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6

7 Stable isotopes reveal food web modifications along the upstream-downstream gradient of a temperate stream.

8

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16

17 Abstract:

18 The upstream-downstream gradient (UDG) is a key feature of streams. For instance food webs are assumed to  
19 change from upstream to downstream. We tested this hypothesis in a small European river catchment (937 km<sup>2</sup>),  
20 and examined whether food web modifications are related to structural (i.e. food web composition) or functional  
21 changes (i.e. alteration of linkages within the web). We adopted a double approach at two levels of organisation  
22 (assemblage and species levels) using two isotopic metrics (isotopic space area and isotopic niche overlap), and  
23 proposed a new hypothesis-testing framework for exploring the dominant feeding strategy within a food web.  
24 We confirmed that the UDG influenced stream food webs, and found that food web modifications were related  
25 to both structural and functional changes. The structural change was mainly related to an increase in species  
26 richness, and induced functional modifications of the web (indirect effect). In addition, the UDG also modified  
27 the functional features of the web directly, without changing the web composition. The proposed framework  
28 allowed relating the direct effect of the UDG to a diet specialisation of the species, and the indirect effect via the  
29 structural changes to a generalist feeding strategy. The framework highlights the benefits of conducting the  
30 double approach, and provides a foundation for future studies investigating the dominant feeding strategy that  
31 underlies food web modifications.

32

33 Keywords: food webs, stable isotopes, longitudinal gradient, species richness, fish density, stream ecology

34 **Introduction:**

35           In most streams water volume, flow velocity, and sediment size vary from upstream to downstream  
36 reaches in a generally progressive fashion (Petts and Calow 1996). All these changes in physical conditions  
37 make up the upstream-downstream gradient (UDG), also called longitudinal or fluvial gradient (Costas and  
38 Pardo 2014, Winemiller et al. 2011). Along this gradient food webs are susceptible to change (Power and  
39 Dietrich 2002). Like Holt (1996) we distinguished changes related to structural and functional features of food  
40 webs.

41           Food web structure is related to the members making up the web, i.e. resources or consumers. In  
42 streams, biological communities are known to respond, at least partly, to the upstream-downstream  
43 modifications in abiotic environmental conditions (Rice et al. 2001) and change accordingly (e.g. Verneaux et al.  
44 2003, Tomanova et al. 2007 for macroinvertebrates in temperate and tropical streams, respectively; Belliard et al.  
45 1997, Ibañez et al. 2007 for fish in temperate and tropical streams, respectively). Sources of organic matter are  
46 also supposed to change (Vannote et al. 1980, but see Sedell et al. 1989 for large rivers), so that the whole food  
47 web structure (resources and consumers) is susceptible to change along the UDG.

48           Functional features of food webs are related to the linkages among the members of the web (e.g.  
49 number, kind or intensity of the linkages). These trophic interactions can be affected by structural modifications  
50 of the web (e.g. changes in species richness, Post and Takimoto 2007), but can also be modified while the food  
51 web structure remains unchanged (e.g. change of the dietary regime of some consumers without changes in  
52 resources or consumers, Holt 1996). Along the UDG, a change in dietary regime can be due to the longitudinal  
53 changes in hydrologic variability (Sabo et al. 2010), habitat volume (MacGarvey and Hughes 2008) and/or  
54 heterogeneity (Townsend and Hildrew 1994), because these modifications of environmental conditions  
55 potentially affect energy requirements of individuals, resource availability or inter- and intra-specific  
56 competition.

57           To date, even if some studies have already focused on the influence of the UDG on stream food webs  
58 (e.g. Winemiller et al. 2011; Chang et al. 2012; Costas and Pardo 2014), the nature of its effect remains poorly  
59 documented. Notably, no study has assessed whether the effect of UDG on food webs is mainly structural or  
60 functional (or both). In this study we deal with this specific issue focusing on fish assemblages. We used fish  
61 because they are known to display various feeding behaviours covering a wide range of trophic levels (from  
62 herbivorous and/or detritivorous to piscivorous species) and a wide range of basal resources (from  
63 autochthonous to terrestrial organic matter) (Matthews 1998; Jepsen and Winemiller 2002; Oberdorff et al.

64 2002). As such, fish assemblages constitute a substantial part of stream food webs and can be used as a model  
65 for testing basic hypotheses on food web functioning. We assess structural modifications using fish assemblage  
66 composition, species richness and density of individuals. Using stable isotopes of carbon and nitrogen, we  
67 evaluate functional modifications with a double approach based on two metrics (i.e. the isotopic space area and  
68 the isotopic niche overlap, see Definition box) related to two levels of organisation (i.e. assemblage and species  
69 levels, respectively).

70 The double approach seems necessary to determine the dominant assemblage feeding strategy  
71 associated with the observed food web modification. Three non-exclusive feeding strategies having different  
72 implications in terms of stable isotope signals, are listed by Bearhop et al. (2004): (1) specialist species (i.e. all  
73 individuals of the species are specialised on the same food type), (2) type A generalist species (i.e. all individuals  
74 of the species are taking a wide range of food types), or (3) type B generalist species (i.e. each individual of the  
75 species is specialising on different but narrow range of food types). Basically, the stable isotope signal of an  
76 individual is the average signal of its diet items (integration in space and time of the signals Rasmussen et al.  
77 2009; Vander Zanden and Rasmussen 1999). From this basic principle we deduce that: (1) individuals should  
78 display highly variable signals among different specialist species (large isotopic space area), but very similar  
79 signals within a specialist species (low isotopic niche overlap); (2) individuals should display very similar  
80 signals among and within different type A generalist species (small isotopic space area and high isotopic niche  
81 overlap respectively); and (3) individuals from type B generalist species should display highly variable signals  
82 (large isotopic space area) but a similar range of isotopic signals among and within the species (high isotopic  
83 niche overlap). The complementary responses of both isotopic metrics led us to propose a hypothesis-testing  
84 framework (Fig. 1) that can determine which feeding strategy is predominant in the assemblage to explain the  
85 observed food web modification. In this study we first examined the effect of the UDG on food web structure  
86 using fish assemblage composition, species richness and density of individuals. We then tested if the UDG  
87 modified the functional features of the web using a linear model and the isotopic space area. We conducted a  
88 path analysis to determine whether the functional changes were related to a direct effect of the UDG on the  
89 trophic linkages, or to an indirect effect induced by structural changes (e.g. modifications of species richness or  
90 density of individuals). Finally we completed our analysis by studying the variations of the isotopic niche  
91 overlap along the UDG and used both isotopic metrics and our hypothesis-testing framework to determine the  
92 feeding strategy of the fish.

## 93 **Material and Methods**

### 94 **Study Sites and estimation of the upstream-downstream gradient (UDG)**

95 We studied twelve sites located in the catchment of the Orge River (937 km<sup>2</sup>), a tributary of the Seine  
96 River, France (Fig. 2). Within this small catchment, climatic conditions and geology are rather homogeneous  
97 (<http://www.geoportail.gouv.fr/accueil>). Sites were chosen to reflect the upstream-downstream gradient (UDG)  
98 and to undergo similar moderate anthropogenic pressures (i.e. mostly forested or agricultural lands with  
99 extensive practices). Upstream catchment areas of the sites ranged from 17 to 210 km<sup>2</sup> (Fig. 2 and Table 1). Site  
100 position along the UDG was quantified using a multivariate combination of four synthetic variables: upstream  
101 catchment area (km<sup>2</sup>), distance from the sources (km), and two geomorphological variables (mean stream width  
102 (m) and mean water depth (m)). Catchment area and distance from the sources were calculated using data from  
103 the Regional Geographic Information System (RGIS) of the "Institut d'Aménagement et d'Urbanisme d'île-de-  
104 France". Stream width was measured at five transects of each sampled site. Water depth was measured at ten  
105 transects (five measurements per transect). Stream width and water depth measurements were averaged to obtain  
106 one mean value for each site. Data for catchment area and stream width were log-transformed for normality.  
107 Finally, a multivariate index was generated using the first axis of a principal component analysis performed on  
108 the four variables (85% of variation explained by the first axis, Table 1); hereafter, we refer to this synthetic  
109 variable as the position along the UDG.

