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► To cite this version:

Clémentine François, Laurent Simon, Florian Malard, Tristan Lefébure, Christophe Jean Douady, et al.. Trophic selectivity in aquatic isopods increases with the availability of resources. *Functional Ecology*, 2020, 34, pp.1078-1090. 10.1111/1365-2435.13530 . hal-02492518

HAL Id: hal-02492518

<https://univ-lyon1.hal.science/hal-02492518>

Submitted on 10 Dec 2020

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Trophic selectivity in aquatic isopods increases with the availability of resources

**Clémentine M. Francois^{a,*}, Laurent Simon^a, Florian Malard^a, Tristan Lefébure^a,
Christophe J. Douady^{a,b} and Florian Mermillod-Blondin^a**

^a Univ Lyon, Université Claude Bernard Lyon 1, CNRS, ENTPE, UMR5023 LEHNA, F-69622, Villeurbanne, France

^b Institut Universitaire de France, Paris, France

* Corresponding author: clementine.francois@univ-lyon1.fr

Keywords

C and N stable isotopes - diet determination - freshwater isopods - resource availability - resource diversity - selective feeding - stoichiometric quality - trophic choices

25 **ABSTRACT**

26 **1.** Trophic selectivity has major influences on consumers' fitness, stability of predator and prey
27 populations, and nutrient fluxes in food webs. Trophic selectivity occurs when the relative
28 abundances of resources in a consumer's diet differ from their relative abundances in the
29 environment. This discrepancy between resources abundance and use has been predicted to
30 increase with the availability of resources in the environment. Trophic selectivity has also been
31 predicted to increase with the heterogeneity of resources quality in the environment. Despite their
32 ecological and evolutionary implications, conclusive *in situ* tests of these predictions are still
33 lacking.

34 **2.** We challenged these two predictions by studying 15 closely related species of isopods
35 distributed along a wide range of resource availability (from 1.3 to 57.6 grams of organic carbon
36 per square meter). The dataset ranged from deep cave systems, considered as some of the most
37 resource-limited environments on Earth, to highly-productive surface streams.

38 **3.** For each species, we quantified the relative abundance of all available resources in the
39 environment and estimated the heterogeneity in the stoichiometric quality of these resources.
40 Isopod diet was determined using C and N stable isotopes and a Bayesian mixing model. The
41 degree of trophic selectivity was then calculated for each species.

42 **4.** By coupling a standardized quantification of trophic resources with a fine determination of
43 diets using stable isotopes, we uncovered a positive relationship between resource availability and
44 trophic selectivity. Contrary to our second prediction, trophic selectivity did not correlate with the
45 heterogeneity of resources quality.

46 **5.** Our results have important implications in trophic ecology by highlighting that resource
47 availability was a main driver of trophic choices in aquatic invertebrates across a broad range of
48 environments. These findings call for further evaluation of the mechanisms (e.g. trophic
49 competition) causing the positive relationship between trophic selectivity and resource

availability, as these mechanisms could be closely linked to those generating the documented relationship between species richness and productive energy.

INTRODUCTION

Trophic selectivity has been recognized to have profound consequences for consumers' fitness (Cruz-Rivera & Hay, 2000; McMurray et al., 2016), for the stability of grazer and prey populations (Strom & Loukos, 1998; Meunier et al., 2016), and for nutrient fluxes and recycling in food webs (Marcarelli et al., 2011; Hood et al., 2014). Trophic selectivity occurs when the relative abundances of resource types in a consumer's diet differ from their relative abundances in the environment (Chesson, 1978). Therefore, selective feeders are consumers discriminating among the available resources in the environment by selecting specific resources (i.e. resources which are more represented in consumer's diet than in the environment; Feinsinger et al., 1981; Peterson & Ausubel, 1984).

The degree of trophic selectivity in organisms has been predicted to vary with the availability of resources in the environment (e.g. Anthony & Kunz, 1977; Tinker et al., 2008; Correa & Winemiller, 2014). According to the predictions of the optimal foraging theory (OFT; Roughgarden, 1972; Pyke et al., 1977; Krebs et al., 1997), an increase in resource availability for organisms feeding on a variety of resources should lead to an increased selectivity on their preferred resources. These OFT predictions have been experimentally demonstrated in rodents and wasps by Lacher (1982) and Armstrong & Stamp (2003), respectively. While informative, these intra-species experimental tests were effectively bound to a narrow range of resource availability change (3-fold) and hardly represented the actual resources distribution in natural ecosystems (controlled laboratory experiment; choice between two artificially-contrasted food

sources). Testing the relationship between resource availability and selectivity in a biologically realistic and relevant framework would thus require to study *in situ* a wide range of environmental trophic conditions, a condition hardly met without working at the species level. Although previous experimental validations of OFT predictions were only obtained at the individual and population levels, we can expect the same kind of relationship at the species level following the hypothesis developed by Evans et al. (2005). These authors predicted that an increase in total resource availability would increase the abundance of rare resources that would then become available in sufficient quantity to enable organisms to selectively feed on them. Consequently, trophic selectivity of species would increase with resource availability in the field.

Trophic selectivity in animals has also been expected to vary with the qualities of resources present in their environment (e.g. Cruz-Rivera & Hay, 2000; Zandona et al., 2011). Indeed, discriminating between the available resources would imply more benefit for the organism in an environment where resources span a wide range of nutritional qualities than in an environment where all resources have similar qualities. Many proxies of resource quality have been proposed, including the widely used C:N and C:P ratios (Hessen et al., 2007; Hillebrand et al., 2008). According to the ecological stoichiometry theory (Sternner & Elser, 2002; Elser, 2006; Hessen et al., 2013; Hood et al., 2014), organisms would select their trophic resources to minimize the stoichiometric mismatch between their elemental composition and that of their consumed resources. They are expected to feed selectively on the resources whose C:N and/or C:P ratios are as close as possible to their own ratios to optimize their nutrient acquisition. This selective stoichiometric choice would be achievable only in environments with resources of differing qualities. As a consequence, trophic selectivity would increase with the diversity (i.e. heterogeneity) of available resources in terms of stoichiometric quality.

101 Although theory predicts that the availability as well as the heterogeneity in the quality of the
102 resources influences trophic selectivity, *in situ* explicit tests of their respective effects on selective
103 feeding are still lacking. The present paper aims to fill this gap by studying *in situ* the trophic
104 selectivity among species living along a gradient of resource availability. In this context, four
105 analytical challenges widely recognized in the literature must be addressed (Evans et al., 2005).
106 First, a wide environmental gradient in resource availability along which many closely related
107 species can be sampled is necessary. Indeed, earlier comparisons between two contrasted
108 environments are instructive (e.g. Huntsman et al., 2011), but lack support for generalization.
109 Second, the quantification of resource availability along the studied gradients was often limited to
110 coarse estimations using various databases and models without precise assessments of available
111 resources in the field (Gende et al., 2001; Kaspari, 2001; Birkhofer & Wolters, 2012). Third,
112 accurate long-term diet estimation requires to integrate resource assimilation over a large period
113 of time. However, estimations of species diet relying on gut content analyses, measurements of
114 resource consumption or visual observations (Lacher, 1982; Armstrong & Stamp, 2003;
115 Huntsman et al., 2011; Zandonà et al., 2011) provide a snapshot of the diet at a given time and do
116 not consider differences in digestibility among resources (e.g. Trumble et al., 2003). Fourth, most
117 broad-scale studies did not account for the phylogenetic relatedness among species even though it
118 is now acknowledged that most biological traits are phylogenetically conserved (Felsenstein,
119 1985; Blomberg et al., 2003; Pienaar et al., 2013).

