stomach content analysis**

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

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

159 Stomach contents were removed and preserved in alcohol (70%) for later identification to
160 the lowest taxonomic level possible using a set of references for several taxonomic groups
161 developed during the commercial trawl haul (including fish otoliths). The relative importance of
162 each prey item was assessed in two ways: (*i*) the numerical index (NI), i.e. the percentage of each
163 prey item relative to the total number of prey items (number of individuals in a prey category /
164 total number of individuals among all prey categories × 100); (*ii*) the occurrence index (OI), i.e.
165 the percentage of each prey item in all non-empty stomachs (number of stomachs containing a
166 prey category / total number of stomachs containing prey × 100). A cumulative prey curve was
167 constructed to assess the adequacy of the number of stomachs sampled. The order of stomachs
168 was randomized 10 times, and the mean ± SE of unique prey items was plotted to minimize a
169 possible bias resulting from the sampling order. The point at which the prey curve achieved an
170 asymptote identified the number of stomachs needed (Ferry et al. 1997). Identifiable prey items

171 that were in good condition were kept at -20º C until isotopic analysis to increase the prey
172 database.
173
174 **Isotopic analyses**
175 Shark tissues, food and prey items (including those collected from stomachs contents) were
176 freeze-dried and ground to a fine powder. For shark muscle, we compared isotopic values before
177 and after lipid extraction. Lipid extraction was performed by rinsing samples with a 2:1
178 chloroform:methanol solvent and then drying them at 60°C for 24 h to remove any residual
179 solvent. Extraction of lipids was not necessary for blood samples because the lipid component in
180 blood is generally low (Caut et al. 2011). For all fish species, we mixed the whole body of the
181 specimen and selected a homogenized subsample. For bivalves, gastropods, and hermit crabs, the
182 shells were removed before analysis. Isotopic analyses were performed on 1 mg subsamples of
183 homogenized materials loaded into tin cups.
184 Stable carbon and nitrogen isotope measurements were carried out using a continuous
185 flow isotope ratio mass spectrometer (Optima, Micromass, UK) coupled to a C-N-S elemental
186 analyser (Carlo Erba, Italy). Stable C and N isotope ratios are expressed as: $\delta^{13}C$ or $\delta^{15}N$=
187 $[(R_{sample}/R_{standard})-1]x1000$, where R is $^{13}C/^{12}C$ or $^{15}N/^{14}N$ for $\delta^{13}C$ or $\delta^{15}N$, respectively. $R_{standard}$ is
188 the ratio of the international references PDB for carbon and AIR for nitrogen. One hundred
189 replicate assays of internal laboratory standards indicate maximum measurement errors (SD) of ±
190 0.20‰ and ± 0.15‰ for $\delta^{13}C$ or $\delta^{15}N$ measurements, respectively.
191
192 **Isotopic turnover and DTDF**

For the two treatments continued on the same diet for 240 days (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:

$y = a + be^{ct}$

Where y is $\delta^{13}C$ or $\delta^{15}N$, 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 ($\delta X_{(\infty)} - \delta X_{(t)}$), 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 α % turnover, we solved the equation (Tieszen et al. 1983):

$T = \ln (1 - \alpha / 100) / c$

Where T is the time in days, α is % 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.

Diet-tissue discrimination factors between a food resource (*food*) and a consumer (*shark*) are described in terms of the difference in delta (δ) values using the Δ notation, where DTDF (Δ) = $X_{(\infty)\ shark}$ (obtained by the fitted model) – X_{food}, where X is $\delta^{13}C$ or $\delta^{15}N$ and were only calculated for sharks held on the same diet for 240 days (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

216 is a weighted combination of the food sources' isotopic values. The weights are made up of
217 dietary proportions (which are given a Dirichlet prior distribution) and the concentration
218 dependencies given for the different food sources. The standard deviation is divided between the
219 uncertainty around the discrimination corrections and the natural variability between target
220 individuals (for more information see Jackson et al. 2008; Moore and Semmens 2008; Parnell et
221 al. 2010). Throughout this paper, the mean dietary proportions from isotope analyses will be
222 followed by their 95% confidence interval, noted C.I. To represent the sharks, we used plasma
223 and muscle tissues because the turnover rates of stable isotopes are different for each, reflecting a
224 short and longer assimilation time, respectively (MacNeil et al. 2006). Isotopic models typically
225 use the mean $\delta^{13}C$ and $\delta^{15}N$ values for each type of diet, corrected by the DTDF. To build our set
226 of different potential prey species, we used isotope values for prey species found in the stomach
227 contents and added values for other species from the literature (Kaiser & Spencer 1994, Olaso et
228 al. 1998, Olaso et al. 2005, Valls et al. 2011, Filipe et al. 2012) to limit the bias due to the
229 sampling size of the stomach analysis. We grouped the different prey species according to taxa
230 and type of consumer (e.g., detritivores) for isotopic model analysis. Because lipids were not
231 extracted from the prey species, we used the general correction for lipid content for aquatic
232 species when the C:N ratio of the tissue being analyzed was > 3.5 (following Post et al. (2007)'s
233 equation: $\delta^{13}C_{normalized} = \delta^{13}C_{untreated} - 3.32 + 0.99\ C{:}N$).

234 Diet tissue discrimination factors depend on several sources of variation (e.g. taxon,
235 environment and tissue). Previous laboratory work had shown significant relationships between
236 $\delta^{13}C$ and $\delta^{15}N$ of diets and the corresponding $\Delta^{15}N$ and $\Delta^{13}C$ of the different tissues of consumers
237 fed on these diets (e.g. reviewed in Caut et al. 2009). Thus, $\Delta^{13}C$ and $\Delta^{15}N$ of plasma and muscle
238 were calculated for each dietary item using regressions between shark $\Delta^{13}C$ and $\Delta^{15}N$ and the

239 corresponding dietary isotopic ratios; these regressions utilized experimental data from our and
240 three other studies on sharks fed a known natural diet (Hussey et al. 2010*b*, Kim et al. 2012*ab*
241 following Caut et al. 2008). Moreover, we ran a SIAR mixing model using the common fish
242 Fixed Discrimination Factors (FDF) of 1‰ for $\delta^{13}C$ and 3.2‰ for $\delta^{15}N$ (Post *et al. 2007*) and
243 compared the outputs with the run of the model using the DTDFs estimated with our regressions.
244
245 **Statistical analyses**
246 We performed Generalized Linear Models to test (*a*) the effect of lipid extraction on the isotopic
247 ratios of shark muscle (captive (*S. stellaris*) and wild individuals (*S. canicula*)) and the two diets
248 (M and S) - values resulting from lipid extraction are noted hereafter as $_{DEL}$; (*b*) the isotopic
249 difference between the two control diets; (c) the effect of the two control diets on the body mass
250 growth; (*d*) the effect of sex and body mass on the isotopic values of *S. canicula*; (*e*) difference
251 in isotope values between tissues (plasma and muscle) in both captive and wild individuals.
252 To compare the isotopic ratios of each tissue (muscle and fin) among the two groups
253 having consumed the same diet (M_{120} vs. M_{240} and S_{120} vs. S_{240}), we performed pairwise
254 comparisons using Kruskal-Wallis nonparametric tests (hereafter KW).
255 Computations were performed with STATISTICA 6.0 (StatSoft Inc 2001) and isotopic
256 incorporation data were fitted using a Marquardt non-linear fitting routine (NLIN, SAS, Cary,
257 NC, USA). The level of significance for statistical analysis was set at p=0.05.
258
259 # RESULTS
260 **Experimental study**
261 *Stable isotopes of the control diets (Smelt and Mussel)*

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

Blood isotopic incorporation

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

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

285

286 *Muscle and fin isotopic incorporation*

287 Lipid extraction had no significant effect on the $\delta^{15}N$ and $\delta^{13}C$ values of muscle (LM: $\delta^{13}C$, $F_{1,50}$
288 = 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
289 ratio (C/N = 2.81 ± 0.01, n = 26). We did not perform lipid extraction on fin samples because
290 their C/N ratio was also very low (fin: C/N = 2.53 ± 0.01).