110

### 111 **Sampling and evaluation of fish species richness and density**

112 The size of each sampling site varied with stream width (site length was 10 to 20 times the stream  
113 width) to encompass complete sets of the characteristic stream form (e.g. pools, riffles and runs: Oberdorff et al.  
114 (2001)). The minimal distance between two sites was more than 2 km, and we considered that fish movements  
115 among sites remained uncommon (cf. Minns 1995). Fish were sampled during July and August 2009 by  
116 conducting a single-pass electrofishing at each site. Species richness of the fish assemblage was the total number  
117 of species sampled by electrofishing at a site, while density of individuals within the assemblage was defined as  
118 the ratio of the number of all fish found at the site divided by the surface of the sampling area. All fish species  
119 were collected for stable isotope analyses (SIA), except those species represented only by a low number of  
120 small-sized juveniles. For one site, recently stocked brown trouts (*Salmo trutta fario*) were discarded from SIA.  
121 For the 12 sites, the number of fish species retained for SIA was closely related to assemblage species richness  
122 ( $\rho = 0.80$ , Spearman's correlation). Whenever possible, a sample consisted of different fin clips from the same

123 individual (from 1 to 3 different fins) to avoid killing fish (Hette-Tronquart et al. 2012). However, two species  
124 were too small to obtain enough fin tissue for analysis (i.e. *Gasterosteus aculeatus* and *Pungitius pungitius*). In  
125 this case, individuals were euthanized and analyses were carried out using the whole fish (after removing head  
126 and viscera) so that a sample consisted mainly of muscle, fin, and bone tissues. Nonetheless, all fish were  
127 handled in accordance with recent ethical standards (American Fisheries Society 2004). After collection, samples  
128 were transported on ice to the laboratory, where they were rinsed the same day with distilled water and kept  
129 frozen for later handling.

130

### 131 **Stable Isotope Analysis (SIA)**

132 We analysed carbon and nitrogen stable isotope ratios for 465 samples (see Table 2) with a Thermo  
133 Electron FlashEA 1112 Series elemental analyser (single reactor setup) coupled with a Thermo Scientific DeltaV  
134 Plus Isotope Ratio Mass Spectrometer (EA-IRMS) (Thermo Scientific, Bremen, Germany). Each sample  
135 underwent the same preparation prior to analysis. After being freeze-dried, samples were ground to powder and  
136 weighed precisely ( $500 \pm 10 \mu\text{g}$ ) in tin capsules. Altogether, isotope analyses were done on eleven fish species  
137 (Table 2). Samples were analysed in consecutive sequences (96 samples per sequence), beginning with 3 empty  
138 tin capsules for blank correction. Three international reference materials (IAEA CH-7, N1 and N2, International  
139 Atomic Energy Agency, Vienna, Austria) were analysed at the beginning and at the end of the sequence for linear  
140 normalisation. One internal reference (muscle from *Squalius cephalus*) was analysed every six samples in order  
141 to compensate for possible machine drift and as a quality control measure. Linearity correction was carried out to  
142 account for differences in peak amplitudes between sample and reference gases ( $\text{CO}_2$  or  $\text{N}_2$ ). Resulting isotope  
143 ratios R (i.e.  $^{13}\text{C}/^{12}\text{C}$  and  $^{15}\text{N}/^{14}\text{N}$ ) were expressed in conventional delta notation ( $\delta$ ), relative to the international  
144 standards, Pee Dee Belemnite limestone (V-PDB  $R = 11180.2 \pm 2.8 \times 10^{-6}$ , Werner and Brand 2001), and  
145 atmospheric air ( $R = 3678 \pm 1.5 \times 10^{-6}$ , Werner and Brand 2001). With the standard deviation of our internal  
146 reference (*S. cephalus*, muscle) we assessed the analytical precision associated with our sample runs for  $\delta^{13}\text{C}$  and  
147  $\delta^{15}\text{N}$  over the whole SIA period (from May 2010 to November 2011), and obtained values of 0.15 and 0.21 ‰,  
148 respectively. Lipid correction was carried out for all samples for which the C:N ratio was greater than 3.5 (395  
149 out of the 465 samples), following recommendations and using the equation for aquatic animals proposed in Post  
150 et al. (2007). All isotope ratios obtained from fin clip analysis were corrected using the general models  
151 developed in Hette-Tronquart et al. (2012) to account for isotope signal differences between fin and muscle  
152 tissues. In addition, this correction strongly reduced the influence of different integration times between the

153 isotope signals of fin clip and whole body, because all signals were related to muscle tissue after correction.

154

### 155 **Determination of the isotopic space area and the isotopic niche overlap**

156 To test our hypotheses, we first used an assemblage-wide metric of the food web: the isotopic space  
157 area occupied by the fish assemblage. The metric is based on the convex hull approach and was adapted from  
158 one metric developed by Layman et al. (2007) initially called Total Area – TA – in the publication (see below  
159 further explanations on the differences between TA and isotopic space area). Hereafter, we define the isotopic  
160 space area as the area of the convex hull encompassing all fish individuals in  $\delta^{13}\text{C} - \delta^{15}\text{N}$  bi-plot space. Like  
161 other studies (e.g. O'Neil and Thorp 2014) we chose the convex hull approach instead of the standard ellipse  
162 approach (proposed by Jackson et al. 2011) because it includes information on every part of the isotopic space  
163 occupied by the assemblage (Layman et al. 2012), which seems more appropriate to measure the isotopic niche  
164 overlap (Hammerschlag-Peyer et al. 2011). Given that the isotopic space is closely tied to the trophic space  
165 (Newsome et al. 2007; Semmens et al. 2009; Jackson et al. 2011), the isotopic space area (like the TA) occupied  
166 by an assemblage gives an integrative measure of the exploited resource diversity (variation in  $\delta^{13}\text{C}$ ) and of the  
167 trophic level richness (variation in  $\delta^{15}\text{N}$ ) in the assemblage. Knowing the isotopic variability in basal resources,  
168 the isotopic space area gives a measure of the trophic diversity displayed by an assemblage. In our case, we  
169 checked that the variability of three basal resources (epilithic biofilm, leaf litter and suspended matter) did not  
170 display any upstream-downstream pattern (Online Resource 1), and we deduced that the variability of the  
171 isotopic space area along the UDG could not be explained by this source of variability. Like the TA, the isotopic  
172 space area is a metric strongly influenced by the number of individuals included in the calculation (Syväranta et  
173 al. 2013), and Layman et al. (2007) emphasized that "between- and among-system comparisons using  
174 community-wide metrics will be most meaningful when food webs are defined in the same fashion". To solve  
175 this problem, Layman et al. (2007) proposed calculating the TA with the mean isotope signal of each species  
176 within an assemblage. In our case however, we analysed only one or two species at the three most upstream sites,  
177 preventing us from calculating a TA from the species' mean isotopic values. Consequently, unlike TA, we chose  
178 to calculate the isotopic space area from individual signals. At one of the three sites, only six individuals were  
179 analysed, so that we calculated the isotopic space area directly, using this reduced number of individuals. For the  
180 other 11 sites, we calculated the isotopic space area according to a three step-method, which was adapted from  
181 the bootstrap-method of Jackson et al. (2012). In a first step we randomly chose one individual per species  
182 collected at the site. In a second step we randomly selected additional individuals from the remaining pool of



183 individuals sampled at the site, to obtain ten individuals. In a third step we calculated the isotopic space area  
184 occupied by the ten selected individuals. We repeated these three steps 1000 times and obtained the isotopic  
185 space area of the site by taking the median of the 1000 calculated areas. Using this bootstrap-method, we  
186 strongly reduced the effect of sampling size on the convex hull area, because we had the same number of  
187 individuals for all but one site. We were aware that 10 individuals were certainly not sufficient to evaluate the  
188 total extent of the realised trophic diversity (Syväranta et al. 2013), but we used the isotopic space areas in a  
189 relative way to compare the trophic diversity among our sites. Examining the isotopic space areas, we tested for  
190 the potential effects of the UDG at the assemblage level, and we quantified the relationships between the UDG,  
191 assemblage species richness, density of individuals within the assemblage, and the isotopic space area by means  
192 of a path analysis (Wootton 1994).