120

121 In the present paper, we addressed the aforementioned challenges through a dedicated sampling
122 design and the use of accurate analytical and statistical methods. We focused on aquatic isopod
123 species of the family Asellidae which represent ideal biological models due to their distribution
124 along a wide environmental gradient ranging from deep cave systems (among the most energy-
125 limited environments on Earth; Poulson & Lavoie, 2000; Gibert & Deharveng, 2002; Venarsky et
126 al., 2014) to highly-productive surface streams. Moreover, their phylogeny has been precisely

described (Morvan et al., 2013), enabling to account for phylogenetic inertia. Based on a deep knowledge of their habitat and ecology (Simon et al., 2003; Leberfinger et al., 2011; Mermillod-Blondin et al., 2013; Eme et al., 2014; Francois et al., 2016a), a standardized sampling design was built to evaluate the abundance as well as the stoichiometric quality (based on C:N and C:P ratios) of all available resources in diverse environments. This precise characterization of trophic resources was combined with diet determination to measure the degree of selectivity of each species. Isopod diets were determined by analyzing the C and N stable isotope ratios of individuals and their resources. The isotopic composition of an individual is a long-term integrator of its trophic choices and reflects what has been actually assimilated in its tissues, summarizing ingestion and digestibility of each resource (Peterson & Fry, 1987; Fry & Sherr, 1989; Traugott et al., 2013).

Based on 15 closely related species of Asellidae, we obtained an original dataset enabling to test two predictions along one of the largest gradient of resource availability existing in natural ecosystems. First, trophic selectivity of aquatic asellids should increase with resource availability following the prediction of Evans et al. (2005). Second, trophic selectivity should increase with the diversity in stoichiometric quality of resources according to the ecological stoichiometric theory.

MATERIALS AND METHODS

Biological model

Our data set comprised 15 species of Asellidae collected in environments spanning a wide range (from caves to surface streams) of freshwater habitats in Southern Europe (Table 1). Species of isopods belonging to this family are known to feed on a wide range of food resources, including coarse and fine particulate organic matter (CPOM and FPOM), filamentous algae, aquatic

153 macrophytes, epilithic biofilm and periphyton (Moore, 1975; Marcus et al., 1978; Rossi & Fano,
154 1979; Basset & Rossi, 1987; Arakelova, 1996; Leberfinger et al., 2011; Francois et al., 2016a).
155 Given this diversity of food resources, we used a commonly accepted procedure which consists in
156 discriminating between broad food compartments (Simon et al., 2003; Leberfinger et al., 2011).
157 These compartments correspond to coarse particulate organic matter (CPOM; 1 mm < particle
158 size < 6 mm), fine particulate organic matter (FPOM; particle size < 1 mm), roots, algae,
159 sedimentary biofilm developed on sand particles (200 μ m < particle size < 1000 μ m), biofilm
160 attached to the debris of animal cuticle (epicuticular biofilm) and thick biofilm attached to
161 submerged rocks (epilithic periphyton).

162

163 **Sampling**

164 The 15 species were sampled from July to October 2011 in caves, springs and streams. Only one
165 focal asellid species was present in each sampling site. For each species, from 9 to 18 individuals
166 were collected (at sight or using the Bou-Rouch method (Bou, 1974) or a Surber sampler), except
167 for the species *P. parvulus* with only 4 sampled individuals (see Table S1 in Supporting
168 Information). In parallel, the trophic resources known to be edible by Asellidae were sampled in
169 each environment. This sampling consisted in 3 replicates per environment, in order to be
170 representative of the spatial heterogeneity. Following the approach of Huntsman et al. (2011),
171 each replicated sampling consisted in collecting on a depth of 1 cm all available resources
172 occurring on a standardized area (256 cm²). After sampling, both samples of trophic resources and
173 isopods were flash-frozen with liquid nitrogen in the field for conservation during the journey
174 back to the laboratory.

175

176 **Trophic resources in the environment**

177 At the laboratory, all collected trophic resources were unfrozen and sorted according to the food
178 categories described above by using nets of different mesh sizes (200 μ m, 1 mm and 6 mm). Food

categories were then frozen, freeze-dried and weighted to obtain a dry mass of each trophic resource per square meter (g of Dry Weight (DW) / m²) for each site (Table S2). All obtained resource samples were then ground using a ball mill grinder (Retsch MM-200) for homogenization before the determination of resource elemental composition (%OC, %N and %P, i.e. the organic carbon (OC), nitrogen and available phosphorus content of each resource, as % of dry mass; Table S2). For sedimentary biofilm and periphyton, samples were treated with 1N HCl before OC analysis to remove carbonates using the ‘capsule method’, as described in Brodie et al. (2011). Elemental composition was then measured alongside the determination of isotopic ratios (see below), allowing to calculate the C:N stoichiometric molar ratio of each resource (Table S2). The quantity of each resource (g of DW / m²) and its elemental composition (%OC) enabled to calculate the OC content for each resource per site (g of OC sequestered in a given resource . m⁻²).

190

191 **Phosphorus content of isopods and trophic resources**

Chemical analyses were performed to determine the phosphorus content of isopods and of their trophic resources (% of P per dry mass). For isopods and trophic resources (except sedimentary biofilm and periphyton), P content was determined on ground samples digested in an autoclave for 30 minutes at 121°C and 15-20 psi with potassium persulfate and sulfuric acid to convert all phosphorus to orthophosphate. Then, orthophosphate content of each sample was determined by the molybdate/ascorbic acid blue method (Murphy & Riley, 1958). For sedimentary biofilm and periphyton, as all fractions of total phosphorus are not available for the biota (Golterman, 2001), the quantity of P available for asellids was determined by measuring the available organic P. Following Ni et al. (2016), available organic P was extracted from resource samples (n = 3 per resource type) with NaOH 1M. After extraction during 16 hours, the supernatant was collected, its pH was stabilized with HCl (3.5M) and the extracted P that has been converted in orthophosphate was quantified using the molybdate/ascorbic acid blue method (Murphy & Riley, 1958).

204

205 **Stable isotope measurements**

206 C and N stable isotope ratios were measured for all isopods (previously freeze-dried) and freeze-
207 dried resource samples, jointly with the measure of their elemental composition (%OC, %N). This
208 joint analysis was not possible for sedimentary biofilm and periphyton samples which were
209 previously treated for carbonate removal using HCl ('capsule method'). For these samples, we
210 performed separate analyses to avoid any damage of the isotope ratio mass spectrometer with
211 chloride. According to Francois et al. (2016a), the 'rinse method' (Brodie et al., 2011) was used for
212 chloride removal before measurement of isotopic ratios. Elemental composition and stable isotope
213 ratios of C and N were measured using an isotope ratio mass spectrometer (Isoprime100, Isoprime
214 Ltd, Manchester, UK) coupled in continuous flow with an elemental analyzer (Thermo FlashEA
215 1112, ThermoElectro, Milan, Italy). In-house standards calibrated against IAEA-N1, IAEA-N2,
216 IAEA-CH6 and IAEA-C3 reference materials were analyzed with the samples, and standard
217 deviations of replicate analyses were lower than 0.20 ‰. C and N stable isotope compositions
218 were expressed as δ in ‰ with V-PDB ($\delta^{13}\text{C}$) and Air ($\delta^{15}\text{N}$) as standards.

