

1 **Determining trophic niche width: An experimental**  
2 **test of the stable isotope approach**

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29 **Abstract**

30 Determining the trophic niche width of an animal population and the relative degree to  
31 which a generalist population consists of dietary specialists are long-standing problems of  
32 ecology. It has been proposed that the variance of stable isotope values in consumer tissues  
33 could be used to quantify trophic niche width of consumer populations. However, this  
34 promising idea has not yet been rigorously tested. By conducting controlled laboratory  
35 experiments using model consumer populations (*Daphnia* sp., Crustacea) with controlled  
36 diets, we investigated the effect of individual- and population-level specialisation and  
37 generalism on consumer  $\delta^{13}\text{C}$  mean and variance values. While our experimental data follow  
38 general expectations, we extend current qualitative models to quantitative predictions of the  
39 dependence of isotopic variance on *dietary correlation time*, a measure for the typical time  
40 over which a consumer changes its diet. This quantitative approach allows us to pinpoint  
41 possible procedural pitfalls and critical sources of measurement uncertainty. Our results  
42 show that the stable isotope approach represents a powerful method for estimating trophic  
43 niche widths, especially when taking the quantitative concept of dietary correlation time into  
44 account.

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## 47 Introduction

48 Trophic niche width is typically measured as the diversity of resource types  
49 consumed by a consumer (e.g., McDonald 2002). It allows researchers to address questions  
50 on the ecology and evolution of various kinds of organisms in their ecological interactions  
51 with predators, prey and competitors (Pigeon et al. 1997, Roughgarden 1972, Van Valen  
52 1965). Conventional diet analysis, which is typically conducted through gut content analysis  
53 (e.g., Hyslop 1980), is nowadays commonly used in conjunction with measurements of  
54 consumer stable isotope ratios. While the former provides more detailed information, the  
55 latter has the advantages of methodological simplicity and of representing the integrated  
56 assimilated diet of the consumer rather than just recent intake. However, consumer isotopic  
57 variance cannot be used as an indicator of individual specialisation without considering the  
58 relative isotopic variation in the available prey, as highlighted by Matthews and Mazumder  
59 (2004). Bearhop *et al.* (2004) proposed a widely-cited approach for the determination of the  
60 trophic (feeding) niche width of a consumer population by using the variance of the  
61 consumers' stable isotope ( $\delta^{13}\text{C}$  and/or  $\delta^{15}\text{N}$ ) ratios among individuals. In general, stable  
62 isotope analysis is a useful method for understanding the trophic ecology of consumers as  
63 the stable isotope ratios in their tissues reflect those of their diet in a generally predictable  
64 manner (Hobson and Clark 1992, Peterson and Fry 1987); there is a mean increase in  $\delta^{13}\text{C}$   
65 by ca. 1 ‰ and in  $\delta^{15}\text{N}$  by ca. 3.4 ‰ per trophic level due to trophic fractionation (Cabana  
66 and Rasmussen 1994, McCutchan et al. 2003). Therefore, if individuals are specialised on  
67 one food type only they will display isotopic values from this food type (adjusted for trophic  
68 fractionation), while individuals that feed on and incorporate many different food items with  
69 different isotopic values will have a mean isotopic value which reflects the different isotopic  
70 contributions in the diet (including fractionation). Reflecting observations from empirical data,  
71 this has been suggested multiple times in the literature (Bolnick et al. 2003, Fry et al. 1978,  
72 Matthews and Mazumder 2004). From this, Bearhop *et al.* (2004) predicted differences in the  
73 distribution of individual isotopic values for dietary specialists (SP; Fig. 1), and for two types  
74 of dietary generalists; generalist populations composed of individuals that all consume a wide  
75 range of resources (Type A generalists, GA; Fig. 1) and generalist populations composed of  
76 individuals that are themselves specialised on different, narrow resource ranges (Type B  
77 generalists, GB; Fig. 1). Distinguishing empirically between GA and GB by using traditional  
78 methods of diet analysis is extremely challenging (Bearhop et al. 2004, Van Valen 1965).  
79 The isotopic approach proposed by Bearhop et al. (2004) is an attractive alternative.  
80 Bearhop et al. (2004) further highlighted the use of consumer stable isotope values to  
81 estimate trophic niche width, as it yields a single measure on a continuous axis common to  
82 all species and provides a temporal integration of prey assimilation. Furthermore, the time  
83 frame of the temporal integration can be adjusted by the choice of the sampled consumer  
84 tissue, as the tissue's isotopic value reflects the diet that was assimilated during the period of  
85 tissue synthesis. For example, in vertebrate consumers muscles or bones integrate diets  
86 over longer times than blood or liver (Hobson 1999).

87 The distinction between Type A and Type B generalists can be seen as two extremes in  
88 a range of temporal continuity in resource usage. True Type A generalist individuals in the  
89 sense of Bearhop et al. (2004) continuously switch between alternative diets, while a true  
90 Type B generalist individual never switches, but constantly consumes the diet it is  
91 specialised on (e.g., Newsome et al. 2009, Vander Zanden et al. 2010). We propose that  
92 continuity in resource usage can be quantified in terms of the *dietary correlation time*, i.e. the  
93 typical length of time after which a consumer changes its diet. To translate the classification  
94 of Bearhop et al. (2004) into this context, we here consider Type B generalists as those  
95 species where the dietary correlation time of individuals is of the order of magnitude of the  
96 natural life-time, or of the duration of the specific life stage considered in an analysis. In  
97 contrast, Type A generalists frequently vary their diet over the full spectrum of available  
98 resources during their life-time or the life-stage considered (Fig. 2). The isotopic pattern  
99 found for Type A generalists will depend on the relative magnitude of the dietary correlation  
100 time and the isotopic integration time of the tissue analyzed. Empirically, one can therefore  
101 distinguish between two kinds of Type A generalists,  $\text{GA}_{\text{short}}$  and  $\text{GA}_{\text{long}}$  corresponding to

102 tissue integration times that are short and long, respectively, compared to dietary correlation  
103 times of the consumers. As indicated in Figure 2, shorter and longer tissue integration times  
104 for Type A generalists will have similar effects on the isotope values as longer and shorter  
105 dietary correlation times, respectively. In this view, expressed also by Bearhop et al. (2004),  
106 the suffixes of  $GA_{\text{short}}$  and  $GA_{\text{long}}$  refer to tissue integration times rather than dietary  
107 correlation time. Type B generalists correspond to species that appear as  $GA_{\text{short}}$  even when  
108 tissues with integration times spanning the full life time of individuals are analyzed.