193         To go a step further, we also examined the effects of the UDG at the species level, by calculating the  
194 isotopic niche overlap of each species. We defined the isotopic niche overlap, as the overlap of the isotopic niche  
195 of one species with the isotopic niches of the other species. To obtain the isotopic niche overlap we considered  
196 each species for which we had at least five individuals at each site where there were at least two species. First we  
197 randomly selected five individuals for each species. Second we determined the niche of each species using the  
198 convex hull that encompassed the five individuals. Third, we calculated the isotopic niche overlap of one species  
199 as the ratio of the area of its niche occupied by the niches of the other species, divided by the area of its niche  
200 (Fig. 3). We repeated these steps 1000 times, and took the median of the resulting distributions to obtain an  
201 isotopic niche overlap value for each species at each site. First, we tested if the isotopic niche overlap values  
202 were strongly influenced by species identity conducting a Kruskal-Wallis rank sum test. Then, we examined the  
203 effects of the UDG, assemblage species richness, assemblage density, and species identity developing a linear  
204 model (isotopic niche overlap ~ UDG + species richness + assemblage density + as.factor(species identity)).

## 205 **Results**

### 206 **Fish assemblage variation along the UDG**

207 Species richness increased from 3 to 12, and density of individuals ranged between 0.10 and 1.69  
208 (individuals m<sup>2</sup>) along the UDG. Species richness and density within the assemblages were not significantly  
209 correlated ( $\rho = -0.32$ , Spearman's correlation). Density was not significantly correlated to the UDG ( $\rho = -$   
210  $0.46$ , Spearman's correlation), whereas species richness was strongly and positively linked to the UDG (linear  
211 model,  $p$ -value  $< 0.001$ ,  $R^2 = 0.71$ ). Overall the composition of the fish assemblage did not exhibit a strong  
212 longitudinal pattern, as most species were present along most of the gradient (Fig. 4).

213

### 214 **Effects of the UDG at the assemblage level: isotopic space area and path analysis**

215 The total (direct and indirect) effect of the UDG on the isotopic space area was significant and positive  
216 (Fig. 5,  $p$ -value = 0.014,  $R^2 = 0.47$ ).

217 The path coefficients of UDG  $\rightarrow$  species richness and UDG  $\rightarrow$  isotopic space area relationships were  
218 both significant and positive ( $p$ -values  $< 0.001$ ). The path coefficient of species richness  $\rightarrow$  isotopic space area  
219 relationship was also significant but negative ( $p$ -value = 0.002). Path coefficients for the relationships including  
220 assemblage density were not significant (Fig. 6).

221

### 222 **Effects of the UDG at the species level: changes in the niche overlap**

223 No significant differences were found among the isotopic niche overlaps of the different species ( $p$ -  
224 value = 0.204, Kruskal-Wallis rank sum test). The linear model combining the three variables – UDG, species  
225 richness and species identity – was significant ( $p$ -value = 0.006, minimization of the Akaike's information  
226 criterion AIC) and explained as much as 70 % of the isotopic niche overlap variations. The UDG seemed to  
227 decrease the overlap, whereas assemblage species richness increased the overlap (Table 3). The effect of fish  
228 species identity was exclusively driven by three species ( $p$ -value  $< 0.050$ , Table 3): *Phoxinus phoxinus*, *P.*  
229 *pungitius*, and *Leuciscus leuciscus*. The three species were rarely found within our 12 sites (Table 2), and we  
230 deduced that species identity was not an important factor acting on the variations of the isotopic niche overlap in  
231 our study. Neither was the density of individuals within the assemblage, because the model was not improved by  
232 adding this factor as a new variable (non-significant effect and greater AIC).

233 **Discussion**

234 **Structural and/or functional effects of the UDG**

235 Our goals consisted in examining the influence of the UDG on a temperate stream food web, and in  
236 clarifying the nature (i.e. structural or functional) of this effect.

237 We first found that the UDG had a structural effect on the food webs. Three potential mechanisms  
238 induce a modification of food web structure: a change in species richness, a change in density of individuals or a  
239 change in species identity. In our case the structural change was mainly due to a change in species richness. In  
240 accordance with previous patterns observed for both temperate (Oberdorff et al. 1993) and tropical streams  
241 (Ibañez et al. 2007; Winemiller et al. 2011), we observed that species richness increased along the UDG.  
242 Concerning the density of individuals, we did not observe any relationship between density and the UDG,  
243 suggesting that the assemblage structure variation along the UDG was not related to density. Last, we observed  
244 that most species were spread over all sampled sites, suggesting a weak longitudinal pattern in fish species  
245 identity.

246 We also found that the UDG had a functional effect on the food webs (both isotopic metrics exhibited  
247 an upstream-downstream pattern), confirming the key role of the longitudinal gradient in ecosystem functioning  
248 (Winemiller et al. 2011; Chang et al. 2012). Using a path analysis, we determined that this functional  
249 modification was related to two antagonistic influences of the UDG: (1) a direct influence modifying the  
250 linkages among the web members and leading to an increase in the isotopic space area, and (2) an indirect  
251 influence generated by its structural effect (increase in species richness) leading to a decrease of the isotopic  
252 space area. The direct effect predominated, so that the total effect of the UDG induced a general increase in the  
253 isotopic space area.

254

255 **A hypothesis-testing framework to determine the dominant feeding strategy of the assemblage**

256 After having determined the nature of the UDG effect on the food webs, we tried to clarify which  
257 feeding strategies of the fish could best explain the functional food web modifications. Following the proposed  
258 hypothesis-testing framework (Fig. 1), we took advantage of our double approach at two levels of organisation  
259 (i.e. assemblage and species levels), and compared the results of both isotopic metrics (i.e. the isotopic space  
260 area, and the isotopic niche overlap). Concerning the direct effect of the UDG on the functional features of the  
261 web, the isotopic space area increased while the isotopic niche overlap decreased from upstream to downstream.  
262 Taken together, the results matched only one feeding strategy of the framework: the “specialist” strategy (Fig. 1),

263 indicating a specialisation of most individuals on a specific narrow range of diet items. For the indirect effect of  
264 the UDG (i.e. via species richness), the isotopic space area decreased, and the isotopic niche overlap increased  
265 when species richness increased. In the hypothesis-testing framework this matched only one feeding strategy: the  
266 “type A generalist” strategy (Fig. 1), meaning that most of the individuals tended to adopt an opportunistic  
267 feeding strategy, taking a similar wide range of diet items.

268         We propose several hypotheses to explain the different feeding strategies adopted by the fish  
269 assemblage that could be tested with further experiments. The diet specialisation associated with the direct effect  
270 of the UDG can be explained by two non-exclusive reasons. First, temperate streams are known to be more  
271 stable from upstream to downstream (e.g. Sabo et al. 2010). Consequently, fish undergo less perturbation stress,  
272 and their energy requirements should decrease along the UDG. With lower energetic demands fish omnivory  
273 tends to decrease, and diet specialisation to increase (Arim et al. 2010). Second, downstream reaches are known  
274 to display higher habitat heterogeneity, offering higher resource diversity (Ibañez et al. 2009). Fish could exploit  
275 this larger range of available resources to focus on preferential food items corresponding to their optimal feeding  
276 requirement (optimal foraging theory, MacArthur and Pianka 1966). In such cases the increase in habitat  
277 heterogeneity along the UDG should lead to an increase in diet specialisation.