291 A comparison of $\delta^{15}N$ and $\delta^{13}C$ in the three tissues (muscle, fin, and whole blood) at 120
292 and 240 days for individuals fed the same diet revealed a different trend for the M and S diets. In
293 the S diet treatment, there were significant differences between the S_{120} and S_{240} groups in $\delta^{15}N$
294 and $\delta^{13}C$ for fin, but no difference was found for muscle (Table 1C). In contrast, in the M diet
295 treatment, there were no significant differences between the M_{120} and M_{240} groups in $\delta^{15}N$ and
296 $\delta^{13}C$ for any of the tissues, except for muscle $\delta^{13}C$ (Table 1C).

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

306

307 **Field study**

308 *Wild shark isotopic values*

309 Over the 67 total hauls, 255 small-spotted catsharks (*Scyliorhinus canicula*) were caught
310 (Fig 1). In total, 39 individuals of *S. canicula* (10♂ and 29♀) were sampled for isotopes and
311 stomach contents with a mean total length and mass of 505 ± 14 mm and 545 ± 41 g,
312 respectively. Among them, 20.5% of the sharks sampled had empty stomachs.

313 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
315 ratio (C/N = 2.74 ± 0.02). Similarly, the C/N ratio of plasma was lower than that of muscle,
316 which meant that no lipid extraction of this tissue was necessary (C/N = 1.48 ± 0.06). There were
317 no significant effects of mass and sex on $\delta^{13}C$ and $\delta^{15}N$ for *S. canicula* ($\delta^{13}C_{MUSCLE}$: mass $F_{1,36} = 1.35$, $P = 0.253$ and sex $F_{1,36} = 1.23$, $P = 0.274$; $\delta^{15}N_{MUSCLE}$: mass $F_{1,36} = 2.78$, $P = 0.104$ and sex
319 $F_{1,36} = 3.06$, $P = 0.089$; $\delta^{13}C_{PLASMA}$: mass $F_{1,36} = 0.82$, $P = 0.371$ and sex $F_{1,36} = 0.86$, $P = 0.361$;
320 $\delta^{15}N_{PLASMA}$: mass $F_{1,36} = 2.30$, $P = 0.138$ and sex $F_{1,36} = 0.77$, $P = 0.386$). However, there was a
321 significant difference between muscle and plasma isotope values ($\delta^{13}C$: $F_{1,76} = 21.24$, $P < 0.001$;
322 $\delta^{15}N$: $F_{1,76} = 43.01$, $P < 0.001$), with muscle having higher $\delta^{15}N$ but lower $\delta^{13}C$ (*S. canicula*:
323 $\delta^{13}C_{MUSCLE} = -16.25$ (0.10) ‰, $\delta^{15}N_{MUSCLE} = 16.11$ (0.14) ‰ vs. $\delta^{13}C_{PLASMA} = -15.47$ (0.18) ‰,
324 $\delta^{15}N_{PLASMA} = 14.87$ (0.15) ‰).

325

326 *Conventional diet analysis*

327 The cumulative prey curve for *Scyliorhinus canicula* reached a well-defined asymptote,
328 indicating that sample size was sufficient to adequately describe the diet (Fig. 3). *S. canicula* had
329 a varied diet based on stomach contents, which was composed of 17 different taxa belonging to 5
330 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, more details see Appendix 1). Teleostei was predominantly represented by two 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 one species, *Buccinum undatum*, which was the second most important prey species after *Liocarcinus depurator* (Appendix 1).

Isotopic diet analysis

Eighty-two different prey items of 6 different orders were caught over a total of 63 hauls (Fig 1, see Appendix 2). We used previous papers (see materials and methods section) and stomach content data from collected sharks to choose likely prey items for isotope analysis and inclusion into the isotope mixing models (see Table 2 and Appendix 1 for list of species). Most of these were collected from trawls but some were from stomach contents (e.g., two different species of Annelida noted [1] and [2]).

Strong significant regressions were found relating shark tissue (plasma and muscle) $\Delta^{13}C$ and $\Delta^{15}N$ to the corresponding dietary isotopic values from controlled natural diet experiments with sharks ($\Delta C_{PLASMA} = -0.12\delta^{13}C + 0.65$, $R^2 = 0.83$; $\Delta C_{MUSCLE} = -0.50\delta^{13}C - 7.87$, $R^2 = 0.82$; $\Delta N_{PLASMA} = -0.37\delta^{15}N + 6.94$, $R^2 = 0.98$; $\Delta N_{MUSCLE} = -0.65\delta^{15}N + 10.82$, $R^2 = 0.82$; Fig. 5). These regression equations allowed for the estimation of $\Delta^{13}C$ and $\Delta^{15}N$ 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 *S. canicula* were found (Fig. 4). Using plasma, the model suggested three principal resources

(mean %): Teleostei (36%), Brachyura (23%), and Annelida^{2} (21%). In contrast, when muscle was used, Caridae (31%), Annelida^{1} (19%), and Teleostei (12%) constituted the majority 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 of the importance of Annelids for both tissues (Fig. 4). Moreover, when we run the SIAR mixing model using the common fish Fixed Discrimination Factors (FDF) 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^{2} (19%) and an underestimation of the contribution of Caridae (5%) and Annelida^{1} (6%). In contrast, when muscle was used, the FDF model strongly overestimated Annelida^{2} (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 been shown to be variable across tissues, species, and dietary isotopic values (Caut et al. 2009, Martnez 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 (Logan & Lutcavage 2010*ab*, Hussey et al. 2010*a*). Because of the

377 unique physiology of sharks, in particular urea retention in tissues for osmoregulation, the
378 estimation of shark-specific DTDFs is even more imperative (Fisk et al. 2002, Hussey et al.
379 2012).

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

394 Previous studies have also found that DTDFs increase with protein content (Pearson et al.
395 2003) and decrease with protein quality (Florin et al. 2010, see quality or quantity hypothesis,
396 Caut et al. 2010). The variation in DTDFs in our study may also be explained by differences in
397 the protein quantity and quality between the invertebrate (M diet) and fish (S diet) used (%N = 8
398 for Mollusca *vs* 12 for fish in wild caught samples; see Appendix 2). Given the strength of
399 DTDF-diet isotope value relationships found across studies that included invertebrate and fish

400 diet items, we feel this relationship is more important. Regardless, these relationships are based
401 on animals that are the potential prey consumed by elasmobranch mesopredators, in the natural
402 environment.

403 In addition to using appropriate DTDFs, it is important to consider the turnover rate of
404 isotopes in different tissues so that the time scale can be considered when interpreting the trophic
405 ecology of the predator. Previous studies on elasmobranch turnover rates estimated that complete
406 nitrogen and carbon turnover differed among tissues, ranging from a minimum of approximately
407 6 months for plasma, 8 months for whole blood, and more than two years for muscle (MacNeil et
408 al. 2006, Logan & Lutcavage 2010*a*, Kim et al. 2012*b*, Malpica-Cruz et al. 2012). Although the
409 physiology of the species and experimental conditions (e.g., temperature) used in this study
410 could be different (e.g., metabolism or size), the turnover rates were in the same range of these
411 previous studies and followed the classical tissue gradient of plasma < RBC < muscle. Moreover,
412 the difference in turnover rate between diets, depends probably on the direction and isotopic
413 amplitude of the diet shift (moving to a lower or higher isotope value), as observed in other
414 studies (e.g. MacNeil et al. 2006, Caut et al. 2011).