109 Obviously, these types (GA and GB) are, in their “pure” forms, idealizations. Differences  
110 between individuals in phenotype, experience, and location are likely to lead to some dietary  
111 variation among individuals in most cases. This is what we intend to capture by introducing  
112 the notion of dietary correlation time (DCT) and speaking of a continuum of different types  
113 spanned by the extreme (meaning idealized) cases GA & GB. Therefore, researchers need  
114 to understand how precisely dietary correlation time would influence the variance of isotope  
115 values within a population in the field in order to reliably interpret stable isotope data from  
116 natural populations. Here we propose a model for the dependence of isotopic variance on  
117 dietary correlation time. The model predicts the isotopic variance of a population from dietary  
118 correlation time and population-level diet width.

119 While the predictions by Bearhop et al. (2004) and the suggestions they derived for using  
120 isotopic variance as a direct measure of trophic niche width have found wide application in  
121 recent years (e.g., Martinez del Rio et al. 2009, Syväranta and Jones 2008), the underlying  
122 ideas have not been subjected to controlled experimental tests, and the interpretation of  
123 results based on them remain largely qualitative. To experimentally address the effects of  
124 dietary correlation time and consumer specialisation or generalisation on the isotopic  
125 variance of a consumer population, we performed controlled laboratory experiments using  
126 the freshwater crustacean zooplankton *Daphnia pulicaria* as the consumer, and the green  
127 alga *Scenedesmus obliquus* (Chlorophyceae) as the food. We considered *Daphnia* as a  
128 useful experimental model for this investigation, since it is one of the most common model  
129 organisms in biological research (Ebert 2011); its physiology is well understood and it grows  
130 and reproduces rapidly. Furthermore, reproduction under favourable conditions is  
131 parthenogenetic, which allows us to exclude effects of genotypic variation on the response  
132 variable, isotopic variance (Lampert 2006). In contrast to Bearhop et al. (2004), we used the  
133 variance of stable carbon ( $\delta^{13}\text{C}$ ) rather than of nitrogen ( $\delta^{15}\text{N}$ ) isotopes. This was done partly  
134 to reduce the effects of isotopic fractionation (McCutchan et al. 2003) on consumer isotope  
135 values, but also because  $\delta^{13}\text{C}$  is typically used to distinguish between different putative  
136 sources of energy fuelling consumers (France 1995).

137 As noted earlier, animals, in particular trophic generalists, do not necessarily feed on one  
138 resource type only throughout their life, but switch between several alternative resources.  
139 Within a population, switching to an alternative diet can occur synchronously for the whole  
140 population ( $GA_{\text{syn}}$ ), e.g. with a habitat shift as it is commonly observed in many group-living  
141 animals ranging from wildebeest and zebra (Ben-Shahar and Coe 1992) to fish (e.g.,  
142 ontogenetic niche shifts in perch, Hjelm et al. 2000). However, diet switches can also occur  
143 asynchronously within a population ( $GA_{\text{as}}$ ), if the population is spread over a heterogeneous  
144 habitat with patchy resource availability and different parts of the population utilize different  
145 food types at a given time (e.g., brown trout, Grey 2001). Information on such diet-switches  
146 of generalist populations can be obtained by examining several different tissues that reflect  
147 long and short-term foraging histories.

148 Following Bearhop et al. (2004), we hypothesized that GB and  $GA_{\text{as}}$  will have a large  
149 within-population isotopic variance as the short-turnover tissues are isotopically most  
150 influenced by the most recent feeding history, which, in turn, is different for individual  
151 members of an asynchronously diet-switching population (Fig. 3). Conversely, synchronously  
152 diet-switching populations would have small variances as all individuals feed on isotopically  
153 identical food sources (Fig. 3).

154 Hence, this study represents the first controlled empirical test of the applicability of the  
155 use of population-level measures of isotopic mean and variance for determining trophic niche  
156 width. This is achieved by (1) experimentally determining means and variances in consumer

157 stable isotope values from specialist populations (SP, experiment 1), Type A generalists  
158 populations ( $GA_{long}$ , long-term integrating tissue, experiment 1), short-term integrating tissue  
159 with synchronous ( $GA_{syn}$ ) and asynchronous ( $GA_{as}$ ) diet switching, and Type B generalist  
160 populations (GB, experiment 2, Fig. 3), and (2) by deriving a model of the influence of dietary  
161 correlation time on variance of isotope values within natural populations. Together, these two  
162 approaches allow us to point to crucial prerequisites and potential caveats in the use of  
163 stable isotope variances to determine trophic niche width. We therefore conclude by  
164 presenting some general prescriptions that should be followed to avoid fallacies when  
165 applying the method of Bearhop et al. (2004), and our extensions of it to field data.

## 166 **Methods**

### 167 *Experimental approach*

169 By cultivating algae in the presence or absence of  $^{13}C$  labelled bicarbonate, we were  
170 able to produce morphologically and qualitatively identical food items with widely differing  
171 isotopic values. Even though *Daphnia* are unselective filter-feeders with no individual food  
172 preferences (Lampert 2006), they can be used as model organisms to investigate the effects  
173 of dietary history on consumer stable isotope signatures by choosing the appropriate  
174 experimental design. By presenting different food sources either at the same or at different  
175 times, we were able to mimic the effects of different kinds of foraging behaviour and different  
176 relative time scales for diet switching and retention of material in consumer tissue. Long  
177 dietary correlation times, corresponding to short-term integrating tissue, were simulated by  
178 feeding *Daphnia* with food of known, fixed stable isotope values over a prolonged period  
179 (experiment 1). Short dietary correlation times, or long-term integrating tissues, were realised  
180 by switching the diet during the experiment (experiment 2). To mimic specialist consumers in  
181 our experiment, we fed groups of *Daphnia* with algae with divergent isotope values, allowing  
182 us to construct artificial populations of *Daphnia* with different feeding histories.