278         The opportunistic feeding strategy related to the indirect effect of the UDG (via structural changes) was  
279 more unexpected. Generally an increase in species richness is associated with a species specialisation on  
280 different trophic niches to reduce competition (niche segregation phenomenon due to the exclusion principle:  
281 Gause 1934; Mason et al. 2008). In our case, the contrary happened: the increase in species richness led all  
282 individuals to share a similar wide range of resources (high niche overlap and trophic redundancy). We explain  
283 this unexpected strategy with a limitation of the available resources. As stated by Matthews (in Fig 9.1., p 459,  
284 1998) resource scarcity makes individuals feed on every possible food items (including previously unused  
285 resources) to meet their energy requirements (Araújo et al. 2011). In our case, resource scarcity could occur if  
286 species richness increases faster than resource availability.

287 **Conclusion**

288           In this study we show that the upstream-downstream gradient has a complex influence on temperate  
289 stream food webs. Its effect concerns both structural and functional features of the webs. From upstream to  
290 downstream, the structural modifications of the webs are mainly related to species richness, while the functional  
291 modifications are due to a direct and an indirect effect (via species richness) of the UDG. We link the direct  
292 effect of the UDG with a diet specialisation of the species, whereas the indirect effect seems related to a “type A  
293 generalist” feeding strategy.

294           We intentionally chose to address our issue with a relatively small catchment (937 km<sup>2</sup>), because it  
295 allowed controlling for environmental factors and isotope baseline variation (see Online Resource 1). The other  
296 side of the coin is that we only considered a relatively small portion of the UDG, and that our results now need  
297 to be confirmed at a larger spatial scale. Anyway, we demonstrate that the UDG influences the functioning of  
298 stream food web. Consequently, integrating the UDG should be a prerequisite for all studies dealing with stream  
299 food webs, and future work should at least ensure, before making comparisons among food webs, that the  
300 different studied sites are situated in a constrained part of the gradient (i.e. similar order of magnitude for  
301 catchment area, stream width...).

302           We also highlight the benefit of conducting a double approach at both assemblage and species levels  
303 with two appropriate isotopic metrics (i.e. isotopic space area and isotopic niche overlap). Discriminating the  
304 three potential feeding strategies is indeed not possible using only one metric. When applying the double  
305 approach it must be kept in mind that the metrics are based on stable isotope analysis. Their interpretation in the  
306 proposed framework should be done with caution in light of isotope limitations (Hoeinghaus and Zeug 2008;  
307 Syväranta et al. 2013). For instance, the isotopic variability of the basal resources must be considered as a  
308 potential confounding factor.

309           Food web modifications can be related to both structural and functional features of the web. The study  
310 of the interactions between these two features has key ecological implications in term of conservation and  
311 management strategy, as directly related to the critical issue of linking structural and functional biodiversity  
312 (Thompson et al. 2012). Our double approach (at assemblage and species level) provides a new hypothesis-  
313 testing framework for exploring the feeding strategies underlying food web modifications, and can help in  
314 understanding the interactions between both kinds (structural or functional) of modifications.

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**Table 1** Sites' description. Upstream catchment area, distance from the sources, and slope were calculated with the RGIS data. Stream width, water depth, current velocity, temperature and oxygen were measured once during October 2009 but not on the same day for all sites. This explains the slightly different temperatures and oxygen concentrations of sites 7 and 12

Stream	Site code	Upstream catchment area	Distance from the sources	Width	Water depth	Slope	Current velocity	Temperature	Oxygen	Position on the UDG
		(km <sup>2</sup> )	(km)	(m)	(m)	(m/m)	(m/s)	(°C)	(mg/l)	1 <sup>st</sup> PCA axis
Rabette	1	17	7.0	1.9	0.07	0.004	0.2	15.0	9.5	-2.11
Aulne	2	24	3.9	2.2	0.16	0.006	0.1	13.0	9.2	-1.64
Yvette	3	28	8.8	2.4	0.26	0.005	0.3	14.0	9.6	-0.73
Vaux	4	19	3.8	2.9	0.29	0.004	0.1	13.5	9.2	-0.90
Aulne	5	58	7.2	2.8	0.17	0.003	0.2	14.5	8.8	-0.64
Yvette	6	153	18.9	7.0	0.25	0.001	0.4	15.0	9.2	1.82
Rémarde	7	178	19.6	6.5	0.39	0.002	0.1	8.8	10.8	2.49
Charmoise	8	20	2.7	1.4	0.13	0.009	0.1	13.0	8.2	-2.35
Prédecelle	9	26	12.5	2.2	0.15	0.005	0.2	12.8	9.7	-1.10
Renarde	10	91	11.3	2.8	0.26	0.004	0.3	12.0	10.2	0.22
Orge	11	115	24.2	4.5	0.44	0.003	0.1	12.0	9.6	2.36
Orge	12	210	26.6	5.8	0.32	0.001	0.2	9.0	11.8	2.57

**Table 2** Species sampled from the Orge River catchment in summer 2009. Nbi is the total number of individuals caught by electrofishing, all sites taken together. Nbs is the number of the different sites where a given species was found. SIA is the number of fish whose stable isotope ratios were determined

Common name	Scientific name	Species code	Nbi	Nbs	SIA
Stone Loach	<i>Barbatula barbatula</i>	Bab	562	12	198
Bullhead	<i>Cottus gobio</i>	Cog	141	6	40
Stickleback	<i>Gasterosteus aculeatus</i>	Gaa	18	6	9
Gudgeon	<i>Gobio gobio</i>	Gog	189	7	83
Common dace	<i>Leuciscus leuciscus</i>	Lel	12	1	6
Perch	<i>Perca fluviatilis</i>	Pef	65	5	33
Minnow	<i>Phoxinus phoxinus</i>	Php	62	2	25
Ninespine stickleback	<i>Pungitius pungitius</i>	Pup	34	5	5
Roach	<i>Rutilus rutilus</i>	Rur	45	5	36
Zander	<i>Sander lucioperca</i>	Sal	2	2	1
Chub	<i>Squalius cephalus</i>	Sqc	46	6	29
Freshwater Bream	<i>Abramis brama</i>	Abb	1	1	No
European eel	<i>Anguilla anguilla</i>	Ana	1	1	No
Crucian carp	<i>Carassius carassius</i>	Cac	2	1	No
European brook lamprey	<i>Lampetra planeri</i>	Lap	2	1	No
Pumpkinseed	<i>Lepomis gibbosus</i>	Leg	6	1	No
Brown trout	<i>Salmo trutta fario</i>	Sat	23	3	No
Rudd	<i>Scardinius erythrophthalmus</i>	Sce	1	1	No

**Table 3** Parameters of the linear model combining – UDG, species richness and species identity – to explain the variations of the isotopic niche overlap. ‘\*’ indicates a significant effect, and ‘\*\*\*’ stands for a highly significant one. Species codes are given in Table2

Variable	Coefficient	p-value	
(intercept)	-31.6	0.014	*
UDG	-7.9	0.037	*
Species richness	8.3	0.0005	***
Bab	0.0	-	-
Cog	-4.0	0.710	
Gaa	20.2	0.246	
Gog	11.0	0.204	
Lel	-40.3	0.037	*
Pef	-4.3	0.732	
Php	25.8	0.049	*
Pup	-48.2	0.015	*
Rur	3.0	0.779	
Sqc	-13.9	0.286	