219

220 **Diet determination**

221 The diets of the 15 species were determined by analyzing the C and N stable isotope signatures of
222 isopods and their available resources with the Bayesian mixing model SIAR (Parnell et al., 2010).
223 The model was run with 1 million of iterations, 50% of burn-in and a thinning of 250. As the diet-
224 tissue discrimination has not been determined for isopod species, we used the commonly accepted
225 values of 2.54 ± 1.27 and 0.4 ± 1.3 for nitrogen ($\Delta^{15}\text{N}$) and carbon isotope discrimination
226 ($\Delta^{13}\text{C}$), respectively (Vander Zanden & Rasmussen, 2001; Post, 2002; Vanderklift & Ponsard,
227 2003). The choice of discrimination factors for non-model organisms is tricky and may impact the
228 results of the mixing model. The isoplots (Fig. S1) suggest that the value chosen for $\Delta^{15}\text{N}$ may be
229 underestimated for some groundwater species, as some individuals fall outside the source polygon
230 in terms of $\delta^{15}\text{N}$ (exceeding by up to 2‰ the expected values) in four species (*P. arthrodilus*, *B.*

231 *molinai*, *P. spelaeus* and *P. parvulus*). Although a higher $\Delta^{15}\text{N}$ would have corrected the
 232 discrepancies in isoplots for these four species, it would not have significantly changed diet
 233 estimations for these species. Thus, the commonly accepted value of 2.54 ± 1.27 for $\Delta^{15}\text{N}$ was
 234 used to be consistent in the analysis of the 15 species. Posterior probability distributions of the
 235 SIAR models were checked for unimodality. Median and 95% credibility interval were reported
 236 for each resource contributing to the diet (Table S3).

237 To determine the sample size of consumers collected in each environment, we followed the
 238 guidelines of Jackson et al. (2011) who recommend a minimum of 10 individuals to properly
 239 quantify the species trophic niche, at the exception of *P. parvulus* for which only 4 individuals
 240 could be collected. For this species, the small sample size implied a higher uncertainty in the diet
 241 reconstruction, and a potential underestimation of trophic niche size.

242 Including too many resources in Bayesian mixing models can lead to confounding results (e.g.
 243 inconsistent or bimodal posterior distributions). In this case, Hopkins & Ferguson (2012) and
 244 Phillips et al. (2014) advised to combine biologically related resources having similar isotopic
 245 signatures to increase the predictive power of the model. Thus, FPOM and CPOM were combined
 246 in a unique resource (hereafter 'FPOM + CPOM') when more than 3 resources were considered in
 247 the diet of isopods and when the average $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of FPOM and CPOM differed by no more
 248 than 1.3‰. This resource combination successfully improved the diet determination for 2 species:
 249 *P. coiffaiti* and *P. ibericus*. For one species (*P. coiffaiti*), we measured differences between the C
 250 isotopic signatures of resources (algae) and consumers that were not consistent with the
 251 discrimination factor ($\Delta^{13}\text{C} = 0.4 \pm 1.3$). This discrepancy did not influence our results as
 252 discussed in the Supporting Note S1. It is worth noting that the bayesian mixing models used for
 253 diet reconstruction usually generate large confidence intervals, as they integrate all biological
 254 uncertainties. If the bayesian mixing model does not properly converge, results are biased towards
 255 the null generalist assumption, i.e. a diet composed of n resources in proportion $1/n$ (Brett 2014).
 256 In our dataset, only 3 out of the 15 reconstructed diets display a pattern close to this

257 equiproportional null scenario (*P. arthrodilus*, *P. granadensis* and to a lesser extent *B. peltatus*),
258 which may indicate uncertainty in diet reconstruction. The 15 isoplots are displayed in the Fig.
259 S1.

260

261 **Estimated variables and data analyses**

262 *Standardization of the availability of trophic resources*

263 Trophic resources had contrasted elemental composition (e.g. the %OC ranged from 0.06% for
264 sedimentary biofilm per mass of dry sediment to 47% for roots). Thus, the absolute quantity of a
265 given resource (in g of DW / m²) is not representative of its energetic significance for a consumer.
266 The OC content sequestered in a given resource provides a much more accurate estimation of this
267 significance and thus the availability of each trophic resource was expressed as amount of OC . m⁻².
268 ².

269 *Indices of trophic selectivity*

270 Several indices have been proposed in the literature to measure the degree of selectivity of a
271 consumer (Hurlbert, 1978; Feinsinger et al., 1981; Smith, 1982). They all rely on a comparison
272 between the proportions of resources in the environment (i.e. here their relative abundances in
273 terms of OC content) and their respective proportions in the consumer diet. If these environment
274 and diet proportions match closely, this consumer is feeding non-selectively on all available
275 resources. If there is a strong mismatch between these proportions (i.e. between the availability
276 and the use of resources), this consumer is feeding highly selectively.

277 We evaluated the degree of selectivity of the 15 studied species by calculating the Proportional
278 Similarity index (PS) proposed by Feinsinger et al. (1981):

$$279 \quad PS = 1 - 0.5 * \sum | q_i - p_i | \quad (1)$$

280 where p_i stands for the availability of the resource i in the environment (i.e. the relative abundance
281 in terms of OC content) and q_i stands for the dietary use of this resource i (i.e. the proportion in
282 the diet, in terms of C and N atoms assimilated in the consumer tissues). This index measures the

283 similarity between the distribution of resource availability and resource use. To highlight the
284 dissimilarity between these distributions (i.e. the selectivity), we modified PS to PS':

285 Trophic selectivity: $PS' = 1 - PS$ (2)

286 The obtained index PS' thus actually reflects the degree of consumer selectivity and ranges from 0
287 (non-selective feeding) to 1 (highly selective feeding). Two other selectivity indices were
288 proposed in the literature: the index B of Hurlbert (1978) and the index FT of Smith (1982). These
289 two indices (described in the Supporting Methods S1 and Table S1) were highly correlated with
290 the PS index (Spearman correlation coefficients $\rho = 0.95$ and 0.98 for B and FT respectively).
291 Thus, we only presented the results obtained with the index PS'. To consider the uncertainty
292 inherent to Bayesian mixing models, for each species 1,000 diet proportion vectors (i.e. $[p_1; p_2; \dots;$
293 $p_n]$ for n resources contributing to the diet) were sampled from the posterior distributions output
294 by SIAR and subsequently used to draw a distribution of PS' values and calculate the 95%
295 credibility interval for PS'.

296 *Resource availability per site*

297 Based on our OC quantifications, the resource availability (RA) in each sampling site was
298 estimated by the mass of OC per surface unit (g of OC . m⁻²), summed over all collected resources
299 edible by asellids (an approach similar to Srivastava & Lawton, 1998; Thompson & Townsend,
300 2005).

301 *Quality of resources*

302 The quality of a given resource was estimated by the strength of the elemental imbalance between
303 this resource and the consumer according to the theory of ecological stoichiometry (Sternner &
304 Elser, 2002; Frost et al., 2005; Hessen et al., 2013). The stronger is this elemental imbalance, the
305 less beneficial it is for the consumer to feed on this resource. For a given resource i , this
306 stoichiometric mismatch ($SM_{i(C:N)}$ or $SM_{i(C:P)}$) was measured by the difference between the C:N (or
307 C:P) ratio of the resource i and the C:N (or C:P) ratio of the consumer (Elser & Hassett, 1994;

308 Sterner & Elser, 2002) (see Table 1 for the C:N and C:P of isopods and Table S2 for the C:N and
309 C:P of resources):

$$310 \quad SM_{i(C:N)} = C:N_{\text{resource } i} - C:N_{\text{consumer}} \quad \text{and} \quad SM_{i(C:P)} = C:P_{\text{resource } i} - C:P_{\text{consumer}} \quad (3)$$

311 The Resource Diversity in terms of Quality for C:N (RDQual_{C:N}) or for C:P (RDQual_{C:P}) was then
312 estimated in each environment by calculating the weighted variance (σ^2) of the stoichiometric
313 mismatches in C:N or C:P of all available resources:

$$314 \quad \sigma^2 = \sum p_i \cdot (SM_{i(C:N \text{ or } C:P)} - \mu^*)^2 \quad (4)$$

315 where μ^* is the weighted mean of the SM of all resources weighted by their relative abundance p_i .
316 As the total number of available resources (RR; Resource Richness) differed between sites, this
317 weighted variance was corrected for sample size and transformed in a weighted standard
318 deviation:

$$319 \quad \text{Resource Diversity in terms of Quality: } RDQual_{(C:N \text{ or } C:P)} = \sqrt{\sigma^2 / ((RR - 1) / RR)} \quad (5)$$

320 This index measures the heterogeneity in the stoichiometric mismatches of all available resources
321 in the environment. Low RDQual_(C:N or C:P) indicates that all available resources in the environment
322 have similar stoichiometric qualities for the consumer. On the opposite, RDQual_(C:N or C:P) increases
323 when the stoichiometric qualities for the consumer increasingly diverge among available
324 resources and when these resources having different stoichiometric qualities are available in even
325 proportions (this latter property is similar to an evenness index which is maximal when all
326 resources are in even proportions and minimal when one resource is predominant). According to
327 our prediction, trophic selectivity would increase when RDQual_(C:N or C:P) increases.