### 183 *Food culture and labelling*

185 The chlorophycean microalga *Scenedesmus obliquus* (TURPIN) KÜTZING was used as  
186 sole food source for *Daphnia pulicaria* (FORBES). *S. obliquus* is commonly used as standard  
187 food for *Daphnia* sp. and allows daphnids to achieve high somatic growth rates (Lampert  
188 2006). Two *S. obliquus* continuous cultures (chemostats) were set up for culturing with a  
189 dilution rate of  $0.5\text{ d}^{-1}$  and with dilute (1:4) Z4 medium (Zehnder and Gorham 1960). The two  
190 chemostats were kept at constant irradiance ( $100\ \mu\text{mol photons s}^{-1}\text{ m}^{-2}$ ) and temperature  
191 ( $20^{\circ}\text{C}$ ). In one of the two chemostats, 0.5% of the added carbonate stock solution (42.0  
192 mg/L  $\text{NaHCO}_3$ , VWR, Darmstadt, Germany) was replaced by  $\text{NaH}^{13}\text{CO}_3$  (Chemotrade,  
193 Leipzig, Germany). This resulted in a shift in mean ( $\pm$  SD)  $\delta^{13}C$  values of *S. obliquus* from  
194  $-15.4\text{ ‰}$  ( $\pm 0.8$ ) in the unlabelled culture to  $8.5\text{ ‰}$  ( $\pm 0.4$ ) in the labelled culture in our first  
195 experiment, and from  $-15.5\text{ ‰}$  ( $\pm 0.3$ ) in unlabelled *S. obliquus* to  $4.1\text{ ‰}$  ( $\pm 0.8$ ) in the  
196 labelled culture in our second experiment. In order to assess whether labelling affected food  
197 quality of *S. obliquus* for the daphnids, the molar C:N ratio and the fatty acid composition of  
198 labelled and unlabelled algae were analyzed, as described by Fink and Von Elert (2006a,  
199 2006b).

### 200 *Pre-experimental Daphnia culture*

202 All experiments were conducted with 4-day old juveniles of a clone of *D. pulicaria*,  
203 originally isolated from a pond near Konstanz, Germany, but kept in laboratory culture with *S.*  
204 *obliquus* as sole food for several years prior to the experiments (Alekseev and Lampert  
205 2001). Juvenile *D. pulicaria* from synchronized third clutches were fed with unlabelled *S.*  
206 *obliquus* for three days after birth prior to each experiment.

### 207 *Experiment 1 – Determination of stable isotope means and variances for SP and $GA_{long}$ populations (long-term integrating tissue)*

210 The experiment was carried out in a temperature-controlled room at  $20^{\circ}\text{C}$  in glass  
211 beakers filled with 1 L of  $0.45\text{-}\mu\text{m}$  filtered lake water (Schöhsee, northern Germany) and ran

212 for three days. *Daphnia* were fed either 100% unlabelled *S. obliquus* (in the following  
213 abbreviated as U), a 1:1 mixture of labelled and unlabelled *S. obliquus* (abbreviated here as  
214 M), or 100% labelled *S. obliquus* (abbreviated as L). In the context of dietary specialisation,  
215 animals feeding on U and L algae can be considered as two populations of specialist  
216 consumers, specialised on two different resources. The animals on the mixed (M) algal diet  
217 can be considered as Type A generalists (GA), as they indiscriminately feed on the mixture  
218 of U and L algae. Each treatment consisted of either 15 (L, M) or 21 (U) beakers (replicates)  
219 with 15 animals per beaker. The 15 *Daphnia* of each beaker were pooled for stable isotope  
220 analysis to reach the required weight for this analysis. Reported isotope values therefore  
221 reflect averages from 15 individuals. Food was supplied *ad libitum* (1.5 mg C L<sup>-1</sup>) and  
222 animals were transferred daily into new food suspensions.

223 At the end of the experiment, *D. pulicaria* were transferred to filtered lake water for  
224 4 h to allow gut clearance in order to avoid isotopic bias. *Daphnia* were then transferred into  
225 pre-weighed tin cups and dried for 24 hours prior to determination of dry mass ( $\pm 0.1 \mu\text{g}$ ).  
226 Since juvenile *Daphnia* grow exponentially with respect to their body mass, and the juvenile  
227 growth rate is a good proxy of fitness (Lampert and Trubetskova 1996), we calculated  
228 somatic growth rates ( $g$ ) using the equation  $g = (\ln W_t - \ln W_0)/t$ , where  $W_0$  is the animals' dry  
229 weight at the beginning and  $W_t$  the dry weight at the end of the experiment and  $t$  the duration  
230 of the experiment in days.  $W_0$  was determined by separately weighing a subsample of the  
231 experimental animals (from the same clutch) at the beginning of the experiment. Carbon  
232 stable isotope ratios and C:N of both algae and *Daphnia* were determined by continuous flow  
233 isotope ratio mass spectrometry (Eurovector EuroEA 3000 Series Elemental Analyser  
234 coupled to a Micromass Isoprime Mass Spectrometer). Stable isotope ratios are given using  
235 the  $\delta$  notation expressed in units per mil as follows:  $\delta (\text{‰}) = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1000$ , and  
236  $R = {}^{13}\text{C}/{}^{12}\text{C}$ . The reference materials used was a secondary standard of known relation to the  
237 international standard of Vienna Pee Dee belemnite. Typical precision for a single analysis  
238 was  $\pm 0.1 \text{‰}$ . All data from the experiments were tested for homoscedasticity (Levene's test)  
239 and comparison between treatments done via one- or two-way analyses of variances  
240 (ANOVAs) using Statistica 6.0 (StatSoft Inc.). Results with a  $p < 0.05$  were considered  
241 significant.

242  
243 *Experiment 2 – Determination of stable isotope means and variances for GA<sub>syn</sub> (short-term*  
244 *integrating tissue; synchronous food source switching) and GA<sub>as</sub> (short-term integrating*  
245 *tissue; asynchronous food source switching)*

246 In this experiment, *Daphnia* encountered three different treatments in which they  
247 received the three food sources with different isotopic values (U, M, L) but in different  
248 sequences (A, B, C; see Table 1). Each treatment consisted of 20 replicates (beakers with 1  
249 L lake water) containing 15 *Daphnia* each. The experiment was run in a temperature  
250 controlled room at 20.7°C for 5 days. All analyses were performed as described above. One  
251 value was identified as an outlier due to an extreme ( $>3$ ) Studentized Residual value of -4.52  
252 but was included in the data analysis as comparative analyses revealed that its inclusion had  
253 no discernible effect on our results. This experiment was designed to create two different  
254 population types: GA<sub>syn</sub> and GA<sub>as</sub> populations. We obtained three different GA<sub>syn</sub> populations  
255 by treating each treatment (A-C, all consisting of 20 beakers, respectively) as a single GA  
256 population that switched the diet synchronously. However, when we consider the individuals  
257 from all 60 beakers as a single population, three sub-populations (corresponding to  
258 treatments A-C) can be distinguished that switched their diets asynchronously (GA<sub>as</sub>).  
259