**Fig. 1** Potential feeding strategies within an assemblage and expected patterns of the isotopic metrics (isotopic space area and niche overlap, abbreviated ISA and INO, respectively). Black dots represent individuals within a species. (1) If the assemblage is mostly composed of specialist species, individuals of a given species would be specialised on the same narrow range of resources (e.g.  $P_1$ ) with the same isotopic signal (e.g.  $\delta_{P_1}$ ), but individuals from different species would consume different resources and display different isotopic signals ( $\delta_{P_1}$ ,  $\delta_{P_2}$ , or  $\delta_{P_3}$ ). Consequently, the assemblage would occupy a large isotopic space area, but the niche overlap between species would be low. (2) In the case of an assemblage composed of type A generalist species, all individuals would consume a similar wide range of resources ( $\bar{P}$ ) and thus have similar isotopic signals ( $\delta_{\bar{P}}$ ). This assemblage would occupy a small isotopic space area, but the niche overlap between species would be high. (3) Last, the assemblage could be mostly composed of type B generalist species. In this case, individuals of the same species would be specialised on different and narrow ranges of resources (e.g.  $P_1, P_2$ , or  $P_3$ ) displaying different isotopic signals ( $\delta_{P_1}$ ,  $\delta_{P_2}$ , or  $\delta_{P_3}$ ), but individuals from different species could share the same resource (e.g.  $P_1$ ) and have the same isotopic signal (e.g.  $\delta_{P_1}$ ). Consequently, the isotopic space area occupied by this assemblage would be large and the niche overlap between species would be high

**Fig. 2** Map of the Orge river catchment showing the location of the 12 sample sites. See Table 1 for a short description of all sites

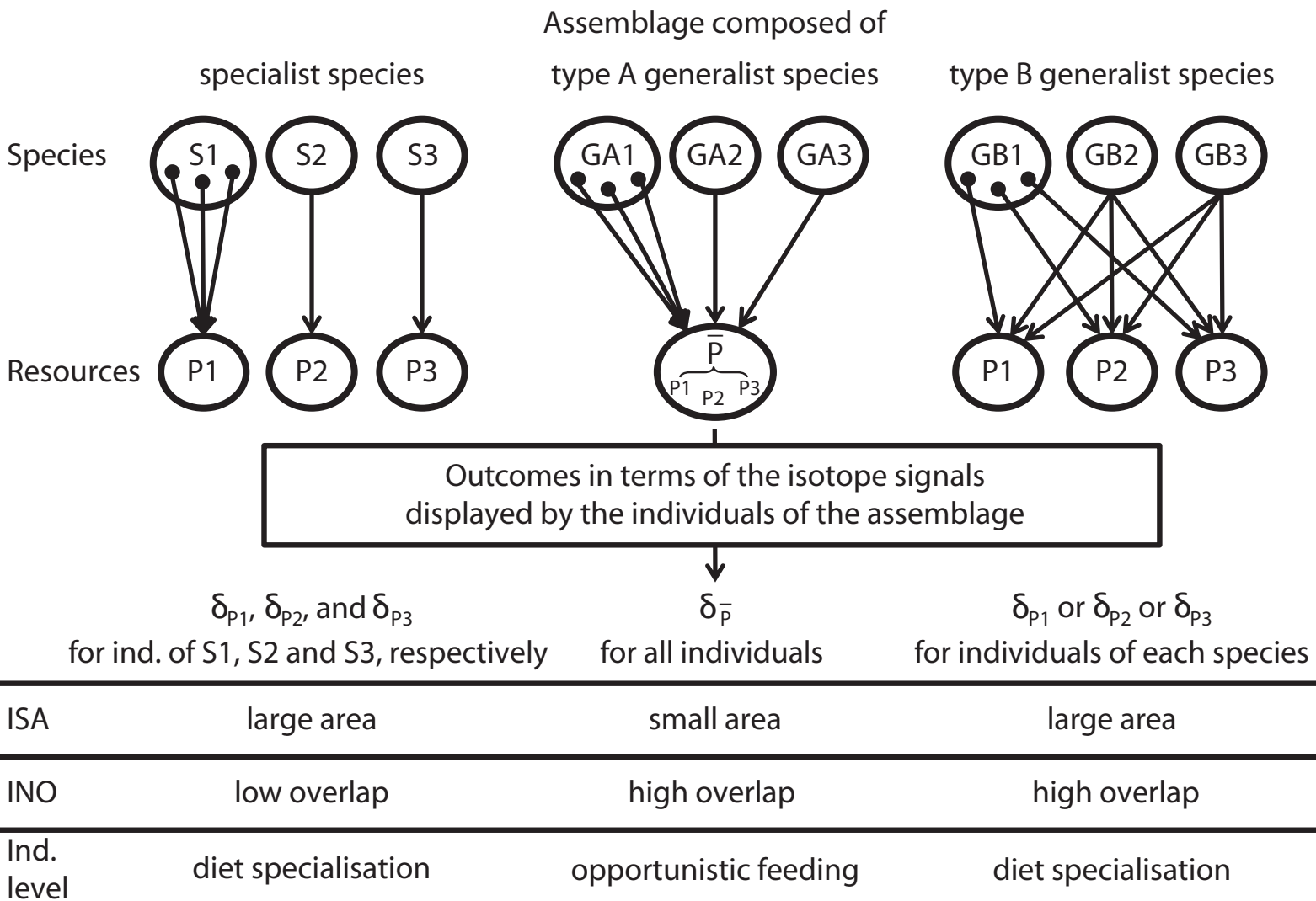
**Fig. 3** Representation of isotopic niche overlap. The different polygons represent the isotopic niches occupied by six different species. The grey area is the niche area of species 3 that is occupied by the other species (2, 4 and 5). Together with the whole niche area of species 3, they give the niche overlap of species 3. Here, species 1 has no niche overlap with other species

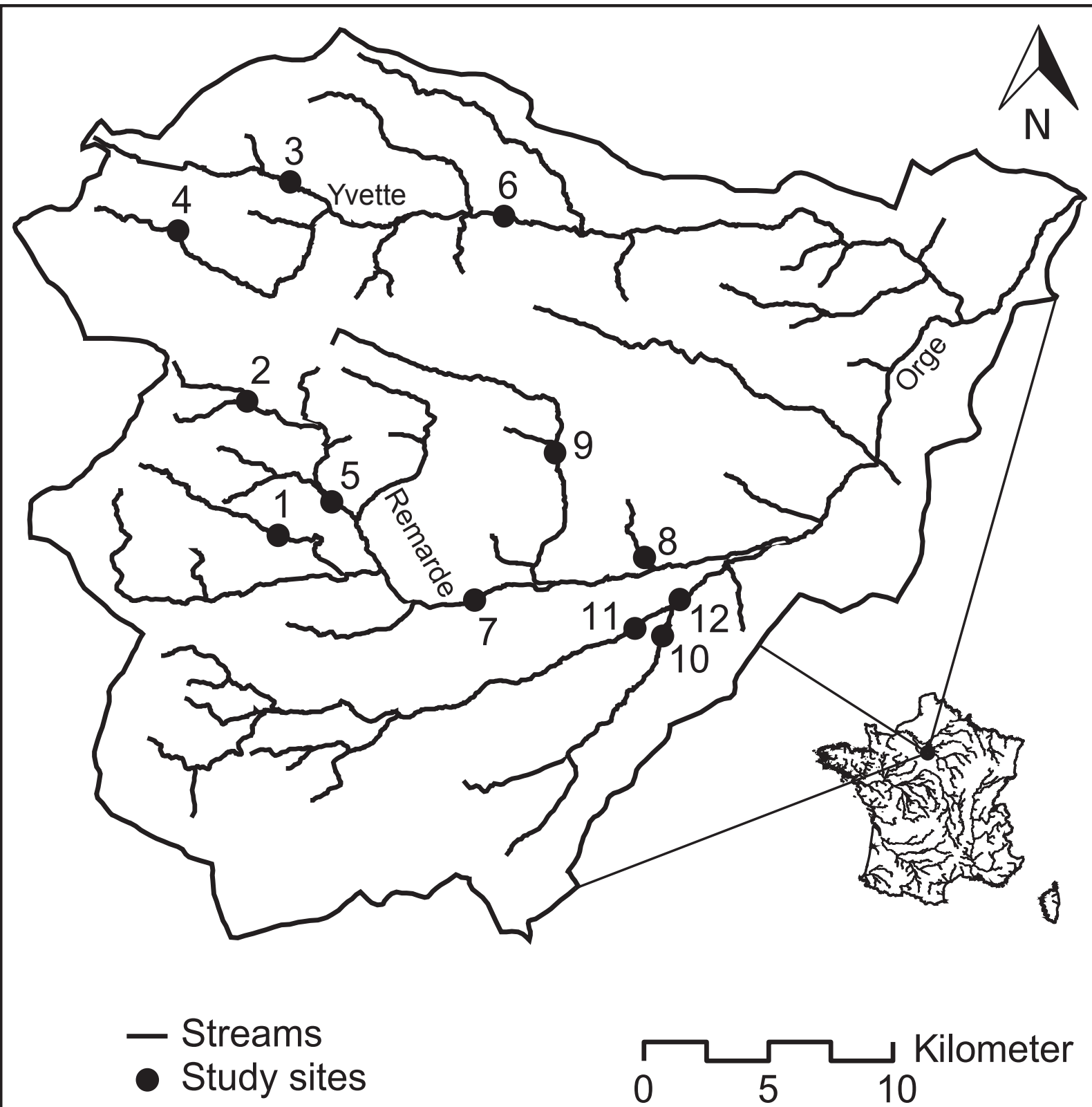
**Fig. 4** Distribution of the fish species along the UDG according to their family. Each point indicates the presence of a species at a given site (12 sites per species maximum). Species codes are given in Table 2. The site position along the UDG is given by a multivariate index that increases from upstream to downstream