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

423 for 240 days (longest time), because the exponential models fitting isotopic incorporation in
424 tissues are extremely sensitive to the duration of the experiment. Indeed, we observed differences
425 between the exponential fit results at 120 days and at 240 days (S_{120} vs. S_{240} or M_{120} vs. M_{240}).
426
427 **Application of diet and tissue-specific DTDFs in mesopredators**
428 Although mesopredators play a key role in marine ecosystems, many isotopic studies focus on
429 top predators, probably because such species are more appealing and challenging to study with
430 traditional methods. Mesopredators link different food webs and trophic levels in marine
431 ecosystems, contributing to system dynamics and stability (Matich et al. 2011). *S. canicula* was
432 caught mainly near the coast and in shallow water (~ 40m), and thus fed on a variety of bottom
433 invertebrates (including polychaetes, crustaceans, and molluscs) and fishes. The prey diversity
434 observed in the shark stomachs in our study was lower than that found in previous studies of
435 stomach contents in this species (Olaso et al. 1998, 2005, Rodriguez-Cabello et al. 2007, Valls et
436 al. 2011, Filipe et al. 2012), which could be due in part to our low sample size. However, this
437 species appears to have low variability in its diet with the same principal prey taxa. As well,
438 Filipe *et al.* (2012) found a stable cumulative trophic diversity from 30-40 stomachs sampled
439 which is both in the range of stomach sampled and consistent with our cumulative prey curve.
440 None of these studies were carried out in the North Sea, but we found the same principal types of
441 prey (fish, Decapoda crustaceans, and molluscs).
442 Using our Δ-diet isotope value relationships for plasma and RBC, specific DTDFs were
443 generated for each potential prey of the wild caught *S. canicula* and used to generate isotope
444 values for incorporation in mixing models (Table 2). These models confirmed our and previous
445 stomach content results, indicating high levels of invertebrate consumption, especially of

446 crustaceans (Decapoda). However, we found differences in the prey proportions that were
447 estimated from muscle versus plasma isotopes. Plasma results, which represent a shorter time
448 scale (170 to 476 d based on $t_{95\%}$), showed a higher proportion of fish in the diet than results
449 from muscle. We do not have a turnover estimate for muscle, but estimates form other studies
450 have suggested higher turnover rate (> 400 days, MacNeil et al. 2006, Logan & Lutcavage
451 2010*a*, Kim et al. 2012*b*). This recent trophic shift could confirm size-related dietary variability
452 observed in this species (Olaso et al. 1998, 2005, Rodriguez-Cabello et al. 2007); when *S.*
453 *canicula* is growing, it decreases consumption of crustaceans and increases that of fish.
454 Individuals caught in our study were in the range of Northeast Atlantic maturity size (52-65 cm
455 and 49-55 cm for females and male, respectively; Ellis and Shackley 1997) and our sampling
456 was outside the egg laying period established during the summer (Capapé et al. 1991; Ellis and
457 Shackley 1997), which would suggest the animals sampled were mature. Thus, the isotopic
458 model results show that the sharks had probably recently undergone a diet shift.
459
460 **Caveats in applying stable isotopes in the study of sharks**
461 Although stable isotope analysis is a powerful tool when used to understand trophic levels, it is
462 not without limitations and potential problems. First, currently this technique should be
463 associated with traditional diet analysis (of stomach contents) if the goal is to identify specific
464 prey. The uncertainty around appropriate DTDFs could lead to false conclusions, and the use of
465 different DTDFs will result in very different results (e.g. Caut et al. 2008, Hussey et al. 2010*a*;
466 Fig 6). Second, if the shark species studied move between areas with different baseline $\delta^{15}N$ or
467 available prey, their tissues will never reach isotopic equilibrium with each habitat's local prey
468 based on our and other turnover rate estimates which suggest approximately 0.5 to 1.5 years to

469 approach equilibrium; instead, their tissues will reflect their average diet over the time of
470 turnover. Thus, turnover makes interpreting resource choices at a given point in time challenging
471 but can provide a broad scale perspective to the feeding ecology of the species. Indeed, it
472 represents the diet over the period of tissue turnover and not only that during the sampling period
473 (e.g., stomachs). Lastly, as we have in this study, it is important to focus on the most important
474 potential prey species, because it is difficult or impossible to make conclusions regarding
475 consumption of specific prey items when a large number of prey with similar stable isotope
476 values are present (Caut et al.2008).

477 Stable isotopes in sharks should be assessed with caution, especially if dietary shifts
478 occur over short time scales. Thus, the type of predator tissue used defines the time scale of the
479 phenomenon studied. Plasma tissue could be used to interpret dietary shifts over the scale of a
480 year, while muscle tissue reflects shifts over many years. However, exceptions may be made if
481 the isotopic amplitude of the phenomenon observed is high, and reaching equilibrium is
482 unnecessary to the interpretation of isotopic data (e.g., a trophic shift between prey with clearly
483 different isotopic values). Although stable isotopes have been successfully used in shark species
484 to examine animal origin and movement (e.g., Abrantes & Barnett 2011, Hussey et al. 2011,
485 2012), it is very difficult to work at a scale of less than six months (minimum turnover time for
486 the plasma), especially if the difference in isotopic values related to trophic shift is small.

487 In conclusion, baseline information on the biology of sharks and other heavily exploited
488 species has recently increased. Information on diet and trophic position can contribute to our
489 understanding of species ecology, management plans for commercial stocks, and conservation
490 plans for endangered species (Shiffman et al. 2012). Published data are too often limited to the
491 qualitative determination of stomach contents over a short time period and provide no sense of

492 the relative contribution of each prey species over the integrated assimilation period. Given the
493 opportunistic feeding behaviour of many sharks, stomach content data are usually insufficient to
494 adequately characterize the trophic position of the various species studied, except in rare
495 instances were regular and longer-term stomach content data seta are available. Conventional
496 methods are, however, complementary to isotopic analysis, because they provide a taxonomic
497 resolution of diet that is necessary before choosing the diet composition of the consumer for
498 isotope mixing models (Caut et al. 2008). Thus, the optimal approach is to combine isotopic
499 analysis with conventional methods. However, it is shark-specific patterns of isotopic
500 incorporation (higher turnover and variable discrimination factors) that may represent an
501 obstacle in trophic interpretations. For example, the use of multi-tissue analyses, which are
502 generally recommended (e.g., Fisk et al. 2002, Kinney et al. 2011), requires information on the
503 turnover rate of each tissue analyzed, the prey consumed during the given time scale, as well as
504 the diet-tissue discriminating factors. Moreover, the accurate interpretation of inter-tissue
505 isotopic difference due to amino acid composition, requires careful consideration of which
506 DTDF value to use, and can help to more accurately elucidate the trophic ecology of the study
507 animals. The use of our DTDFs that are scaled to the diet isotope values could be the first step
508 towards more accurate mixing models, especially those utilized for mesopredators, which are
509 known to consume a variety of potential prey with a wide range of isotopic values.
510
511
512 ## Acknowledgements
513 This work was supported by the University of Liège and CSIC (Consejo Superior de
514 Investigaciones Científicas) contracts to S.C. We are grateful to K. Das and E. Parmentier for

515 their scientific advice and comments, Y. Verrin for her scientific assistance with the data set and
516 this permission to sample during the IBTS 2008 campaign, and to C. Michel, director of Liège
517 aquarium, and the Liège aquarium staff for their daily help and permission to sample. We also
518 thank Jessica Pearce-Duvet for her English editing services. All authors have applied appropriate
519 ethics and other approval for the research; S. Caut was authorized for animal experimentation
520 (R-45GRETA-F1-04) by the French Minister of Agriculture.

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658 **Table 1. (A)** Effect of lipid extraction on the nitrogen and carbon isotopic values of the two diet treatments (M and S, values resulting
659 from lipid extraction are noted hereafter as $_{DEL}$). **(B)** Exponential (with R^2) and statistics of convergent equations of stable isotope
660 incorporation in plasma and RBC of dogfish under controlled conditions. Nitrogen and carbon diet tissue discrimination factors (Δ, ‰)
661 and turnover rates ($t_{50\%}$ and $t_{95\%}$) in days in different dogfish tissues (calculated from lipid-extracted control diet samples for $\delta^{13}C$ and
662 non-lipid-extracted samples for $\delta^{15}N$) are listed. **(C)** Difference between the isotopic ratios of each tissue (muscle and fin) among the
663 two groups having consumed the same diet (M_{120} vs. M_{240} and S_{120} vs. $S_{240,}$ Kruskal-Wallis test). Nitrogen and carbon discrimination
664 factors (Δ, ‰) were calculated at time 240 days.