260 *Model of consumer generalism and dietary correlation time*

261 In modelling changes in the  $\delta^{13}\text{C}$  value of *Daphnia* in response to changes in the  $\delta^{13}\text{C}$   
262 value of its diet, we assume that the effect of consumed food on somatic  $\delta^{13}\text{C}$  decays  
263 exponentially over time. This assumption holds exactly if, for example, C atoms in the body  
264 tissue are replaced at random with C atoms of newly assimilated material, or when  
265 individuals grow exponentially in size, as is the case for juvenile *Daphnia* (Lampert and  
266 Trubetskova 1996), and body tissue is mostly added rather than replaced, as demonstrated

267 for broad whitefish (*Coregonus nasus*) by Hesslein et al. (1993). Phenomenologically, we  
 268 expect the assumption of exponential dilution of tissue to be valid over a wide range of  
 269 situations. It leads to a simple model for *Daphnia*  $\delta^{13}\text{C}$  values of the form

$$270 \quad (1) \quad \delta^{13}\text{C}_{\text{daph}}(t) = r \int_{-\infty}^t e^{-r(t-\tau)} \delta^{13}\text{C}_{\text{food}}(\tau) d\tau + \Delta_C$$

271 , where  $r$  is the tissue dilution rate (i.e. the rate at which tissue and the associated isotopic  
 272 values are diluted through addition and replacement),  $\delta^{13}\text{C}_{\text{daph}}(t)$  is the  $\delta^{13}\text{C}$  value of *Daphnia*  
 273 at time  $t$ ,  $\delta^{13}\text{C}_{\text{food}}(\tau)$  is the  $\delta^{13}\text{C}$  value of the food assimilated at time  $\tau$  (assuming that the  
 274 uptake rates are approximately constant over times of duration  $1/r$ ), and  $\Delta_C$  represents the  
 275 effect of fractionation (fractionation constant).

276 Our experimental protocol (see above) resolves time in units of days only. Model equation (1)  
 277 is therefore appropriately re-written in the corresponding time-discrete form

$$278 \quad (2) \quad \delta^{13}\text{C}_{\text{daph},t} = (1 - e^{-r}) \sum_{\tau=-\infty}^t e^{-r(t-\tau)} \delta^{13}\text{C}_{\text{food},\tau} + \Delta_C,$$

279 , where the replacement of the factor  $r$  by  $(1 - e^{-r})$  guarantees that, as expected, in the case of  
 280 continuously feeding on a single resource the  $\delta^{13}\text{C}$  value is simply shifted by the fractionation  
 281 constant.

282 Treatments A, B, and C of experiment 2 differ from treatment M (mixed diet) of  
 283 experiment 1 only in the two days where either unlabelled or labelled algae are fed to  
 284 *Daphnia* (Table 1). Equation (2) therefore implies the following statistical models for the  
 285 measured isotope values of treatments M, A, B, and C, which we fitted to the experimental  
 286 results using the non-linear least-square fitting function `nls` of the software package R (R  
 287 Development Core Team 2010):

$$288 \quad (3a) \quad \delta^{13}\text{C} = b + \epsilon$$

289 for treatment M, where  $b$  represents the baseline result for feeding on mixed samples and  $\epsilon$  a  
 290 random residual, and

$$291 \quad (3b) \quad \delta^{13}\text{C} = b + a(1 - e^{-r})(-0.5e^{-rd} + 0.5e^{-r(d+1)}) + \epsilon$$

292 for treatments A, B, C (Table 1). The experimental parameter  $d$  represents the delay  
 293 between the day of feeding on unlabelled algae and of sampling, which is varied in the three  
 294 treatments. The parameter  $a$ , scaling the effect strength, would ideally equal the difference in  
 295 isotope values between labelled and unlabelled algae (17.6), however, to absorb  
 296 uncertainties in the effective time of sampling (i.e. the effective delay  $d$ ), we treat  $a$  as a free  
 297 model parameter, to be fitted to the data, just as baseline value  $b$  and dilution rate  $r$ .

298

## 299 Results

### 300 Labelling and food quality

301 The  $^{13}\text{C}$ -labelling resulted in slightly altered molar C:N ratios ( $F_{(2,34)} = 108.6$ ,  
 302  $p < 0.001$ ) in algae (mean  $\pm$  SD  $4.7 \pm 0.1$  for labelled algae,  $5.0 \pm 0.1$  for unlabelled *S.*  
 303 *obliquus*). However, as it is to be expected for a stoichiometrically homeostatic consumer  
 304 (Persson et al. 2010), the daphnids' molar C:N ratios were not significantly affected by the  
 305 different food sources (mean  $\pm$  SD  $4.5 \pm 0.1$  for L, M and U;  $F_{2,48} = 0.16$ ,  $p = 0.86$ ) and the  
 306 C:N variance of the animals was stable between food treatments (Levene's test:  $F_{2,46} =$   
 307  $0.827$ ,  $p = 0.44$ ). The fatty acid composition (% of total fatty acids) of *S. obliquus* was also  
 308 not affected by labelling (two-way ANOVA: "label"  $F_{(1,24)} = 0.0$ ,  $p = 1.0$ , "fatty acid"  $F_{(5,24)} =$   
 309  $3577.5$ ,  $p < 0.001$ ). Furthermore, there were no significant differences in *D. pulicaria* growth  
 310 rates between feeding regimes in the first experiment (one-way ANOVA,  $F_{(2,48)} = 2.7$ ,  $p = 0.08$ ).  
 311 Mean ( $\pm$  SD) juvenile growth rate was  $0.40 \pm 0.02 \text{ d}^{-1}$  in the daphnids fed unlabelled algae,  
 312  $0.41 \pm 0.02 \text{ d}^{-1}$  with labelled algae, and  $0.40 \pm 0.02 \text{ d}^{-1}$  in those fed the mixture of labelled  
 313 and unlabelled *S. obliquus*. In experiment 2, the somatic growth rates in treatment A ( $0.51 \pm$

314 0.02 d<sup>-1</sup>) were slightly, but significantly (one-way ANOVA:  $F_{(2, 57)} = 6.8$ ,  $p < 0.01$ ) higher than  
315 in treatments B and C (both  $0.50 \pm 0.02$  d<sup>-1</sup>). This was probably due to slight imbalance in the  
316 temperature distribution within the experimental chamber, since treatments B and C were  
317 situated closer to the cooling system, resulting in slightly (0.11 °C) reduced water  
318 temperatures. Nevertheless, the differences were very small and we therefore feel that there  
319 is little evidence for differences in food quality between the treatments.