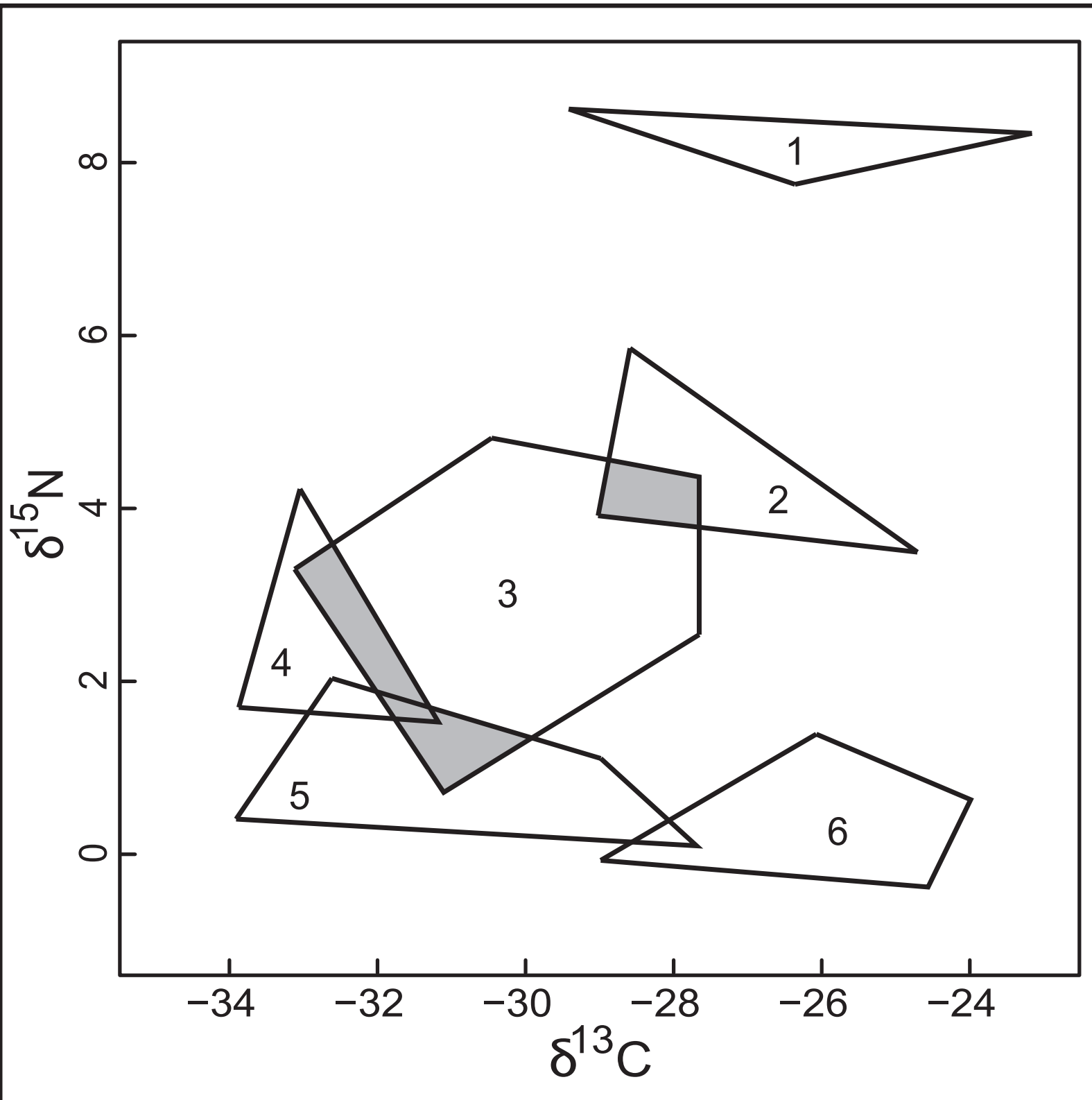
**Fig. 5** Influence of the UDG on the isotopic space area: a significant linear model is found (black line). Dashed lines represent the confidence limits of the model at the 0.95 level ( $p = 0.012$ ,  $R^2 = 0.48$ )

**Fig. 6** Results of the path analysis quantifying the relationships between the UDG, species richness, and density of individuals within the assemblage. Solid lines indicate significant paths and dashed lines indicate not significant paths. Numbers are path coefficients