328 *Number of trophic resources*

329 In addition to Resource Availability (RA) and Resource Diversity in terms of Quality (RDQual_{(C:N}
330 or C:P)), the number of trophic resources present in each environment can also influence trophic
331 selectivity by a purely probabilistic effect. Indeed, an increase in the number of resources
332 enhances the trophic choice and influences the probability of selective feeding by organisms,
333 independently of resources quality. To include this probabilistic hypothesis in our data analyses,

the Resource Diversity in terms of Quantity (RDQuant, Table S1) was estimated in each environment with a Shannon index that integrates resource number and evenness (relative abundance) across all available resources:

$$\text{Resource Diversity in terms of Quantity: RDQuant} = - \sum p_i \cdot \ln(p_i) \quad (6)$$

where p_i is the relative abundance of the resource i in the environment (relative to the total amount of OC).

Phylogenetic analyses

Evaluating the relative influences of the Resource Availability (RA) and the Resource Diversity in terms of Quality (RDQual_(C:N or C:P)) and in terms of Quantity (RDQuant) on trophic selectivity needs to take into account the phylogenetic relationships among the 15 species. Taking advantages of whole transcriptome sequencing for the 15 species considered in the present study (Francois et al., 2016b), we extracted a set of 386 1-to-1 orthologous gene alignments present in all species. The concatenated alignment was used to reconstruct a phylogram with phyML with a GTR+G+I model of evolution (Guindon et al., 2010). Using this topology, we estimated a chronogram with mcmctree (Yang 2007) using default parameters, two independent runs to check chains convergences and setting the divergence between the *Bragasellus* and *Proasellus* to be not older than 150 MYA.

Statistical analyses

According to the recommendations of Warton & Hui (2011) for proportion data, the trophic selectivity index PS' has been logit-transformed prior to statistical analyses. RA and RDQual_{C:P} have been log-transformed prior to statistical analyses to correct for non-normality of the data. We checked for multi-collinearity among the four environmental variables (RA, RDQual_{C:N}, RDQual_{C:P} and RDQuant) using variance inflation factors (VIF) and found them to be in acceptable range (all VIF < 2.4; see Zuur et al., 2010). We used Phylogenetic Generalized Least-Squares models (PGLS; Martins & Hansen, 1997) to evaluate the influence of the four environmental variables on trophic selectivity while accounting for the phylogenetic relationships

among the focal species (using the R package caper). Blomberg's K was estimated using the function phylosig in phytools R package (Revell, 2012). As a dataset based on 15 asellid species did not enable to statistically test the influence of four environmental variables and their interactions on trophic selectivity, we decided to perform two separate analyses with RA, RDQual_{C:N} and RDQuant as explaining variables in one hand and RA, RDQual_{C:P} and RDQuant as explaining variables in another hand. For each analysis, we performed a procedure of model selection based on information theory (Burnham & Anderson, 2002) and the ranking of models according to Akaike's Information Criterion corrected for small sample size (AICc, MuMIn R package), starting from the most comprehensive model (including all interactions). We checked that the order of entry of each variable into the PGLS models did not change significantly the results. The adjusted McFadden's pseudo-R² (McFadden, 1974) was computed for all tested models to provide for a comparable estimation of the amount of explained variance among models with different number of parameters. The normality of the residuals of the final 'best' model (with the smallest AICc) was checked.

All data analyses were performed with R 3.4 software (R Development Core Team 2016).

RESULTS

Trophic resources, isopod diets and trophic selectivity

Our dataset comprised 15 environments characterized by a wide range of resource availability (RA) with amounts of OC . m⁻² varying from 1.3 g to 57.6 g (Fig. 1, Tables S1 & S2). The Resource Diversity in terms of stoichiometric Quality was also contrasted among environments with RDQual_{C:N} indexes ranging between 1.1 and 20.1, and RDQual_{C:P} indexes ranging between 4.0 and 633.4 (Table S1). There was no collinearity between the four predictor variables RA, RDQual_{C:N}, RDQual_{C:P} and RDQuant (VIF = 1.65, 1.60, 2.44 and 1.49 respectively). Seven trophic resources were collected and quantified in the environments. Three resources (epicuticular

386 biofilm, periphyton and algae) were found in only one or two environments whereas three
387 resources (fine and coarse particulate organic matter, and sedimentary biofilm) were present in
388 almost all environments (Table 2, Fig. 2). Each trophic resource was dominant ($> 50\%$) in at least
389 one environment, at the exception of periphyton and algae (Table 2).

390 Isotopic measurements and analyses using Bayesian mixing models showed that all available
391 trophic resources were found in the diets of at least one asellid species (Fig. 2, Table 2). For the
392 three resources found in almost all environments (fine and coarse particulate organic matter, and
393 sedimentary biofilm), their contributions to animal diets widely varied across species (Fig. 2). For
394 example, the sedimentary biofilm could be minor ($< 10\%$) or dominant ($> 50\%$) in the diets of
395 asellid species.

396 The PS' trophic selectivity indexes based on the match between isopod diets and resource
397 availabilities ranged from 0.042 to 0.919 (Fig. 1, Table S1). The dataset thus comprised non-
398 selective feeding (PS' close to 0) and highly selective feeding (PS' close to 1) species of isopods.

399

400 **Relationships between trophic selectivity and environmental variables**

401 The first procedure of model selection starting from the comprehensive model including resource
402 availability (RA), resource diversity in terms of quantity (RDQuant) and in terms of quality based
403 on C:N ratios (RDQual_{C:N}) and all interactions as predictors of trophic selectivity (Table 3)
404 resulted in the selection of a model with the amount of available resources (RA) as unique
405 predictor of trophic selectivity (PS') (PGLS; P-value = 0.0031 and $R^2 = 0.46$; equivalent to Fig.
406 3a). A comparable result was obtained when resource diversity in terms of quality based on C:P
407 ratios (RDQual_{C:P}) replaced RDQual_{C:N} as predictor of trophic selectivity (Table S4). PS' was
408 positively correlated with RA (Fig. 3a). PS' was not correlated with the resource diversity in terms
409 of quality for C:N (RDQual_{C:N}, Fig. 3c) or for C:P (RDQual_{C:P}, Fig. 3d), neither with the resource
410 diversity in terms of quantity (RDQuant, Fig. 3b). Trophic selectivity showed no phylogenetic
411 signal among the 15 species (Blomberg's $K = 0.09$, P-value = 0.94; Blomberg et al. 2003).

412 Among the 15 species studied, we found that 4 out of the 5 species living in environments with
413 more than 25 g of OC . m⁻² were selective-feeders (i.e. with a PS'>0.5, Fig. 1): *P. solanasi*, *P.*
414 *beticus*, *P. ibericus* and *B. peltatus*. These species always fed selectively on resource types that
415 were not dominant in the environment. For example, the diet of *P. beticus* comprised 56% of
416 sedimentary biofilm whereas this resource type represented less than 2% of OC resources in the
417 environment (Fig. 2). There was a tendency across species for feeding selectively on sedimentary
418 biofilm but other resource types were also selected. If *P. solanasi* and *P. beticus* selected solely
419 sedimentary biofilm in the environment, the two other selective-feeding species fed selectively on
420 2 or more resources: sedimentary biofilm and roots for *P. ibericus*, and CPOM, FPOM and
421 sedimentary biofilm for *B. peltatus*.