320

321 *Experiment 1 – Experimentally determining stable isotope means and variances for SP and*  
322 *GA<sub>long</sub> (long-term integrating tissue)*

323 Experiment 1 resulted in two “specialist” populations (*sensu* Bearhop *et al.* 2004): one  
324 fed unlabelled (U), one labelled (L) algae. The isotopic difference in resource resulted in a  
325 marked difference in the consumers’ carbon isotope values: At the beginning of the  
326 experiment, all *Daphnia* had the same mean ( $\pm$  SD;  $n = 4$ )  $\delta^{13}\text{C}$  of  $-17.4$  ‰ ( $\pm 0.1$ ). After  
327 three days fed with either U ( $\delta^{13}\text{C}$   $-15.4$  ‰  $\pm 0.8$ ) or L ( $\delta^{13}\text{C}$   $8.5$  ‰  $\pm 0.4$ ) *S. obliquus*,  
328 *Daphnia*’s mean  $\delta^{13}\text{C}$  ( $\pm$  SD) was  $-16.3$  ‰ ( $\pm 0.16$ ;  $n = 21$ ) in U and  $1.1$  ‰ ( $\pm 0.27$ ;  $n = 15$ ) in  
329 the L treatment. Mean  $\delta^{13}\text{C}$  differed between treatments (Fig. 4:  $F_{1,34} = 59972$ ,  $p < 0.001$ ). In  
330 addition to the two specialist populations, this experiment also yielded one GA population fed  
331 a 1:1 mixture of labelled and unlabelled food (treatment M). The mean  $\delta^{13}\text{C}$  ( $\pm$  SD;  $n = 13$ ) of  
332 the mixed algal resource was  $-3.4$  ‰ ( $\pm 0.54$ ). The mean  $\delta^{13}\text{C}$  ( $\pm$  SD;  $n = 15$ ) of the *Daphnia*  
333 fed with this mixture for three days was  $-7.3$  ‰ ( $\pm 0.24$ ). Mean  $\delta^{13}\text{C}$  differed between all  
334 three populations (one-way ANOVA:  $F_{2,48} = 27901$ ,  $p < 0.0001$ ), but  $\delta^{13}\text{C}$  variance was  
335 always small and did not differ between populations (Levene’s test:  $F_{2,48} = 2.38$ ,  $p = 0.104$ ;  
336 Fig. 4).

337

338 *Experiment 2 – Experimentally determining stable isotope means and variances for GA<sub>short</sub>*  
339 *(short-term integrating tissue)*

340 *Daphnia* were subject to three dietary treatments by feeding them either unlabelled  
341 (U) or labelled (L) *S. obliquus*, or a 1:1 mixture of U and L algae having an intermediate  $\delta^{13}\text{C}$   
342 (M). Mean algal  $\delta^{13}\text{C}$  ( $\pm$  SD) was  $-15.5$  ‰  $\pm 0.3$  for U ( $n = 25$ ),  $4.1$  ‰  $\pm 0.8$  for L ( $n = 25$ ) and  
343  $-5.5$  ‰  $\pm 0.4$  for M ( $n = 24$ ). Mean ( $\pm$  SD)  $\delta^{13}\text{C}$  of *Daphnia* was  $-18.1$  ‰ ( $\pm 0.5$ ,  $n = 2$ ) at the  
344 beginning of the experiment.

345 After five days mean ( $\pm$  SD) *Daphnia*  $\delta^{13}\text{C}$  was  $-9.8$  ‰ ( $\pm 0.2$ ,  $n = 20$ ) in treatment A,  $-8.7$  ‰  
346 ( $\pm 0.4$ ,  $n = 20$ ) in treatment B and  $-8.2$  ‰ ( $\pm 0.2$ ,  $n = 20$ ) in treatment C, *i.e.* the variance  
347 (squared SD) was relatively small within each GA population, if food switching occurred  
348 synchronously as compared to a GA<sub>as</sub> population (Fig. 4). If these three treatments were  
349 considered as three generalist subpopulations that fed asynchronously on different  
350 resources, then the calculated mean ( $\pm$  SD) was  $-8.9$  ‰ ( $\pm 0.7$ ,  $N = 60$ , Fig. 4). This revealed  
351 that variance within GA<sub>as</sub> populations was large when a rapid turnover tissue and  
352 asynchronous feeding was considered.

353 Comparison of the isotopic variance in this GA<sub>short</sub> population (Experiment 2) with the GA<sub>long</sub>  
354 population where we consider a sampling of long-term integrating tissue (Experiment 1),  
355 showed greater variance in Experiment 2 (Levene’s test:  $F_{1,73} = 30.6$ ,  $p < 0.0001$ ), following  
356 the predictions of Bearhop *et al.* (2004, Fig. 3, 4).

357

358 *Fitting of the model for tissue dilution*

359 Model parameters in Eqs. (3a, b), estimated from the experimental results, their  
360 standard errors, and confidence intervals are listed in table 2. The residual standard  
361 deviation, quantifying the empirical accuracy, was 0.27. Residuals were normally distributed  
362 according to the Lilliefors (adjusted Kolmogorov-Smirnov) test ( $D = 0.06$ ,  $p = 0.747$ ). As  
363 noted above, a Q-Q-plot of residuals indicates that one sample in treatment B of experiment  
364 2 was an outlier. However, since removing this data point shifts the confidence intervals only  
365 by small amounts, and its classification is not entirely clear, the data point was retained.

366

367 **Discussion**

368 Following earlier observations by e.g. Fry et al. (1978), Bolnick et al. (2003),  
369 Matthews and Mazumder (2004) and others, Bearhop *et al.* (2004) developed a hypothesis  
370 that the variance in stable isotope ratios of consumer tissues can be used to determine the  
371 trophic niche width of consumer populations. For this approach to be valid, the authors stated  
372 the following conditions: (i) the possible food sources must be isotopically distinct; (ii) each  
373 food source must be relatively invariable over time; and (iii) the tissue analysed must reflect  
374 the period over which the niche width is expressed. If these assumptions are fulfilled,  
375 Bearhop *et al.* (2004) predicted that it is possible to distinguish between specialist  
376 populations (SP), and two types of generalist populations (GA and GB, Fig. 1, 3) by  
377 analysing the variance of stable isotope ratios of appropriate consumer's tissues.