		Nitrogen							Carbon						
(A)															
	Diet	$\delta^{15}N \pm SD$	dn,dd	F	P				$\delta^{13}C \pm SD$	dn,dd	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_{DEL}	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_{DEL}	17.59 ± 0.27‰							-22.26 ± 0.33‰						
(B)															
Tissue	Diet	Equation (R^2)	dn,dd	F	P	Δ	$t_{50\%}$	$t_{95\%}$	Equation (R^2)	dn,dd	F	P	Δ	$t_{50\%}$	$t_{95\%}$
Plasma	M_{240}	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_{240}	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_{240}	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_{240}	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)															
Tissue	Effect		dn,dd	H	P	Δ				dn,dd	H	P	Δ		
Muscle	M_{120} *vs* M_{240}		1,7	1.13	0.289	3.49	M diet			1,7	4.58	0.032	0.52	M diet	
	S_{120} *vs* S_{240}		1,7	2.00	0.157	-1.81	S diet			1,7	0.00	1	4.28	S diet	
Fin	M_{120} *vs* M_{240}		1,6	0.86	0.335	0.49	M diet			1,6	3.43	0.064	1.06	M diet	

S_{120} *vs* S_{240}	1,7	4.50	0.034	-1.95	S diet		1,7	4.50	0.034	5.14	S diet	

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

		ISOTOPIC VALUES			ESTIMATED DTDFs			
Species		*n*	$\delta^{13}C_{DEL}$	$\delta^{15}N$	$\Delta^{13}C_P$	$\Delta^{15}N_P$	$\Delta^{13}C_M$	$\Delta^{15}N_M$
S. caniculata	Muscle	*39*	-16.15 (0.09)	16.11 (0.14)				
	Plasma	*39*	-15.47 (0.18)	14.87 (0.15)				
Items								
Annelida								
	Annelida[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[2]	*1*	-17.43	11.61	2.81	2.53	0.91	3.27
Arthropoda (Decapoda)								
	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)

671 **FIGURE LEGENDS**

672 *Figure 1.* (A) Map of the North Sea, where the annual French International Bottom Trawl Survey
673 of 2008 (1-20 February) was conducted using randomized trawl hauls. One haul was randomly
674 performed in each rectangle; the trawl hauls are represented with white circles. (B) Locations
675 where *Scyliorhinus canicula* were collected, the number next to the black circles is the total
676 number of individuals caught and the "exponent" indicates the number of samples analysed (n =
677 39).

678 *Figure 2.* Nitrogen and carbon isotopic values (mean ± SD) of plasma and red blood cells (RBC)
679 of *Scyliorhinus stellaris* for the different diet treatments: (*i*) $S_{120}M_{120}$ = switch from smelt (S) to
680 mussel (M) diet at 120 days (Diet Shift. *DS*); (*ii*) $M_{120}S_{120}$ = switched from M to S diet at 120
681 days; (*iii*) M_{240} and S_{240} = remained on the same diet (M or S) for 240 days. The diet treatments
682 M_{120} and S_{120} represent the first part of the experiment (0-120 days), before the diet shift occurred
683 (*DS*).

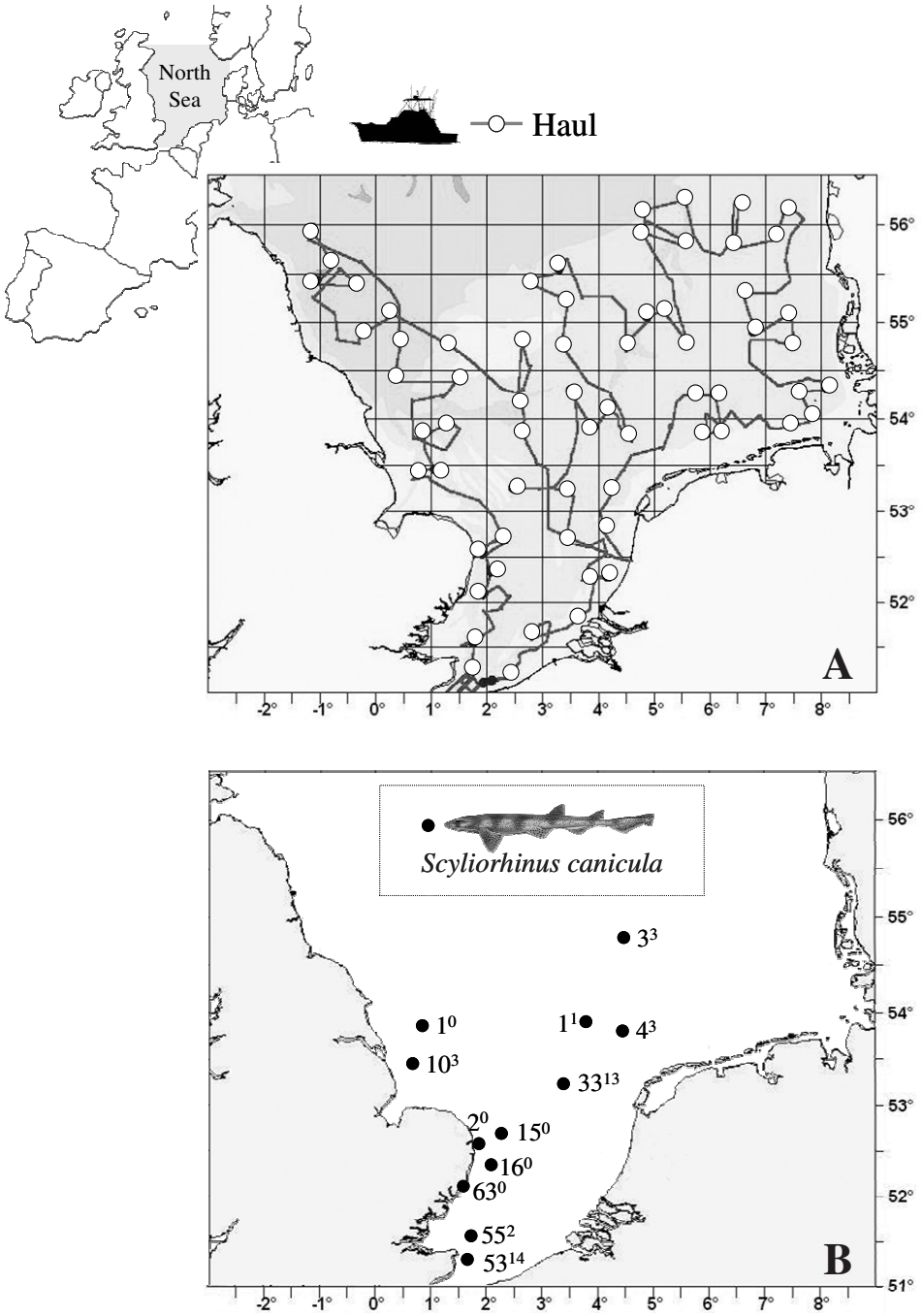
684 *Figure 3*. Randomized cumulative prey curve for *Scyliorhinus canicula*. Mean values of 10
685 randomizations are presented ± SE.

686 *Figure 4.* Proportional contribution of different potential prey to the diets of *Scyliorhinus*
687 *canicula* based on plasma and muscle isotopes (*SIAR* model) and stomach contents (NI:
688 mean±SD). Boxplots (x-axis) show the distribution of possible contributions from each prey
689 source to the diet of *S. canicula* that result from the application of the *SIAR* isotopic model.
690 Values shown are the 25, 75 and 95%, credibility internals respectively for these distributions.
691 Abbreviations for *S. canicula* prey group are as follows: AN^1 Annelida group 1; AN^2 Annelida
692 group 2; ANO Anomura; BRA Brachyura; CAR Caridae; TEL Teleostei; ECH Echinodermata;
693 and MOL Mollusca.

694 *Figure 5*. Relationship between the mean values of (A) nitrogen isotopic ratios ($\delta^{15}N$) and diet
695 tissue discrimination factors ($\Delta^{15}N$) and (B) carbon isotopic ratios ($\delta^{13}C$) and diet tissue
696 discrimination factors ($\Delta^{13}C$) for the different tissues sampled (black = muscle and white =
697 plasma) for laboratory derived DTDFs. The number at the top of each point identifies the shark
698 study (1 = this study, 2 = Kim et al. 2012*a*. 3 = Hussey et al. 2010*b*, and 4 = Kim et al. 2012*b*).
699 Equations, regression coefficients, and fits are shown for the significant models.
700 *Figure 6.* Mean proportional contribution of different potential prey to the diets of *Scyliorhinus*
701 *canicula* based on plasma and muscle isotopes (*SIAR* model) with Fixed Discrimination Factors
702 (FDF, $\Delta^{13}C$ = 1‰ and $\Delta^{15}N$ = 3.2‰) and Diet Tissues Discrimination Factors estimated by
703 regressions (DTDF, Fig 4). Abbreviations for *S. canicula* prey group are as follows: AN^1
704 Annelida group 1; AN^2 Annelida group 2; ANO Anomura; BRA Brachyura; CAR Caridae; TEL
705 Teleostei; ECH Echinodermata; and MOL Mollusca.