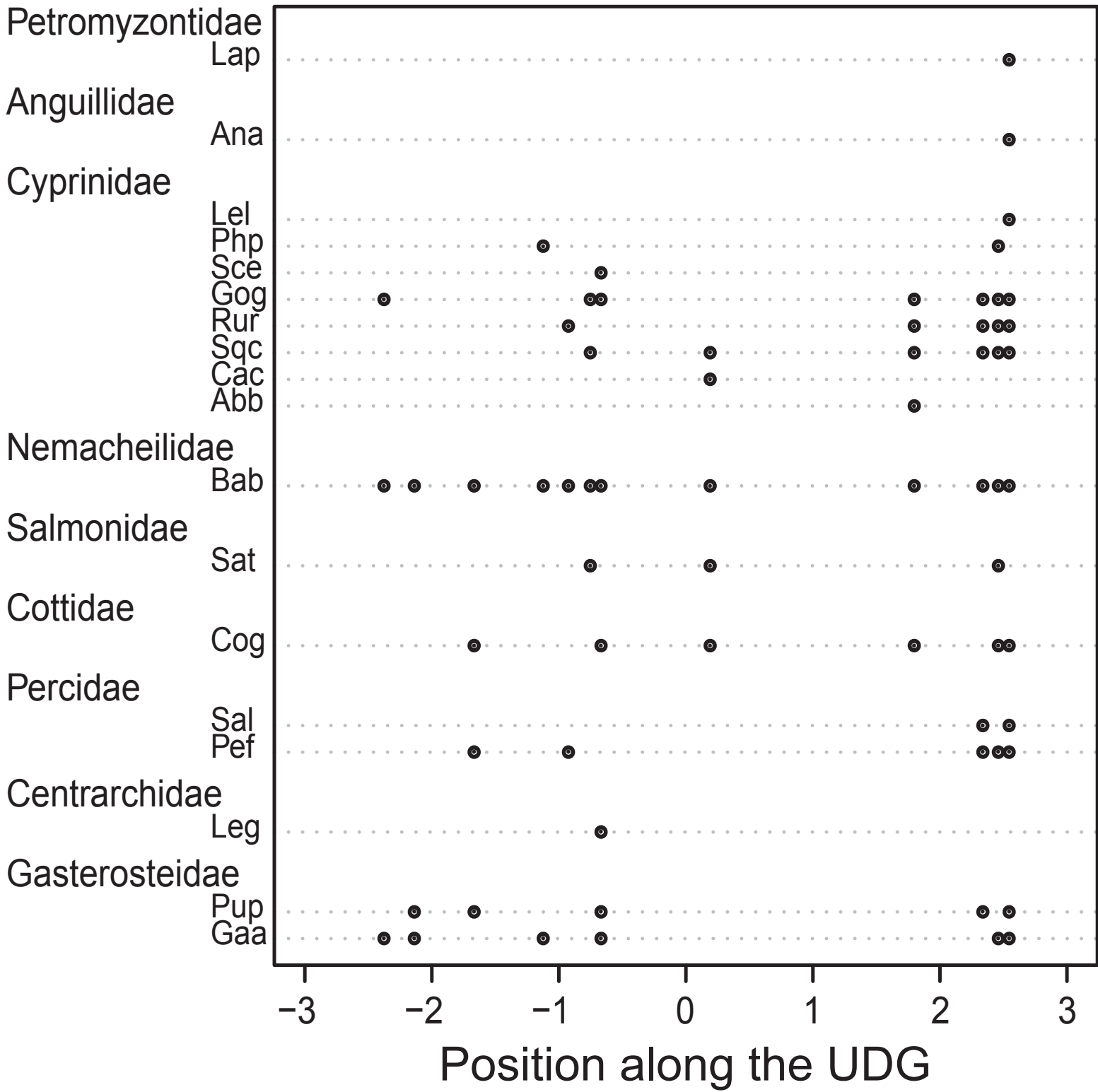
**Online Resource 1** Isotopic variations in basal resources along the UDG