378 More formally, the distinction between generalists and specialists can be regarded as  
379 two extremes in a range of dietary correlation times. In these extreme cases, the generalist  
380 switches diets continuously while the specialist never switches. Field ecologists require  
381 information that allows them to assess the relative importance of assimilated prey on the  
382 isotopic variance recorded from wild populations. Here we have determined the factors  
383 driving dietary correlation time, which in turn influences population-wide isotopic variance. To  
384 facilitate this, we here created a model of isotopic variance dependent on dietary correlation  
385 time within a (model) population. The model makes the assumption that the impact of food  
386 on consumer isotope ratios decays approximately exponentially with the time since  
387 assimilation. We find our experimental data consistent with this model and that the decay  
388 rate can be measured to good precision.

389 As predicted, our results from Experiment 1 clearly demonstrated specialist  
390 populations (Fig. 4 A, B) had reduced isotopic variance compared to generalist populations  
391 (Fig. 4 C, G). However, one should note that the variances were measured under laboratory  
392 conditions that largely eliminated environmental and genetic variability. Variances were  
393 further reduced by pooling 15 individuals in each sample. In the field, all variance will be  
394 enhanced due to environmental and genetic variability among individuals. In principle, this  
395 should act as a constant offset, keeping differences between variances unaffected.

396 During our experiments, the *Daphnia* populations were not in equilibrium with the  
397 food, as our experimental period was too short to permit full tissue turnover (Grey 2000).  
398 Thus, our experiment imitated a natural situation, e.g. following a marked isotopic shift. Such  
399 a shift would occur for example during lake turnover, when lake stratification breaks down in  
400 the autumn/winter, and previously distinct water masses are mixed. This makes new food  
401 materials available to zooplankton (e.g. Harrod and Grey 2006, Perga and Gerdeaux 2006,  
402 Zohary et al. 1994). Variance in stable isotope values was also small in Type A generalist  
403 populations (GA<sub>long</sub>), when we imitated sampling of long-term integrating tissue. We imitated  
404 this Type A generalist population by feeding the *Daphnia* with two isotopically different food  
405 sources. As *Daphnia* are non-selective feeders and as the isotopically different food particles  
406 did not differ in any feature, every beaker represented a generalist individual within a  
407 population (treatment). Our analysis shows that the variances are equal in the two SP and  
408 GA, again in accordance with the prediction of Bearhop *et al.* (2004). Thus, taking only the  
409 variances of the stable isotope ratios into account, it would not have been possible to  
410 differentiate between these two types of populations. However, by taking also the mean  
411 stable isotope ratio into account and by knowing the stable isotope values of the potential  
412 food sources, we can reliably distinguish between the two population types. This is due to the  
413 fact that the mean stable isotope ratio of consumers corresponds well to the mean stable  
414 isotope value of the food source. If more than one food source is ingested, then the mean  
415 also depends on the proportion of the ingested food sources.

416 Many animal species, both trophic generalists but also species with pronounced  
417 ontogenetic diet shifts, do not feed on only one resource type throughout their life, but switch  
418 between several alternative resources. Within a population, switching to an alternative diet  
419 can occur synchronously for the whole population, e.g. with a habitat shift (e.g., Ben-Shahar  
420 and Coe 1992, Hjelm et al. 2000); or asynchronously within a population, if different parts of  
421 the population utilise different food patches at a given time (e.g., Harrod et al. 2010).  
422 Information on such diet-switches of generalist populations can be obtained by examining

423 several different tissues that reflect long- and short-term foraging histories. We studied this in  
 424 our second experiment in which we simulated two different GA population types: If each  
 425 beaker within a treatment (A, B or C) was taken as a GA population we simulate three  
 426 populations (A-C) that underwent a synchronous diet switch. In contrast, all beakers together  
 427 can be interpreted as belonging to a single population consisting of three generalist  
 428 subpopulations (the three treatments) that switched their food source asynchronously. While  
 429 our synchronously feeding GA population ( $GA_{syn}$ ) had a small variance, the  $GA_{as}$  population  
 430 feeding asynchronously yielded a large variance (Fig. 4), permitting the two different  
 431 population types to be differentiated.

432 Combining the results from the two experiments, we suggest that one can not only  
 433 reliably differentiate between SP and GA populations, but also between asynchronous and  
 434 synchronous diet-switching modes in GA populations. However, this is limited to situations  
 435 where short-term integrating tissues are available (Fig. 3).  
 436

#### 437 *Modelling the $\delta^{13}C$ response function*

438 The central result of fitting the response function [Eq. (1)] to the experimental data is  
 439 an estimate of the tissue dilution rate (with standard error in parentheses)  $r = 0.549 \text{ d}^{-1}$   
 440 (0.041) which is, next to the constant fractionation offset, the only parameter entering this  
 441 generic model. We find the dilution rate to be equal or slightly larger than the growth rate  $g =$   
 442  $0.4\text{-}0.5 \text{ d}^{-1}$ . A small difference of the order of  $0.1 \text{ d}^{-1}$  can indeed be expected in view of the  
 443 tissue turnover rates of  $0.1 \text{ d}^{-1}$  found by Grey (2000) for adult *Daphnia* which show only  
 444 limited growth.

445 From Eq. (1), it is immediately clear that *Daphnia* individuals that feed on all potential  
 446 food items within time intervals much shorter than the dilution time of  $r^{-1} \approx 2$  days have a  $\delta^{13}C$   
 447 value that simply represents the isotopic average of their diets, offset by trophic fractionation.  
 448 In the opposite case, when individuals continuously feed on a single food type over time  
 449 intervals that are long compared to  $r^{-1}$ , their  $\delta^{13}C$  value corresponds with the food type they  
 450 are currently feeding on, again offset by trophic fractionation. However, Eq. (1) also makes  
 451 quantitative predictions for intermediate cases. Assume, for example, that the  $\delta^{13}C$  values of  
 452 the food items of *Daphnia*, weighted by diet proportions, are approximately normally  
 453 distributed with variance  $V_{food}$ , and that, as an individual browses different food categories,  
 454 the momentary  $\delta^{13}C$  value of its diet can be modelled by a mean-reverting random walk  
 455 (Ornstein-Uhlenbeck process) with reversion rate  $k$ . The correlation time of this process,  $1/k$   
 456 (see, e.g. Gardiner 1983), gives the dietary correlation time. Equation (1) effectively  
 457 describes a linear filter, smoothing the time series of  $\delta^{13}C_{food}(t)$  with a filter kernel  $r e^{-rt}$ , which  
 458 has a power gain (the squared modulus of its Fourier transform) that depends on angular  
 459 frequency  $\omega$  as  $r^2/(r^2 + \omega^2)$ . The variance  $V_{cons}$  of the  $\delta^{13}C$  value of individuals can  
 460 therefore be computed as the integral, over all radial frequencies  $\omega$ , of the product of the  
 461 power spectrum of the Ornstein-Uhlenbeck process (given by  $\pi^{-1}kV_{food}/(k^2 + \omega^2)$ ) and the  
 462 power gain of the filter kernel. This yields