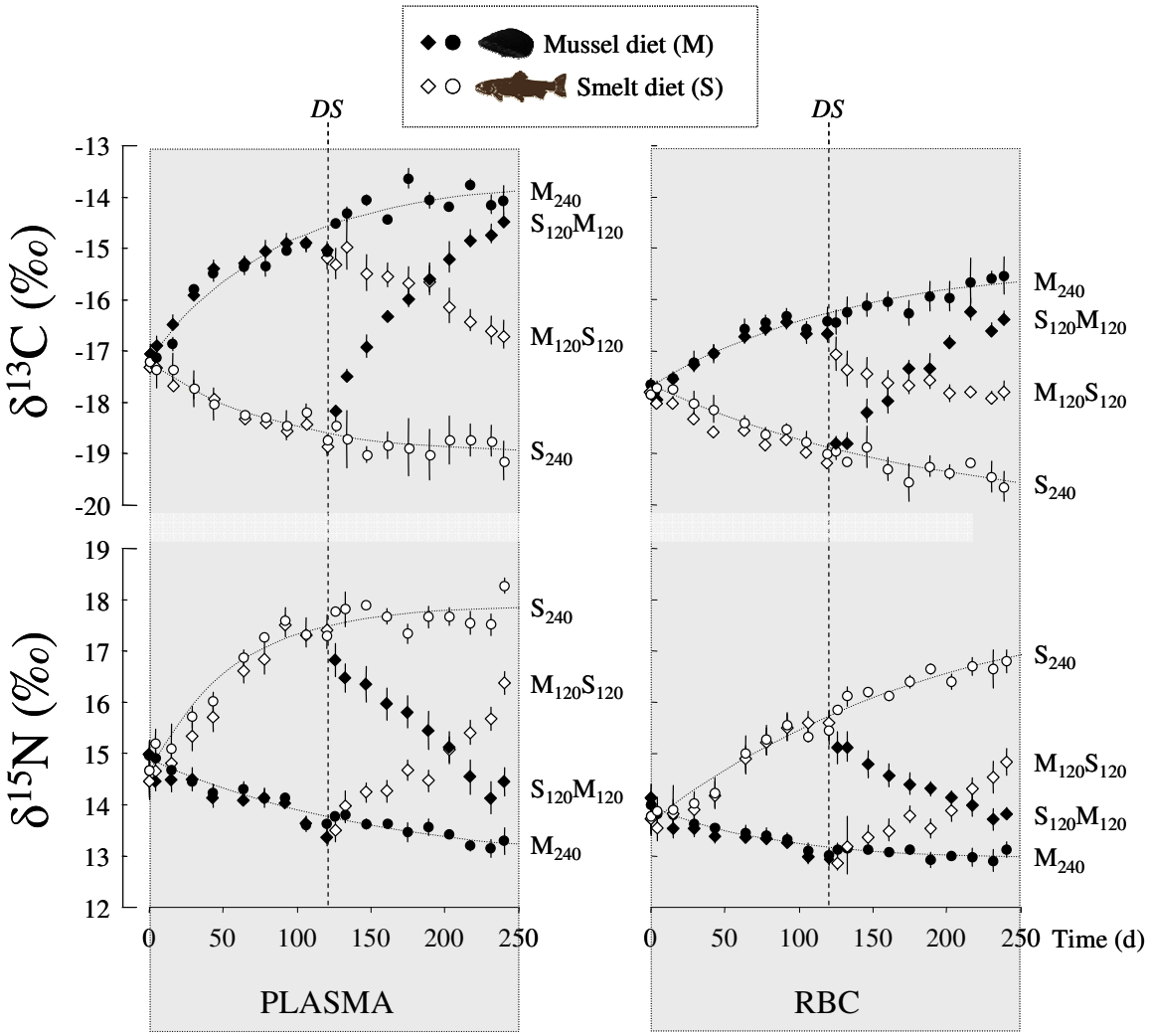
706 Figure 1.