422

423

424 **DISCUSSION**

425 The dataset collected for the present study allowed to test the influence of trophic environmental
426 conditions (resource availability and diversity) on the trophic selectivity of aquatic species across
427 a broad range of environments. In this dataset, the absence of collinearity between the predictor
428 variables allowed to discriminate the relative influences of trophic conditions on selectivity.
429 Coupling an accurate quantification of trophic resources in a wide range of environments with a
430 fine determination of animal diets using stable isotopes showed that the amount of available
431 resources rather than the diversity of resource (in terms of quality and quantity, respectively
432 RDQual_(C:N or C:P) and RDQuant) increases trophic selectivity of aquatic isopod species. The
433 positive relationship between resource availability and trophic selectivity was probably uncovered
434 in this study thanks to the wide range of resource availability studied (from 1.3 to 57.6 g of OC .
435 m⁻²) which incorporated both surface and cave streams, these latter being considered as one of the
436 most energy-limited environments on Earth (Poulson & Lavoie, 2000; Simon et al., 2003;
437 Venarsky et al., 2012, 2014). By contrasting environmental conditions, we demonstrated that

species tend to feed non-selectively at lower bounds of resource availability whereas selective-feeding species were detected in resource-rich environments. Intraspecific niche partitioning (*sensu* Bolnick et al., 2002) could theoretically affect this pattern, as non-selective species can be composed of individuals feeding selectively on different resources. However, non-selective species at the lower bound of resource availability do not display particularly dispersed individuals (see the isoplots in Fig S1; except for *B.molinai*), neither do they display distinct groups of individuals feeding on (i.e. isotopically close to) different resources, suggesting that the observed selectivity pattern should be similar at the species and individual levels. The analysis also suggested that trophic selectivity was not phylogenetically conserved among the focal species.

One major finding of the present study is the demonstration of a positive relationship between resource availability and trophic selectivity in aquatic isopods. This relationship supports the hypothesis that trophic selectivity was low in resource-poor environments probably because species cannot fulfill their energy requirements by foraging on a reduced set of the available resources (Thompson & Colgan, 1990; Tinker et al., 2008). Nevertheless, the mechanisms explaining selective feeding by isopods in resource-rich environments remain elusive. Our results did not validate the mechanism proposed by Evans et al. (2005) to explain the positive relationship between resource availability and trophic selectivity. These authors hypothesized that an increase in total resource availability could increase some rare resources which would then become available in sufficient quantity for species to selectively feed on them. However, sedimentary biofilm was selected by many selective-feeding isopods in resource-rich environments even when the amount of this trophic resource was lower than in resource-poor environments (eg. see *P. ibericus*, Fig. 2). These results did not give a strong support to the mechanism presented by Evans et al. (2005) to explain the positive relationship between resource availability and trophic selectivity. The mechanisms leading to increased trophic selectivity with

464 increasing resource availability might also have been associated with other ecological factors. For
465 instance, the species-energy theory indicates that species richness increases with resource
466 availability in the environment (Bonn et al., 2004). In such condition, interactions among species
467 would be stronger in resource-rich than in resource-poor environments, which could increase
468 niche partitioning among species (MacArthur & Levins, 1967). This niche partitioning for food
469 and/or habitat would therefore enhance the mismatch between the relative abundances of
470 resources in species diet and their relative abundances in the environment (i.e. leading to an
471 increased trophic selectivity). For example, the microhabitat partitioning reported for marine
472 amphipods to avoid competitive exclusion and predation (Cruz-Rivera & Hay, 2000; Best &
473 Stachowicz, 2014) indicates that each amphipod species does not have access to the whole range
474 of resources present in the environment. We can expect the same mechanism to occur in our
475 experiment for isopod species living in resource-rich environments where biotic interactions are
476 expected to be strong. Demonstrating this mechanism will represent a significant advance in our
477 understanding of ecosystem structure and functioning, but represents a difficult task as it requires
478 to finely describe the spatial distributions of all species in each environment but also the biotic
479 interactions among these species.

480

481 Our results demonstrated that trophic selectivity was associated with resource availability but not
482 with the diversity (i.e. heterogeneity) in resource quality. We expected that the stoichiometric
483 constraints would have determined the resource used by consumers in accordance with the
484 stoichiometric ecological theory (Sternner & Elser, 2002). In other words, a high diversity of
485 resource stoichiometric qualities would have generated selective feeding whereas non-selective
486 feeding was expected in environments harboring resources of comparable stoichiometric qualities.
487 These predictions were not validated because asellid species fed selectively or non-selectively
488 regardless of the diversity in resource quality in their environment. For example, three species of
489 selective feeders ($PS' > 0.5$) were observed in environments displaying comparatively low

490 diversity in resource quality for C:N ($RDQual_{C:N} < 5$ in our dataset, figure 3b), whereas no
491 selective feeding was expected under the stoichiometric hypothesis in these environments.
492 Admittedly, some criticisms can be drawn concerning the use of stoichiometric C:N or C:P ratios
493 to evaluate resource quality. Measuring C:N and C:P ratios of a given trophic resource as a ‘bulk’
494 can skew our analyses, as detritivores can selectively ingest and/or assimilate only a subfraction of
495 the considered pool (e.g. the conditioned leaves colonized by fungi; Constantini and Rossi, 1995).
496 Besides, detrimental chemicals such as phenolic compounds may reduce the palatability of
497 trophic resources without affecting their C:N and C:P ratios (Oates et al., 1980; Dorenbosch &
498 Bakker, 2011) and compounds which cannot be synthesized by animals such as sterols or essential
499 fatty acids may influence resource selectivity in a different way than C:N and C:P ratios (Wagner
500 et al., 2013; Sperfeld et al., 2017). However, our results gave support to the idea of a dietary
501 mixing strategy in asellids because most selective-feeding species (eg. *P. ibericus* and *B. peltatus*)
502 selected at least 2 trophic resources that were largely contrasted in terms of C:N and C:P (e.g.
503 averaged C:N : 10.2 ± 4.2 for sedimentary biofilms, 21.4 ± 8.2 for FPOM, 22.4 ± 11.8 for roots,
504 and 29.3 ± 18.9 for CPOM; Table S2). This suggests that asellid species need to mix trophic
505 resources for nutrient acquisition (nutrient complementation hypothesis; Hägele & Rowell-Rahier,
506 1999). Thus, we can assume that trophic selectivity in aquatic isopod species was not driven by
507 the stoichiometric diversity of available resources because of the need for essential compounds
508 that were not contained in the trophic resources having the lowest C:N or C:P mismatches with
509 organisms (ie. best resources in terms of stoichiometric quality). To address this topic, use of
510 specific markers such as amino acids and fatty acids and their isotopic compositions would be
511 pertinent to have more precise reconstructions of asellid diets and more precise quantifications of
512 the diversity of trophic resources in the environment than those obtained from bulk analyses of N
513 and C (Bec et al., 2011).