$$463 \quad (4) \quad V_{cons} = \int_{-\infty}^{\infty} \frac{1}{\pi} \frac{kV_{food}}{k^2 + \omega^2} \frac{r^2}{r^2 + \omega^2} d\omega = \frac{V_{food}}{1 + \frac{k}{r}}$$

464 Figure 5 illustrates the dependence of  $V_{cons}$  on  $k/r$  predicted by this model for a  
 465 hypothetical field study. Equation (4) contains the previously discussed limiting cases: when  
 466 food sources are varied quickly ( $k \gg r$ ) all individuals have similar  $\delta^{13}C$  values and  $V_{cons}$  is  
 467 much smaller than  $V_{food}$  ( $V_{cons} \approx r V_{food}/k \ll V_{food}$ ), while in the opposite case ( $k \ll r$ ) the two  
 468 variances are practically identical.

469 Together with an example for the expected dependence of  $V_{food}$  on dietary correlation  
 470 time, Fig. 5 also shows estimates of expected measurement errors and biases. The curve is  
 471 computed from Eq. (4) with  $r$  set to the value and  $V_{food} = 2.2 \text{ ‰}$ , which corresponds to the  
 472 variance of  $\delta^{13}C$  values among phytoplankton taxa reported by Vuorio et al. (2006) for a  
 473 freshwater lake. As a simple estimate of additional contributions to  $V_{cons}$  due to measurement

474 uncertainty and non-trophic variation among individuals, we used the mean over the cases  
475 U, L, and M in Experiment 1 of the products of observed sample variances (Table 3) and the  
476 numbers of daphnia per sample, giving  $0.8 \times 10^{-6}$ . Expected standard errors of empirically  
477 determined variances were then computed for a hypothetical sample size of  $n = 100$ , using  
478 the formula  $2^{1/2} (n-1)^{-1/2} V_{\text{cons}}$ , which is the exact result for the standard error of a variance,  
479 estimated using the standard formula from normally distributed sample values.

480 Araújo et al. (2007) performed a related analysis, in which the degree of individual  
481 specialisation of frogs in a field study was estimated from isotopic variance among  
482 individuals and compared against the degree of individual specialisation that would have  
483 been deduced from the momentary stomach content of individuals sampled in the field.  
484 Depending on the species studied, agreements between the two estimates were found, but  
485 also deviations in either direction. The approach of Araújo et al. differs from ours in that their  
486 model for isotopic variance implicitly assumes diets to be fixed over times of the order of  
487 magnitude of the tissue integration time (Type GB or  $GA_{\text{short}}$ ), but instead contains a  
488 parameter controlling the degree of individual variation in the composition of these diets. The  
489 difference between the two models corresponds to different models of foraging behaviour.  
490 While our approach assumes that a consumer's next prey choice is biased to be similar to  
491 recent prey choices (and DCT parameterises this bias), the model of Araújo et al. (2007)  
492 implies that variation in individual prey preference are in effect statistically independent of the  
493 temporal order of previous intakes. Instead, they could be controlled by phenotypic variation  
494 among individuals (Fermon and Cibert 1998, Robinson et al. 1993, Svanbäck and Bolnick  
495 2005). Both behavioural models are plausible as they stand. Only detailed studies of  
496 foraging behaviour can reveal the relative degree to which they are realised in nature, and  
497 hence which analytic approach of isotopic variation is more appropriate.

498

#### 499 *Consequences for the determination of trophic niche width*

500 The stable isotope approach for determining trophic niche width proposed by Bearhop et  
501 al. (2004) is a very appealing method, as it is less labour intensive than conventional gut  
502 content analysis and depending on the tissues sampled can reflect longer periods of  
503 consumer feeding behaviour. It has been used in a series of recent field studies which  
504 confirmed its successful applicability (e.g., Jaeger et al. 2010, Willson et al. 2010).  
505 Additionally, by choosing the appropriate tissue, this method delivers a good time-integrated  
506 measurement of niche width, which is difficult to achieve with the conventional methods. This  
507 is of particular importance when seasonally synchronized diet shifts of specialist populations  
508 occur which can only be interpreted when analyzing tissues with different integration times  
509 (Martinez del Rio et al. 2009). When Syväranta and Jones (2008) investigated their field data  
510 from fish with respect to the predictions of Bearhop et al. (2004), they found that the  
511 variances in stable isotope ratios in fish changed after mass removal of fish from an originally  
512 overpopulated lake and they interpreted the results as a broadening of the niche due to  
513 competitive release. However, none of these previous studies gave any direct evidence that  
514 the niches actually widened but only made indirect conclusions based on the prediction of  
515 Bearhop et al. (2004). While these previous studies are important starting points to indirectly  
516 assess the validity of the model in natural systems, our work aims to present more direct  
517 evidence from a controlled laboratory system. Our data clearly demonstrate that variation in  
518 isotopic variance between different population types is closely associated with the trophic  
519 history of the populations.

520 In closing, we propose a standard workflow for the use of dietary correlation time as a  
521 useful tool to quantify the position of a population on the “specialist-/generalist-individuals”  
522 axis. We point out important issues and potential caveats that isotope ecologists should  
523 consider:

- 524 1. Determine the diet of the target population of consumers. To avoid circularity of the  
525 analysis, this will generally require the use of traditional methods (e.g., observations  
526 of foraging, analyses of gut/stomach content, faeces).
- 527 2. Determine the population-level isotopic variance of the diet  $V_{\text{food}}$ , weighted by relative  
528 contributions.