707



708 Figure 2.

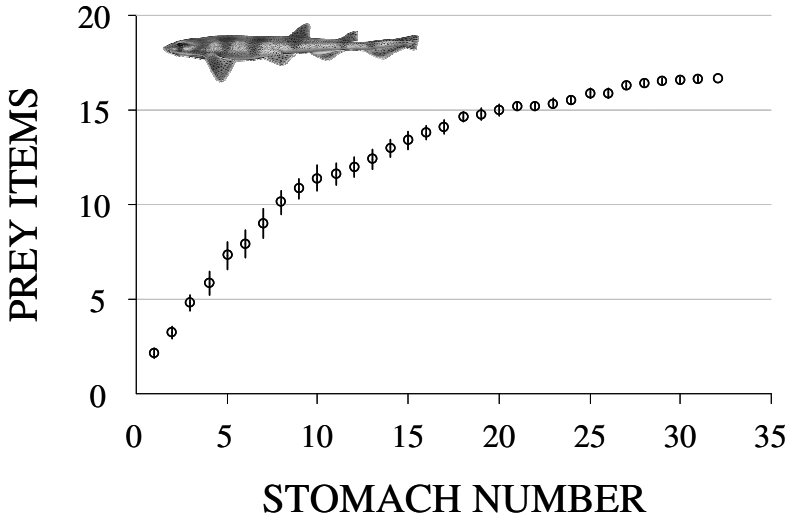
709



710 Figure 3.

711

712



713 Figure 4.

714

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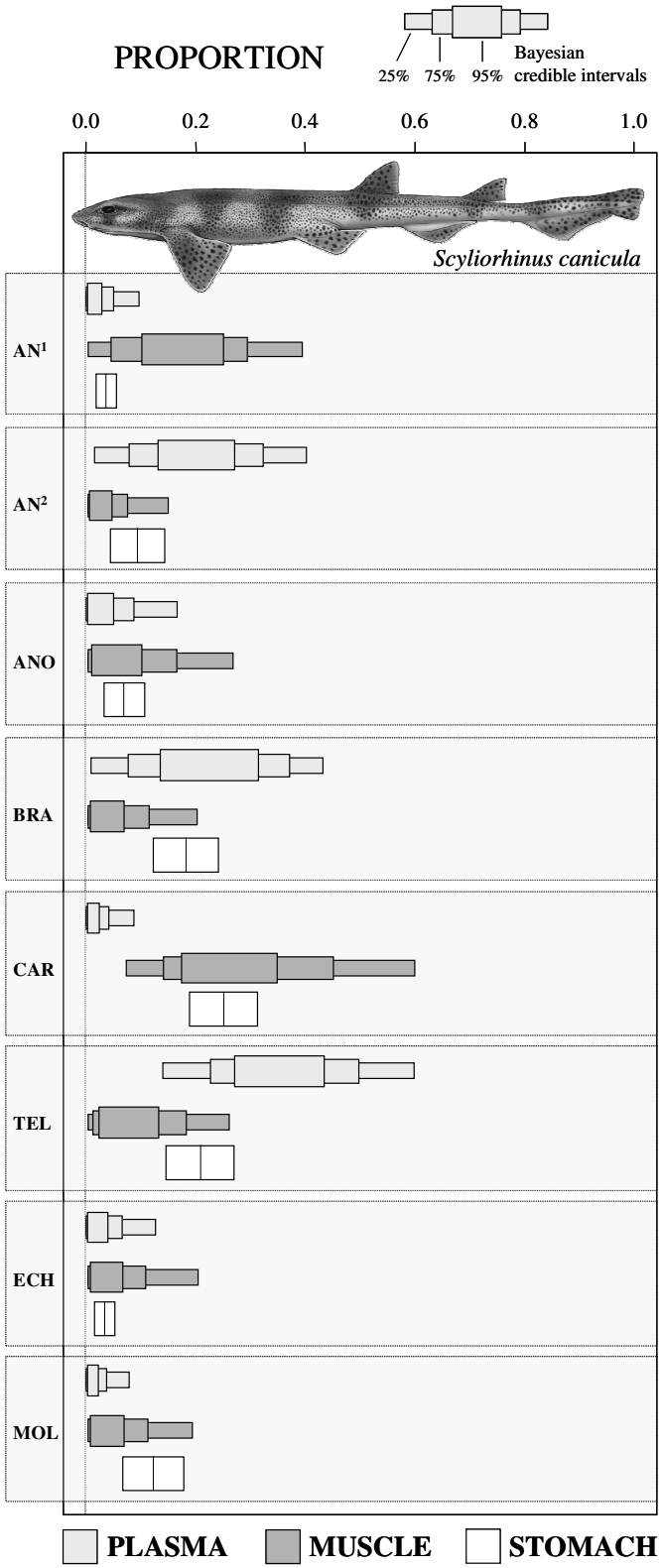
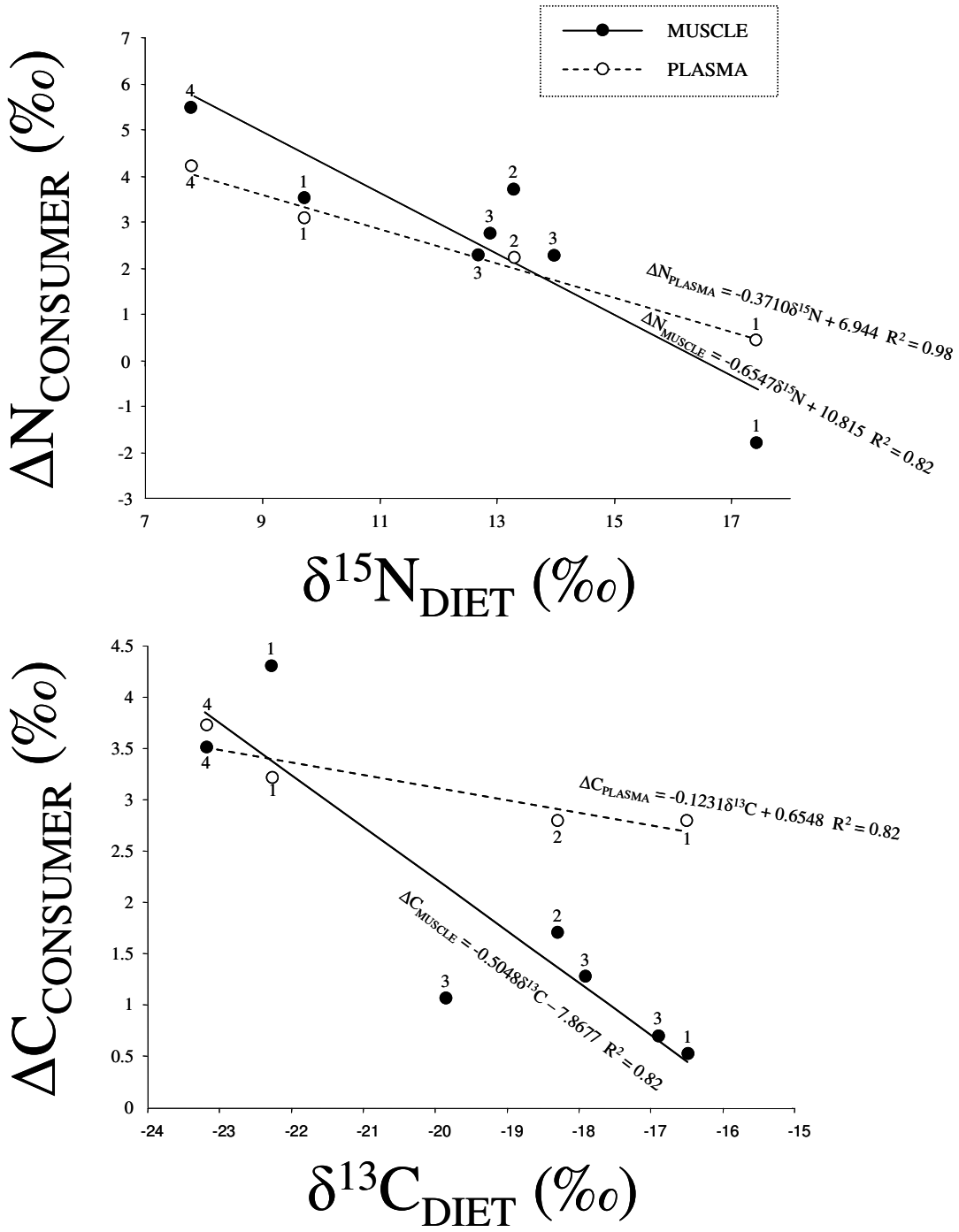


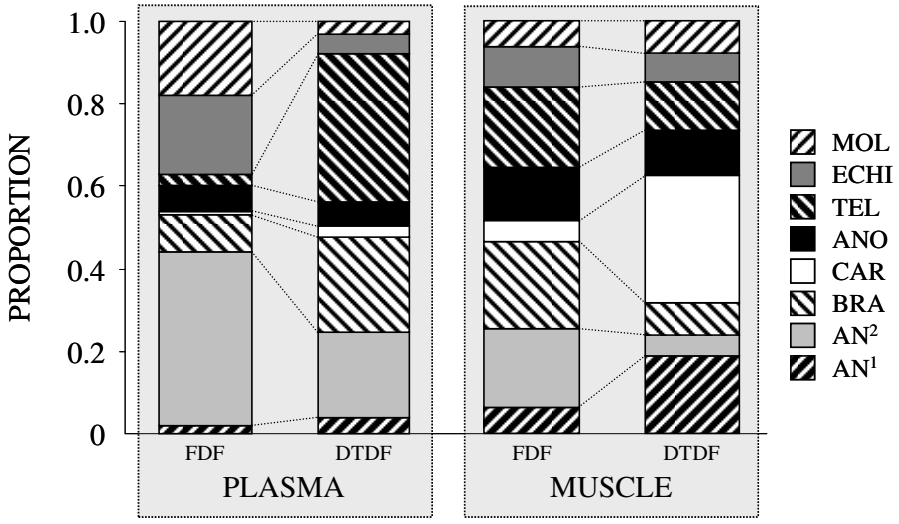
716 Figure 5.

717



718 Figure 6.

719



720 **Appendix 1.** Stomach contents of *Scyliorhinus canicula* (SCY), summarized as occurrence (OI)
721 and numeric (NI) indices. Mean isotopic values of carbon ($\delta^{13}C_{DEL}$, lipid extracted) and nitrogen
722 ($\delta^{15}N$) of the prey items found in the North Sea, either directly observed in the stomach contents
723 or identified from the literature (see the materials and methods section). We calculated the mean
724 of the different prey groups (in blood: for *S. caniculata* = Annelida[1], Annelida[2], Anomura,
725 Brachyura, Caridae, Chordata, Echinodermata, and Mollusca).

Species of prey item		OI_{SCY}	NI_{SCY}	*n*	$\delta^{13}C_{DEL}$	SD	$\delta^{15}N$	SD
Annelidae		28	13					
Polychaeta								
Group1					**-16,47**	**0,20**	**14,99**	**0,62**
Aphroditidae	*Aphrodita aculeata*			*3*	-16,54	0,33	14,52	0,90
Nephtyidae	*Nephtys hombergii*	13	6	*2*	-16,37	0,18	15,69	0,76
Group2								
Indeterminate	*sp1*	13	6	*1*	**-17,43**	**-**	**11,61**	-
Indeterminate		3	1					
Arthropoda		63	45					
Malacostraca								
Decapoda								
Anomura		13	5		**-16,67**	**0,44**	**13,14**	**0,82**
Paguroidae	*Pagurus bernhardus*	3	1	*4*	-15,97	0,51	14,63	0,69
Paguroidae	*Pagurus prideaux*	9	4	*3*	-17,61	0,15	11,14	0,54
Brachyura		31	14		**-17,67**	**0,12**	**12,39**	**0,48**
Atelecyclidae	*Atelecyclus rotundatus*			*5*	-17,44	0,28	10,67	0,47
Carcinidae	*Liocarcinus depurator*	22	10	*6*	-17,90	0,17	11,83	0,69
Carcinidae	*Liocarcinus holsatus*			*6*	-17,64	0,19	14,39	0,31
Indeterminate		9	4					
Caridae		41	26		**-16,62**	**0,21**	**16,07**	**0,24**
Crangonidae	*Crangon allmanni*	9	7	*3*	-16,10	0,36	15,97	0,44
Crangonidae	*Crangon crangon*	16	7	*4*	-16,54	0,33	16,66	0,42
Palaemonidae	*Palaemon serratus*	3	1	*2*	-15,67	0,12	15,70	0,52
Pandalidae	*Pandalus montagui*	13	8	*5*	-17,38	0,10	15,81	0,46
Indeterminate		3	3					
Chordata		34	19		**-18,44**	**0,19**	**13,79**	**0,21**
Actinopterygii								
Clupeidae	*Sardina pilchardus*			*3*	-19,23	0,28	13,15	0,04
Gadidae	*Trisopterus minutus*	6	3	*3*	-18,33	0,32	14,36	0,49
Merlucciidae	*Merluccius merluccius*			*3*	-18,35	0,40	13,62	0,93
Ammodytida	*Ammodytes tobianus*	13	7	*9*	-18,29	0,45	13,06	0,40
Callionymidae	*Callionymus lyra*	3	1	*3*	-19,35	0,51	14,38	0,85
Callionymidae	*Callionymus maculatus*			*3*	-18,79	0,29	13,57	0,75