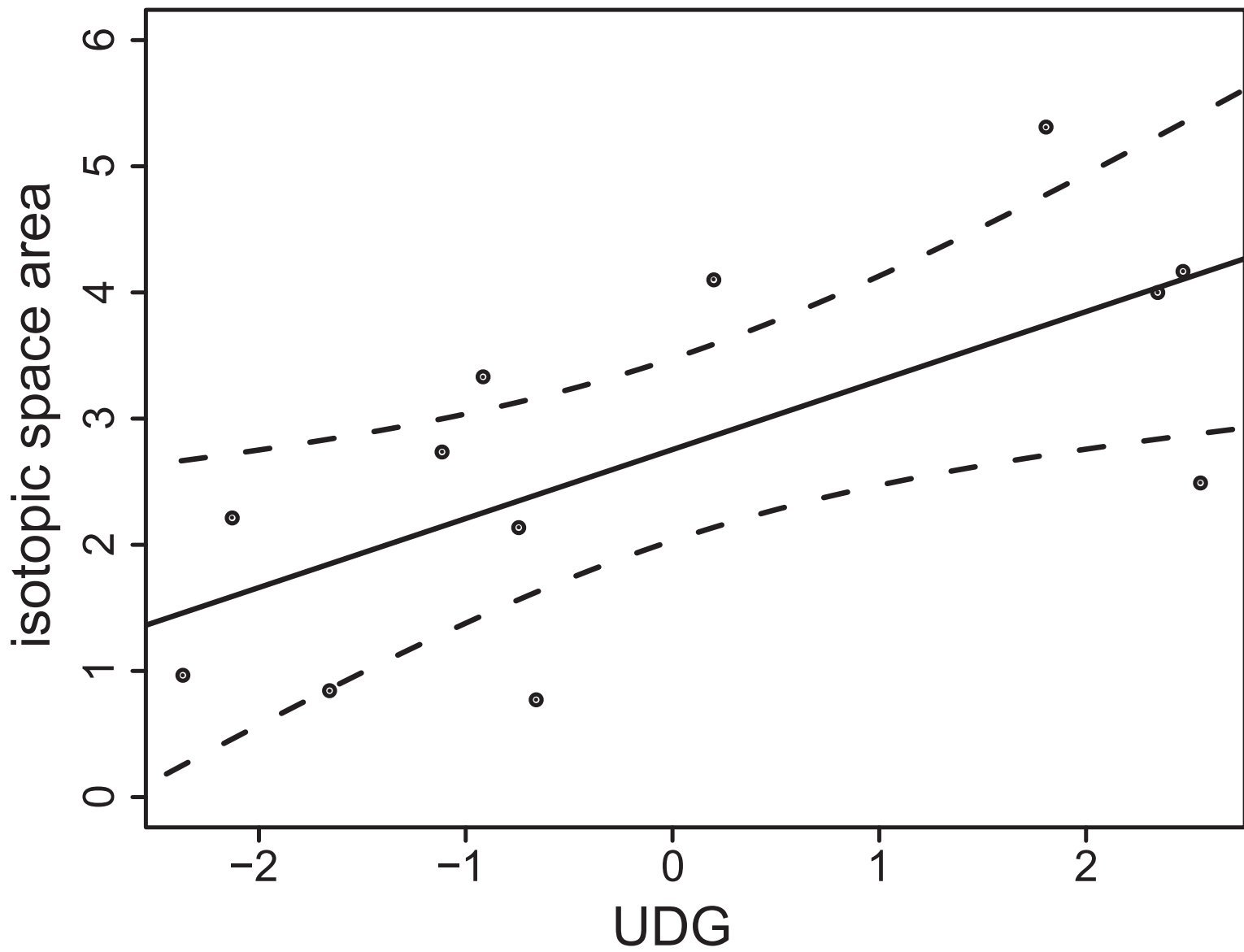


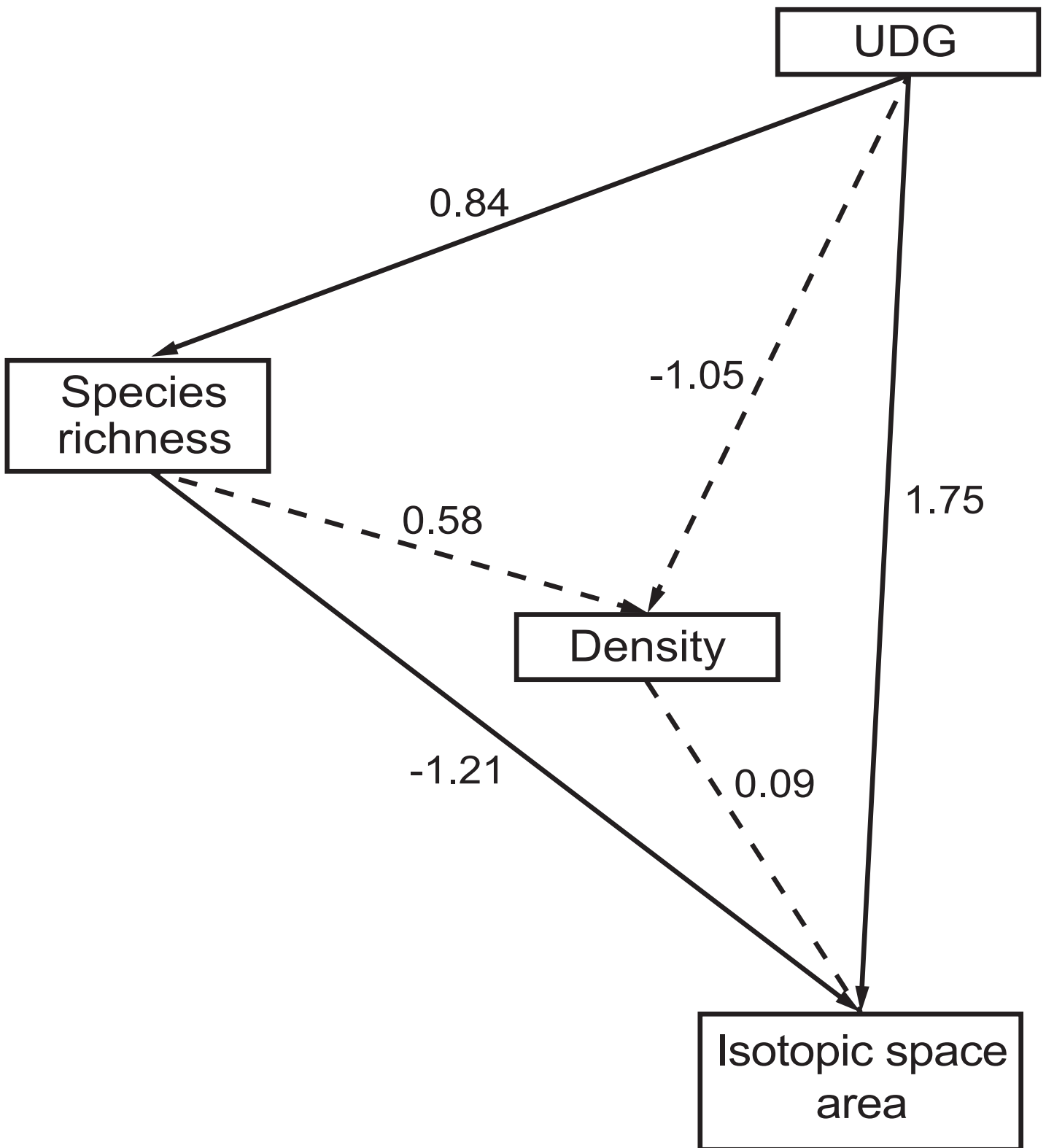












# Isotopic variations in basal resources along the Upstream-Downstream Gradient (UDG)

Online resource of the article: *Stable isotopes reveal food web modifications along the upstream-downstream gradient of a temperate stream*

Aquatic Sciences

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**Context** The variability of the isotopic signals within a food web depends on the diversity of resources available at the base of the food web, and on the isotopic variability among the different food items (Matthews and Mazumder 2004). As a result, an increase of the isotopic variability in the food web does not necessarily mean that the diversity of the resources consumed by the web increases ; it can also be the result of a greater isotopic variability within the same set of resources.

In our study, we find that the isotopic space area increases along the UDG, and before interpreting the result we checked if the isotopic variability at the resource level contributed to the increase of the isotopic space area. We first examined if the isotopic variation in basal resources increased along the UDG. For this, we took advantage of the carbon and nitrogen isotope signals of three basal resources : epilithic biofilm, leaf litter and suspended matter.

**Method** Three independent samples of each source of organic matter were collected at each site approximately one month before fish sampling occurred. Biofilms were collected by gently brushing stones found in the river with a toothbrush ; leaf litter sample consisted in old leaves decomposing in the stream ; and suspended matter were obtained by

filtering stream water on glass fiber filter ( $\varnothing = 0.7 \mu\text{m}$ ). Leaf litter samples were rinsed with distilled water at laboratory, before being freeze-dried like the biofilm and suspended matter samples. Samples were ground to powder if necessary, weighed in tin capsules and analysed with EA-IRMS following the same method than fish samples (see “stable isotope analysis SIA“ section in the “Material and Methods” part). We evaluated the mean isotope signal of each source for carbon and nitrogen using the isotope ratios of the three independent samples (see Table A1). Based on the average values, we determined the isotopic variability in basal resources calculating the range of the signals at each site for both carbon and nitrogen. We tested if the isotopic variation in basal resources increases from upstream to downstream, calculating Spearman’s rank correlation coefficients between the range of the isotope signals and the UDG for both carbon and nitrogen.

Table A1 – Carbon and nitrogen isotope ratios of three basal resources. For each source, three independent samples were collected at each site

Site code		leaf litter			epilithic biofilm			suspended matter		
		1	2	3	1	2	3	1	2	3
1	$\delta^{13}\text{C}$	-30.4	-29.6	-29.6	-26.9	-26.3	-27.4	-28.4	-28.3	-28.4
2		-28.5	-29.5	-28.6	-29.2	-28.7	-29.2	-28.6	-28.7	-28.5
3		-28.9	-29.5	-29.6	-33.9	-33.3	-33.1	-29.1	-29.3	-29.5
4		-28.1	-28.7	-28.9	-26.9	-28.8	-29.6	-28.8	-29.0	-28.9
5		-28.8	-27.6	-30.5	-29.6	-29.4	-29.1	-28.3	-28.4	-28.5
6		-27.4	-29.7	-29.8	-34.1	-33.2	-31.3	-28.9	-28.7	-29.1
7		-30.1	-31.7	-29.5	-22.8	-20.3	-18.5	-28.2	-28.4	-28.3
8		-29.7	-29.4	-28.7	-27.5	-27.7	-27.1	-27.9	-27.7	-27.8
9		-29.1	-29.0	-29.6	-27.1	-30.6	-31.7	-26.2	-27.8	-28.1
10		-28.3	-29.2	-29.5	-28.3	-30.2	-21.4	-25.9	-25.8	-26.0
11		-27.3	-28.7	-26.1	-26.4	-25.9	-26.3	-27.9	-27.8	-27.9
12		-31.0	-29.3	-27.4	-28.3	-28.2	-27.6	-28.0	-27.6	-27.7
1	$\delta^{15}\text{N}$	0.6	0.9	1.7	3.5	3.1	3.3	2.8	3.0	3.0
2		-0.1	1.5	-0.2	-0.2	-0.6	1.5	2.2	2.9	2.2
3		-1.7	0.7	2.1	12.9	11.6	13.0	5.8	5.8	6.0
4		4.3	2.5	3.6	7.4	5.8	6.0	4.1	4.5	4.7
5		0.9	1.1	0.6	5.6	5.6	8.5	4.8	4.2	4.5
6		3.9	2.8	3.3	7.3	8.6	10.9	6.3	6.9	6.4
7		1.8	1.9	5.3	6.6	5.8	5.2	6.4	6.2	6.4
8		2.4	2.1	2.7	7.1	6.7	5.8	7.7	7.6	8.0
9		-0.6	-0.5	1.2	4.7	4.5	4.7	5.3	4.9	5.6
10		1.5	-0.2	0.5	5.0	4.7	4.8	3.9	4.0	4.3
11		0.6	3.0	0.7	6.2	5.3	6.8	4.2	4.5	4.4
12		-0.7	-0.8	0.6	5.1	4.7	6.5	4.9	5.1	5.4

**Result and Conclusion** Results of both correlation tests (see Table A2) show that there are no correlation between the isotopic variability in basal resources and the UDG. We can confidently deduce that the isotopic variability at the resource level does not

contribute to the increase of the isotopic space area. Consequently, the diversity of the exploited resources shall be the main reason why the isotopic space area increases from upstream to downstream.

Table A2 – Results of the two Spearman’s correlation tests between the range of the resource isotope ratios (calculated with the mean signals of each source) and the UDG

Spearman's correlation test between UDG and...	rho coefficient	p-value
carbon isotopic range	0.24	0.444
nitrogen isotopic range	0.35	0.266

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