514

Although we observed that asellid species could selectively feed on several trophic resources, sedimentary biofilm was generally found in higher proportion in the diet of selective-feeding isopods than in their environment. Besides, a previous study by Francois et al. (2016a) demonstrated that two asellid species exhibited a strong specialization (i.e. a higher performance; Irschick et al., 2005; Whitfield et al., 2009; Devictor et al., 2010; Poisot et al., 2011) on sedimentary biofilm. Feeding experiments in the laboratory showed that both species assimilated about 10 times more carbon and at least 4 times more nitrogen from sedimentary biofilm than from coarse and fine particulate organic matter. Thus, the fact that selective feeding on sedimentary biofilm was common in the present study might have resulted from a positive fitness consequence for isopods to use this resource. Nevertheless, we cannot conclude from the present experiment that selective feeding on sedimentary biofilm conferred a better performance to asellid species than feeding on other resources without measurements of the fitness of all isopod species when feeding on individual resources present in their environments. Coupling our original dataset on trophic selectivity of asellid species with measures of their performance on the available resources would provide a unique opportunity to document and understand the relationship between trophic specialization and selectivity in regard to resource availability.

FIGURES CAPTION

Figure 1: Phylogenetic tree of the 15 isopod species, plotted along with the resource availability in their environments and their trophic selectivity PS'. Resource availability in the environment is expressed in grams of Organic Carbon (OC) per square meter.

Figure 2: Relative abundance and use of trophic resources in the 15 sampling sites.

541 For each site, the upper panel presents the relative abundance of all available resources in the
542 environment (in %; see Methods for details on the underlying calculations), while the lower panel
543 presents the contribution of these resources to the diet of isopods (in %).

544 For a given environment, the total resource availability (indicated on the left hand side of the
545 barplots, in grams of Organic Carbon per square meter) is summed over all available resources.
546 Environments (and their corresponding species) are sorted by increasing resource availability.

547

548 **Figure 3: Relationships between the trophic selectivity PS' and (a) the Resource Availability**
549 **(RA) as grams of OC . m⁻²; (b) the Resource Diversity in terms of Quantity (RDQuant); (c)**
550 **and (d) the Resource Diversity in terms of stoichiometric Quality based on C:N and C:P**
551 **ratios respectively (RDQual_{C:N} and RDQual_{C:P}).**

552 These plots present biological data prior to transformation. Only significant regression lines are
553 drawn. See M&M for details on the credibility intervals drawn for PS'.

554

555

556 **ACKNOWLEDGEMENTS**

557 We thank the following collaborators and organizations for their precious help in collecting
558 biological material: Alhama de Granada municipality, M. Isabel Carreira, T. Datry, D. Eme, Cueva
559 de Valporquero, Junta de Andalucía (Hundidero – Gato) and Rafael Orozco. We are grateful to
560 David Eme for valuable comments on statistical analyses and Pierre Marmonier for a careful
561 reading and editing of the manuscript. We thank the editors and the anonymous reviewers for their
562 valuable comments on an earlier version of this manuscript.

563

564 **AUTHORS' CONTRIBUTIONS**

565 All authors conceived the ideas and designed methodology; CF, LS, TL, FM and CD collected
566 field data; CF, FMB and LS carried out the elemental and stable isotope analyses, analysed the

data and wrote the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

DATA ACCESSIBILITY

Datasets supporting this article are available in the electronic supplementary material. Raw isotopic data are available from the Dryad Digital Repository (<https://doi.org/10.5061/dryad.v9s4mw6qw>).

COMPETING INTERESTS

We have no competing interests.

FUNDING

This work was supported by grants from the Agence Nationale de la Recherche (ANR-08-JCJC-0120-01 DEEP) and the Centre National de la Recherche Scientifique (APEGE 2012 'Stoichiogenomics' No. 70632 and EC2CO 2013-2014 'Ecosphere Continentale et Cotiere' - 'CoCoNutS' project).

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911 **SUPPORTING INFORMATION**

912 Additional supporting information may be found in the online version of this article.

913 Figure S1: Isoplots (carbon and nitrogen stable isotope compositions of trophic resources and
914 individuals) for the 15 isopod species.

915 Methods S1: Calculation of the selectivity indices of Hurlbert and Smith.

916 Note S1: Discussion of the isotopic analyses (for *P. coiffaiti*).

917 Table S1: Environmental variables and indices of trophic selectivity.

918 Table S2: Environment characterization: abundance and elemental composition of available
919 trophic resources.

920 Table S3: Diet determination: median and 95% credibility interval of resource contributions to the
921 diet.

922 Table S4: Procedure of model selection with $RDQual_{C:P}$ (Resource Diversity in terms of Quality,
923 based on C:P ratios).