	Carangidés	*Trachurus trachurus*			*3*	-18,35	0,42	12,88	0,46
	Scombridae	*Scomber scombris*			*2*	-18,30	0,68	14,24	1,00
	Soleidae	*Solea solea*	3	1	*5*	-18,09	1,11	14,12	0,53
	Soleidae	*Buglossidium luteum*	9	7	*4*	-17,99	0,20	15,35	0,26
Echinodermata									
	Echinoidae	*Psammechinus miliaris*	9	4	*3*	**-16,04**	**0,40**	**12,47**	**0,64**
Mollusca									
	Buccinidae	*Buccinum undatum*	19	17	*5*	**-15,04**	**0,46**	**12,80**	**0,38**
Number of species				17					
Average length (cm)				50,5±1,4					
Average mass (gr)				545±41					
Number of empty stomachs				8					
Number of total stomachs				40					

726

727

728 **Appendix 2.** C/N ratio. $\delta^{13}C$ and $\delta^{15}N$ (Means ± SD) of the different species caught during the annual French International Bottom

729 Trawl Survey 2008 (1-20 February) in the northern North Sea (see Fig 1).

Phylum	Class	Order	Familly	Species	*n*	C/N	SD	$\delta^{13}C$	SD	$\delta^{15}N$	SD
Annelidae	Polychaeta	Phyllodocida	Aphroditidae	*Aphrodita aculeata*	*3*	3.84	0.27	-17.01	0.29	14.52	0.90
Arthropoda	Malacostraca	Decapoda	Atelecyclidae	*Atelecyclus rotundatus*	*3*	4.29	0.06	-18.01	0.17	11.05	0.69
Arthropoda	Malacostraca	Decapoda	Carcinidae	*Carcinus maenas*	*1*	4.57	-	-19.14	-	15.77	-
Arthropoda	Malacostraca	Decapoda	Carcinidae	*Liocarcinus depurator*	*3*	4.80	0.30	-19.04	0.42	13.00	0.71
Arthropoda	Malacostraca	Decapoda	Carcinidae	*Liocarcinus holsatus*	*6*	5.28	0.33	-19.55	0.36	14.39	0.31
Arthropoda	Malacostraca	Decapoda	Carcinidae	*Liocarcinus mamoreus*	*2*	5.63	1.93	-19.48	0.75	14.67	0.41
Arthropoda	Malacostraca	Decapoda	Carcinidae	*Liocarcinus vernalis*	*1*	4.93	-	-18.66	-	14.21	-
Arthropoda	Malacostraca	Decapoda	Corystidae	*Corystes cassivelaunus*	*2*	4.63	0.03	-17.49	0.58	13.71	0.43
Arthropoda	Malacostraca	Decapoda	Crangonidae	*Crangon allmanni*	*2*	3.27	0.05	-16.07	0.67	16.29	0.52
Arthropoda	Malacostraca	Decapoda	Crangonidae	*Crangon crangon*	*3*	3.32	0.05	-16.30	0.38	16.58	0.59
Arthropoda	Malacostraca	Decapoda	Goneplacidae	*Goneplax rhomboides*	*1*	3.72	-	-17.53	-	13.17	-
Arthropoda	Malacostraca	Decapoda	Inachidae	*Macropodia tenuirostris*	*3*	3.96	0.65	-17.21	0.95	12.79	0.45
Arthropoda	Malacostraca	Decapoda	Macropipidae	*Necora puber*	*1*	3.59	-	-19.15	-	15.86	-
Arthropoda	Malacostraca	Decapoda	Nephropidae	*Nephrops norvegicus*	*1*	3.13	-	-16.70	-	13.70	-
Arthropoda	Malacostraca	Decapoda	Paguroidae	*Pagurus bernhardus*	*4*	3.23	0.04	-15.97	0.51	14.63	0.69
Arthropoda	Malacostraca	Decapoda	Paguroidae	*Pagurus prideaux*	*2*	3.48	0.16	-17.59	0.22	11.24	0.91
Arthropoda	Malacostraca	Decapoda	Palaemonidae	*Palaemon serratus*	*2*	3.38	0.01	-15.69	0.13	15.70	0.52
Arthropoda	Malacostraca	Decapoda	Pandalidae	*Pandalus montagui*	*3*	3.47	0.02	-17.50	0.16	15.33	0.66
Arthropoda	Malacostraca	Decapoda	Processidae	*Processa sp.*	*1*	3.14	-	-15.15	-	15.00	-
Chordata	Teleostei	Clupeiformes	Clupeidae	*Alosa fallax fallax*	*3*	4.58	0.45	-18.82	0.11	16.40	0.29
Chordata	Teleostei	Clupeiformes	Clupeidae	*Clupea harengus harengus*	*4*	4.45	0.61	-20.45	0.88	12.13	0.63
Chordata	Teleostei	Clupeiformes	Clupeidae	*Sardina pilchardus*	*3*	3.47	0.07	-19.34	0.21	13.15	0.04
Chordata	Teleostei	Clupeiformes	Clupeidae	*Sprattus sprattus sprattus*	*6*	5.08	0.50	-20.60	0.47	13.05	0.38
Chordata	Teleostei	Clupeiformes	Engraulidae	*Engraulis encrasicolus*	*5*	3.37	0.06	-18.40	0.12	14.03	0.28
Chordata	Teleostei	Gadiformes	Gadidae	*Gadus morhua*	*3*	3.45	0.05	-18.01	0.27	15.81	0.47
Chordata	Teleostei	Gadiformes	Gadidae	*Melanogrammus aeglefinus*	*4*	3.74	0.09	-19.07	0.31	12.78	0.13
Chordata	Teleostei	Gadiformes	Gadidae	*Merlangius merlangus*	*4*	3.41	0.11	-18.37	0.65	16.38	0.69

Chordata	Teleostei	Gadiformes	Gadidae	*Trisopterus esmarkii*	*3*	4.04	0.14	-20.06	0.14	12.73	0.57
Chordata	Teleostei	Gadiformes	Gadidae	*Trisopterus minutus*	*3*	3.45	0.17	-18.43	0.20	14.36	0.49
Chordata	Teleostei	Gadiformes	Lotidae	*Ciliatus mustela*	*3*	3.83	0.31	-18.27	0.30	14.19	0.82
Chordata	Teleostei	Gadiformes	Lotidae	*Enchelyopus cimbrius*	*3*	3.72	0.02	-18.18	0.16	15.42	0.07
Chordata	Teleostei	Gadiformes	Merlucciidae	*Merluccius merluccius*	*3*	3.95	0.34	-18.94	0.14	13.62	0.93
Chordata	Teleostei	Perciformes	Ammodytida	*Ammodytes tobianus*	*7*	3.37	0.04	-18.60	0.51	12.66	0.46
Chordata	Teleostei	Perciformes	Ammodytida	*Hyperoplus lanceolatus*	*6*	3.45	0.13	-18.87	0.56	14.06	0.20
Chordata	Teleostei	Perciformes	Callionymidae	*Callionymus lyra*	*3*	3.88	0.27	-19.88	0.62	14.38	0.85
Chordata	Teleostei	Perciformes	Callionymidae	*Callionymus maculatus*	*3*	3.63	0.13	-19.06	0.38	13.57	0.75
Chordata	Teleostei	Perciformes	Carangidés	*Trachurus trachurus*	*3*	3.29	0.07	-18.35	0.42	12.88	0.46
Chordata	Teleostei	Perciformes	Moronidae	*Dicentrarchus labrax*	*1*	3.96	-	-18.51	-	14.69	-
Chordata	Teleostei	Perciformes	Mullidae	*Mullus surmuletus*	*3*	5.01	0.49	-19.88	0.26	14.39	0.38
Chordata	Teleostei	Perciformes	Pholidae	*Pholis gunnellus*	*1*	3.28	-	-16.70	-	16.70	-
Chordata	Teleostei	Perciformes	Scombridae	*Scomber scombris*	*2*	4.12	0.31	-19.07	0.38	14.24	1.00
Chordata	Teleostei	Perciformes	Stichaeidés	*Lumpenus lumpretaeformis*	*3*	3.27	0.03	-17.95	0.16	13.06	0.12
Chordata	Teleostei	Perciformes	Trachinidés	*Echiichthys vipera*	*3*	3.73	0.10	-18.11	0.18	15.11	0.31
Chordata	Teleostei	Perciformes	Trachinidés	*Trachinus draco*	*3*	3.39	0.10	-18.06	0.27	14.29	0.11
Chordata	Teleostei	Pleuronectiformes	Bothidae	*Arnoglossus laterna*	*3*	3.68	0.29	-17.65	0.41	15.26	0.33
Chordata	Teleostei	Pleuronectiformes	Pleuronectidae	*Glyptocephalus cynoglossus*	*1*	3.35	-	-17.53	-	11.97	-
Chordata	Teleostei	Pleuronectiformes	Pleuronectidae	*Hippoglossoides platessoides*	*3*	3.36	0.07	-17.97	0.37	13.40	0.08
Chordata	Teleostei	Pleuronectiformes	Pleuronectidae	*Limanda limanda*	*5*	3.85	0.12	-19.17	0.61	14.63	0.25
Chordata	Teleostei	Pleuronectiformes	Pleuronectidae	*Microstomus kitt*	*3*	3.63	0.06	-18.07	0.25	13.81	0.59
Chordata	Teleostei	Pleuronectiformes	Pleuronectidae	*Platichthys flesus*	*3*	3.64	0.16	-14.83	0.70	16.21	0.56
Chordata	Teleostei	Pleuronectiformes	Pleuronectidae	*Pleuronectes platessa*	*3*	3.59	0.08	-17.92	0.43	14.33	0.23
Chordata	Teleostei	Pleuronectiformes	Scophthalmidae	*Scophthalmus rhombus*	*1*	3.16	-	-17.10	-	14.79	-
Chordata	Teleostei	Pleuronectiformes	Soleidae	*Buglossidium luteum*	*3*	4.34	0.21	-19.05	0.40	15.60	0.08
Chordata	Teleostei	Pleuronectiformes	Soleidae	*Microchirus variegatus*	*2*	3.42	0.13	-18.63	0.02	12.80	0.12
Chordata	Teleostei	Pleuronectiformes	Soleidae	*Solea solea*	*5*	3.34	0.17	-18.09	1.11	14.12	0.53
Chordata	Teleostei	Scorpaeniformes	Agonidae	*Agonus cataphractus*	*3*	3.94	0.13	-17.86	0.33	13.81	0.81
Chordata	Teleostei	Scorpaeniformes	Cyclopteridae	*Cyclopterus lumpus*	*1*	4.43	-	-18.69	-	15.24	-
Chordata	Teleostei	Scorpanaeniformes	Cottidae	*Myoxocephalus scorpius*	*3*	3.72	0.05	-18.26	1.37	12.50	0.29
Chordata	Teleostei	Scorpanaeniformes	Cottidae	*Taurulus bubalis*	*3*	3.84	0.17	-17.29	0.36	15.81	0.95
Chordata	Teleostei	Scorpanaeniformes	Cottidae	*Zeugopterus punctatus*	*2*	3.84	0.39	-18.75	0.24	14.75	0.80
Chordata	Teleostei	Scorpanaeniformes	Cyclopteridae	*Liparis liparis liparis*	*3*	3.53	0.02	-16.23	0.09	15.02	0.51
Chordata	Teleostei	Scorpanaeniformes	Triglidae	*Aspitrigla cuculus*	*3*	3.96	0.18	-18.56	0.27	13.88	0.05
Chordata	Teleostei	Scorpanaeniformes	Triglidae	*Chelidonichthys lucerna*	*3*	4.33	0.24	-19.05	0.26	15.16	0.24

Chordata	Teleostei	Scorpanaeniformes	Triglidae	*Eutrigla gurnardus*	*3*	5.05	0.31	-19.30	0.38	15.00	0.44
Chordata	Teleostei	Scorpanaeniformes	Triglidae	*Triglia lyra*	*1*	4.54	-	-19.44	-	15.48	-
Chordata	Teleostei	Syngnathiformes	Syngnathidae	*Syngnathus acus*	*1*	4.17	-	-19.78	-	8.62	-
Chordata	Teleostei	Zeiformes	Zeidae	*Zeus faber*	*1*	4.10	-	-19.30	-	15.35	-
Chordata	Myxinii	Myxiniformes	Myxinidae	*Myxine glutinosa*	*1*	5.96	-	-20.84	-	13.08	-
Echinodermata	Asteroidea	Forcipulatida	Asteroidae	*Asterias rubens*	*1*	4.91	-	-18.60	-	13.48	-
Echinodermata	Asteroidea	Forcipulatida	Asteroidae	*Luida sarcis*	*1*	5.20	-	-16.62	-	8.97	-
Echinodermata	Echinoidea	Echinoida	Echinoidae	*Psammechinus miliaris*	*3*	5.48	0.38	-17.48	0.48	12.47	0.64
Mollusca	Bilvavia	Ostreoida	Pectinidae	*Aequipecten opercularis*	*1*	3.23	-	-19.73	-	7.06	-
Mollusca	Bivalvia	Arcoida	Glycymeridídae	*Glycimeris glycimeris*	*1*	5.17	-	-20.27	-	10.02	-
Mollusca	Bivalvia	Mytiloida	Mytilidae	*Mytilus edulis*	*1*	4.78	-	-20.01	-	9.16	-
Mollusca	Bivalvia	Ostreoida	Pectinidae	*Pecten maximus*	*1*	3.38	-	-19.39	-	10.19	-
Mollusca	Bivalvia	Veneroida	Cardiidae	*Laevicardium crassum*	*1*	3.68	-	-19.91	-	8.84	-
Mollusca	Bivalvia	Veneroida	Mactracea	*Lutraria lutraria*	*1*	3.88	-	-18.50	-	12.77	-
Mollusca	Bivalvia	Veneroida	Pharidae	*Ensis arcuatus*	*2*	3.27	0.05	-18.41	0.22	12.47	0.46
Mollusca	Cephalopoda	Sepiida	Sepiidae	*Sepia officinalis*	*3*	3.46	0.01	-18.47	0.28	15.01	0.19
Mollusca	Cephalopoda	Sepiolida	Sepiolidae	*Sepiola atlantica*	*4*	4.06	0.04	-18.78	0.31	13.39	0.65
Mollusca	Cephalopoda	Teuthida	Loliginidae	*Loligo vulgaris*	*4*	3.54	0.09	-18.77	0.19	14.63	0.23
Mollusca	Cephalopoda	Teuthida	Loliginidae	*Alloteuthis media*	*3*	4.05	0.05	-19.32	0.50	14.44	0.31
Mollusca	Cephalopoda	Teuthida	Ommastrephidae	*Todaropsis eblanae*	*1*	3.95	-	-19.45	-	14.36	-
Mollusca	Gastropoda	Cephalaspidea	Scaphandridae	*Scaphander lignarius*	*1*	4.33	-	-18.12	-	10.09	-
Mollusca	Gastropoda	Neogastropoda	Buccinidae	*Buccinum undatum*	*3*	4.52	0.29	-16.20	0.49	12.30	0.38

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