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Figure 1

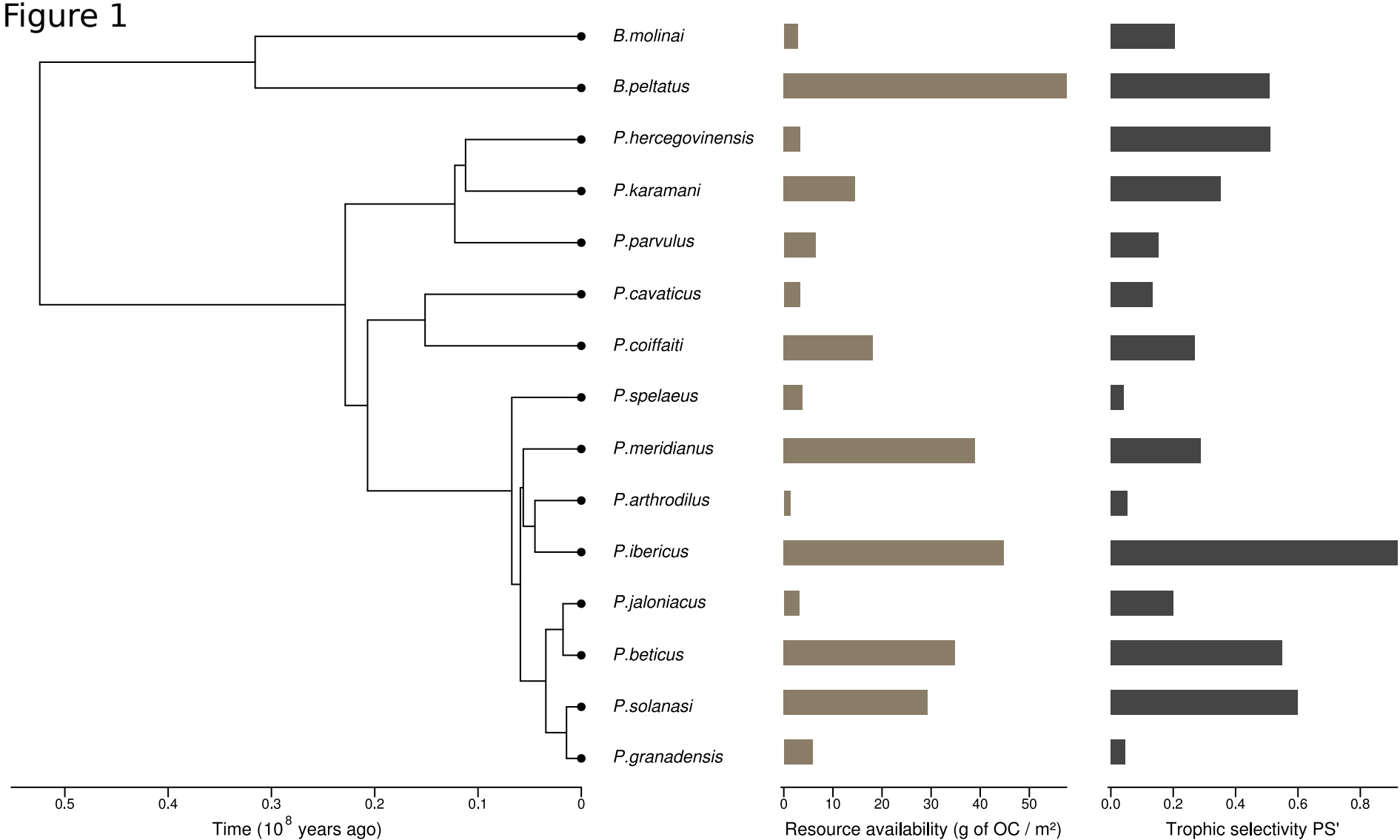
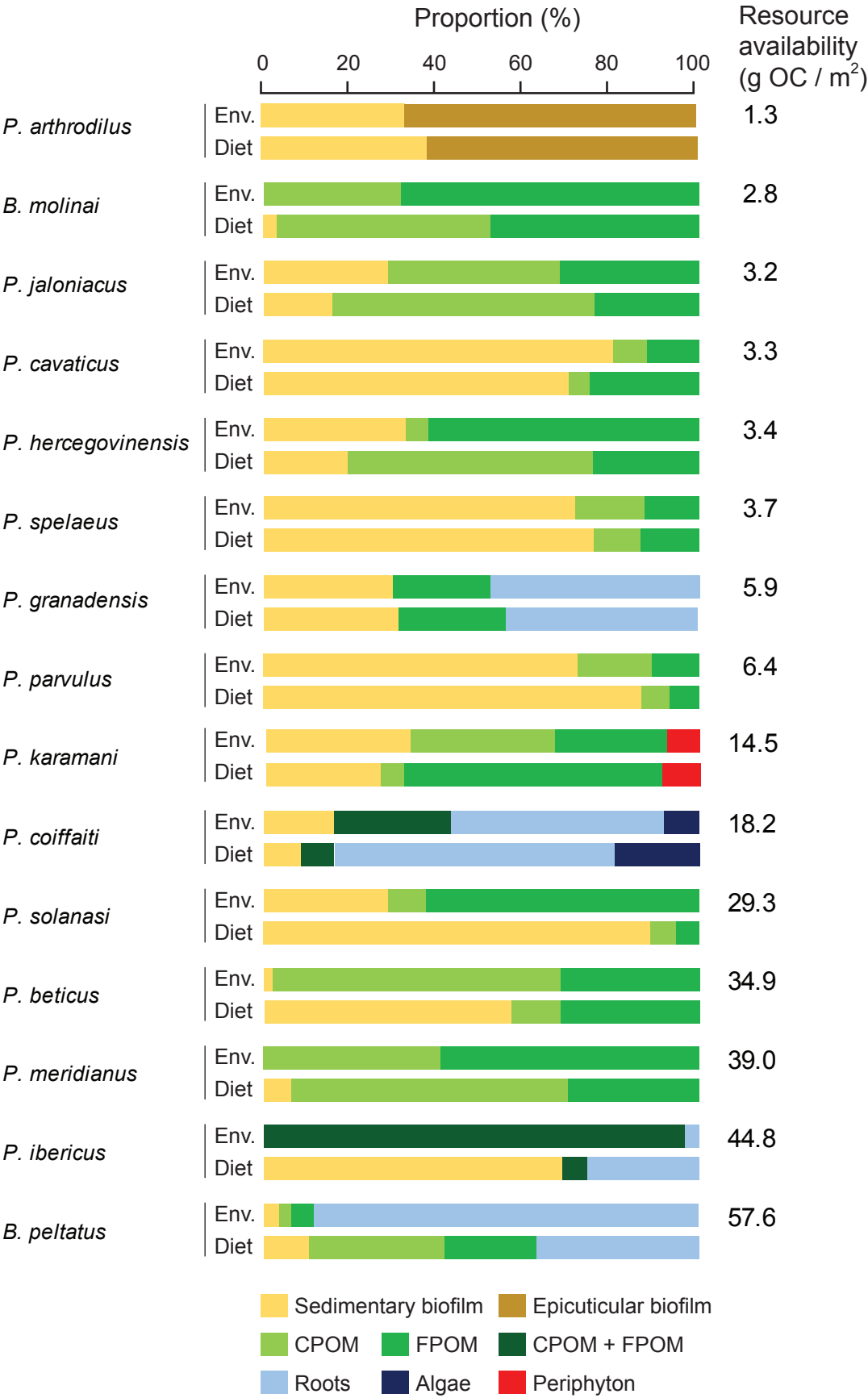
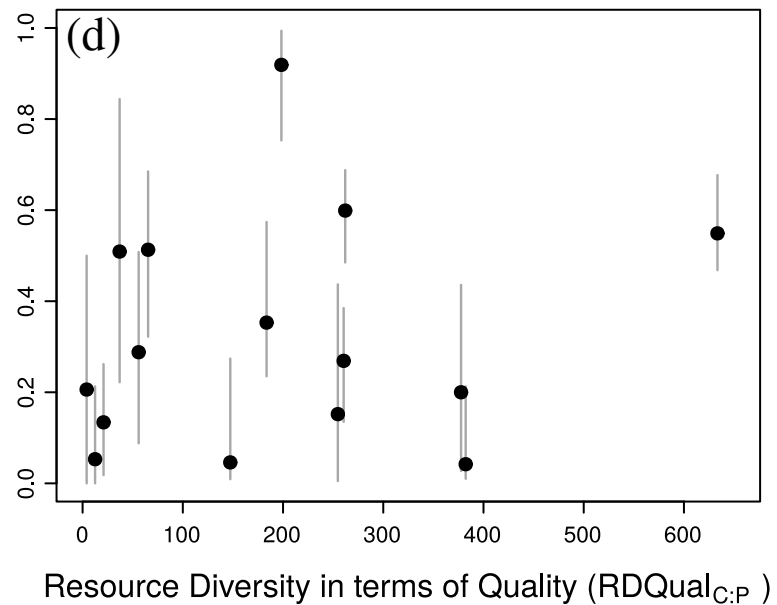
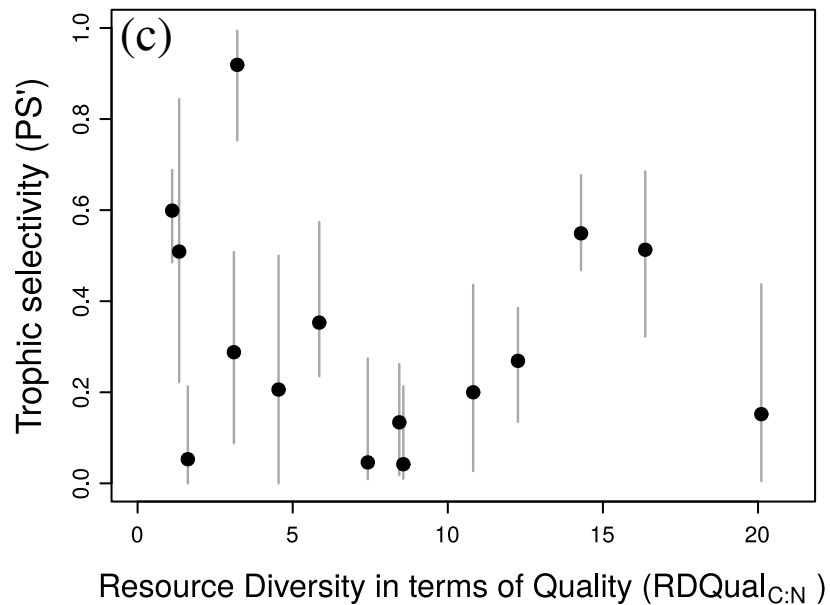
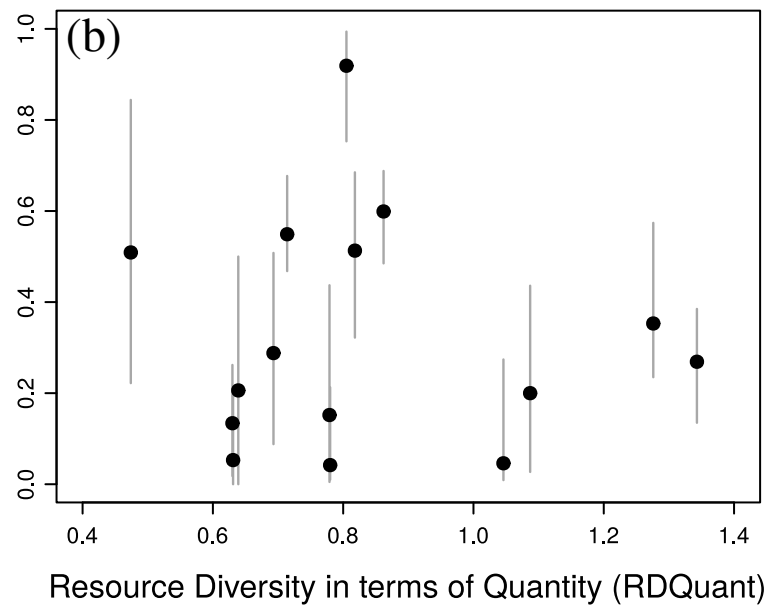
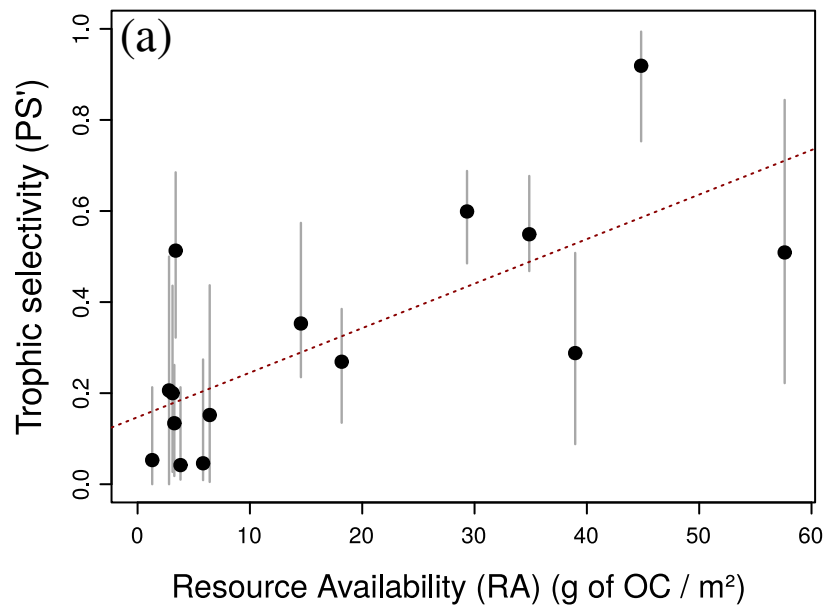


Figure 2





Species		Site	Habitat	C:N _{org}	C:P _{org}	Locality	CO	LA	LO
<i>Proasellus arthrodilus</i>	(Braga, 1945)	1	cave stream	8.86	158.9	Rabacal	PT	40.03	-8.47
<i>Bragasellus molinai</i>	(Henry & Magniez, 1988)	2	cave pool	9.18	152.1	Vegacervera	ES	42.91	-5.56
<i>Proasellus jaloniacus</i>	(Henry & Magniez, 1978)	3	hyporheic zone	5.06	101.5	Benichembla	ES	38.76	-0.10
<i>Proasellus cavaticus</i>	(Leydig, 1871)	4	cave stream	5.41	105.3	Thoiria	FR	46.53	5.73
<i>Proasellus hercegovinensis</i>	(Karaman, 1933)	5	cave pool	5.74	94.1	Zavala	BA	42.85	17.98
<i>Proasellus spelaeus</i>	(Racovitza, 1922)	6	cave stream	4.86	97.8	Ancille	FR	43.14	-1.20
<i>Proasellus granadensis</i>	(Henry & Magniez, 2003)	7	spring	4.43	64.8	Alhama de Granada	ES	37.05	-4.06
<i>Proasellus parvulus</i>	(Sket, 1960)	8	cave stream	4.57	75.6	Crnomelj	SI	45.60	15.17
<i>Proasellus karamani</i>	(Remy, 1934)	9	surface stream	5.45	88.0	Kljuc	BA	43.09	18.49
<i>Proasellus coiffaiti</i>	(Henry & Magniez, 1972)	10	surface stream	5.41	108.0	Cauneille	FR	43.54	1.04
<i>Proasellus solanasi</i>	(Henry & Magniez, 1972)	11	cave entrance	5.89	80.9	Benajoan	ES	36.73	-5.24
<i>Proasellus beticus</i>	(Henry & Magniez, 1992)	12	sinkhole	5.29	108.5	Vallada	ES	38.88	-0.69
<i>Proasellus meridianus</i>	(Racovitza, 1919)	13	surface stream	4.97	78.6	Alfoz de Lloredo	ES	43.35	-4.18
<i>Proasellus ibericus</i>	(Braga, 1946)	14	surface stream	5.97	76.8	Ponte de Lima	PT	41.85	-8.57
<i>Bragasellus peltatus</i>	(Braga, 1944)	15	surface stream	5.65	102.0	Lousada	PT	41.28	-8.31

Table 1: Description of the 15 isopod species.

Species are sorted by increasing resource availability in their environment. C:N_{org} and C:P_{org} refers respectively to the molar carbon-to-nitrogen and carbon-to-phosphorus ratios of isopods (whole organism). CO stands for the ISO country code and LA / LO stand for the latitude / longitude (in decimal degrees) of the sampling site location.

	Minimum	Maximum
<i>Proportion of resources in the environment (%)</i>		
Fine particulate organic matter (FPOM)	0	68
Coarse particulate organic matter (CPOM)	0	66
Sedimentary biofilm	0	80
Epicuticular biofilm*	0	67
Periphyton*	0	7
Algae *	0	8
Roots	0	88
<i>Proportion of resources in isopod diet (%)</i>		
Fine particulate organic matter (FPOM)	5	57
Coarse particulate organic matter (CPOM)	5	63
Sedimentary biofilm	3	87
Epicuticular biofilm *	-	62
Periphyton*	-	8
Algae *	-	19
Roots	25	64

Table 2: Overview of the range of resources abundance and use across the 15 sampling sites.

This table presents the minimum and maximum abundances of each trophic resource across the 15 surveyed environments (as % of the total OC / m²).

The minimum and maximum proportions of each resource in the diet of isopods are presented in the same way (considering only the sites where the given resource was available).

* denotes the 3 resources present in only one of the 15 sampling sites.

PGLS models	AICc	adjusted R ²
PS' ~ RA * RDQuant * RDQual _{C:N}	73.71	0.47
PS' ~ RDQuant * RDQual _{C:N}	65.19	-0.24
PS' ~ RDQuant + RDQual _{C:N}	61.49	-0.15
PS' ~ RDQuant	58.52	-0.08
PS' ~ RDQual _{C:N}	58.31	-0.06
PS' ~ RA + RDQuant + RDQual _{C:N}	54.90	0.37
PS' ~ RA * RDQuant	54.76	0.38
PS' ~ RA * RDQual _{C:N}	54.10	0.41
PS' ~ RA + RDQuant	51.21	0.42
PS' ~ RA + RDQual _{C:N}	51.20	0.42
PS' ~ RA	48.10	0.46

Table 3 : Procedure of model selection.

Models are ranked by decreasing Akaike's information criterion corrected for small sample size (AICc). A difference of AICc between two models is considered as a significant improvement in favour of the model with the lowest AICc (Burnham & Anderson, 2002). PS', RA, RDQual_{C:N} and RDQuant represent the trophic selectivity, the Resource Availability (as g of OC / m²), the Resource Diversity in terms of Quality (based on C:N ratios), and in terms of Quantity, respectively (see main text for details). The 'adjusted R²' (modified R² that has been adjusted for the number of predictors in the model) is indicated here as an estimation of the proportion of variance explained by the corresponding model.