- 529 3. Determine the tissue dilution rate  $r$  in laboratory studies or from the literature, or, for  
530 juveniles, estimate it from the somatic growth rate  $g$  as  $r \approx g$ , either for the targeted  
531 organisms or for similar species.
- 532 4. Determine the population-level isotopic variance among individuals  $V_{\text{cons}}$  of the target  
533 population in the field.
- 534 5. Estimate the dietary reversion rate  $k$  by solving Eq. (4), i.e.,  $k = r[(V_{\text{food}}/V_{\text{cons}}) - 1]$ . The  
535 dietary correlation time equals  $k^{-1}$ .
- 536 6. Understand potential errors. The dependence of dietary correlation time on  $r$  is rather  
537 robust: according to the standard propagation-of-errors law, the relative error in  
538 dietary correlation time contributed by errors in  $r$  is of the same magnitude as the  
539 relative error in  $r$  itself. The dependence of  $k$  on  $V_{\text{food}}$  and  $V_{\text{cons}}$  is more sensitive. For  
540 pronounced individual generalism, that is,  $V_{\text{food}} \approx V_{\text{cons}}$  and  $V_{\text{food}}/V_{\text{cons}} \approx 1$ , only the semi-  
541 quantitative conclusion that  $k \ll r$  will be possible, because  $k$  is then given by the  
542 difference between two similar values. Use of tissues with shorter integration time  
543 might mitigate this constraint. As illustrated in Fig. 5, statistical errors in  $V_{\text{cons}}$  can be  
544 substantial, even with a sample size of 100 individuals. Besides,  $V_{\text{cons}}$  will generally  
545 contain an unintended additive contribution of non-dietary origin (e.g., genetic  
546 variation). This contribution can be estimated in laboratory experiments, hence  
547 allowing determination of bounds on the resulting biases on dietary reversion rate  $k$   
548 and dietary correlation time  $k^{-1}$ .

549 In summary, our results support Bearhop *et al.*'s (2004) proposed use of stable isotope ratios  
550 to distinguish between populations that differ in their trophic niche. However, we urge that  
551 users also take mean stable isotope ratio values into account, as this will permit  
552 differentiation between SP and GA populations, when only short-term integrating tissues are  
553 available. This is particularly important in the case of small-bodied animals that are short  
554 lived, where only whole-body samples can be obtained. The stable isotope method provides  
555 a valuable means to distinguish between different consumption patterns ranging from true  
556 specialists through to true generalists. Nevertheless, it should be considered that it gives no  
557 information about the actual food items consumed, which can be investigated using the  
558 traditional approach of gut content analyses, or estimated from isotope mixing models, where  
559 the isotopic values of different prey resources are known (Phillips and Gregg 2001). Further  
560 studies are required to test the reliability of the method in a range of ecological settings,  
561 combining niche width estimates derived from stable isotope values and conventional gut  
562 content analysis.

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**Table 1:** Sequential feeding scheme for *Daphnia* in treatments A-C of experiment 2, following the suggestion of Bearhop *et al.* (2004). U=unlabelled food, L=labelled food, M= mixed food (1:1 U:L).

Day	Treatment A	Treatment B	Treatment C
1	M	M	L
2	M	L	U
3	L	U	M
4	U	M	M
5	M	M	M

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**Table 2:** Maximum likelihood fit of the model given by Eqs. (3a,b) to laboratory results.

<b>Variable</b>	<b>Unit</b>	<b>Best fit</b>	<b>Std. error</b>	<b>2.5%</b>	<b>97.5%</b>
a	‰	82.7	4.2	75.0	92.2
b	‰	-7.33	0.07	-7.47	-7.19
r	day <sup>-1</sup>	0.549	0.041	0.472	0.638

692 **Table 3** Isotopic ( $\delta^{13}\text{C}$ ) values of *Daphnia pulicaria* (experiment 1) at the start and the end of  
 693 the experiment. Throughout the experiment, *Daphnia* were fed either unlabelled  
 694 *Scenedesmus obliquus* (U),  $^{13}\text{C}$ -labelled *S. obliquus* (L) or a 1:1 mixture of U and L (M); The  
 695 last column gives the expected  $\delta^{13}\text{C}$  signature of *Daphnia* when in equilibrium with their new  
 696 food; Note that animals fed U and L represent specialist populations (SP), while animals fed  
 697 M represent a Type A generalist population (long-term tissue turnover,  $\text{GA}_{\text{long}}$ ).  
 698

Treat- ment	Food label	Non-equilibrium <i>Daphnia</i> $\delta^{13}\text{C}$ (measured) $\delta^{13}\text{C}$ ‰		Equilibrium <i>Daphnia</i> $\delta^{13}\text{C}$ (expected from food $\delta^{13}\text{C}$ ) $\delta^{13}\text{C}$ ‰
			variance	
	Start	-17.4	0.080	
SP	Unlabelled (U)	-16.3	0.025	-15.4
SP	Labelled (L)	1.1	0.070	8.5
$\text{GA}_{\text{long}}$	Mixed (M)	-7.3	0.057	-3.4

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702 *Figure legends*

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704 **Fig. 1:** Distinction between specialist populations (SP), Type A generalist populations  
705 composed of generalist individuals (GA), and Type B generalist populations composed of  
706 specialist individuals (GB); classification after Bearhop et al. (2004).

707

708 **Fig. 2:** Schematic illustration of the distinctions between  $GA_{short}$ ,  $GA_{long}$  and GB.  $GA_{short}$  and  
709  $GA_{long}$  distinguish situations where tissue integration time is short or long compared to the  
710 dietary correlating time. GB describes generalist populations where dietary correlation time is  
711 of the order of magnitude of the natural life time of individuals.

712

713 **Fig. 3:** Expected isotopic frequency distributions for mean variances of tissue  $\delta^{13}C$  values for  
714 the three types of consumers, if two isotopically distinct food types are consumed: specialist  
715 population (SP), Type A generalist population (GA), and Type B generalist population (GB).  
716 Broken lines represent  $\delta^{13}C$  values from food items, solid lines represent consumer  $\delta^{13}C$   
717 values. The upper panels (I) represent  $\delta^{13}C$  values in slow-turnover, long-time period  
718 integrating tissue; the lower panels (II) represent  $\delta^{13}C$  signatures in fast-turnover tissues. The  
719 split panel for GA shows the expected variances for synchronously and asynchronously  
720 feeding GA in short-term integrating tissue; the shift of the consumer's  $\delta^{13}C$  signatures to the  
721 right is due to trophic fractionation.

722

723 **Fig. 4:** Frequency histograms of  $\delta^{13}C$  values (from the experiments) of two specialist  
724 populations (SP, fed unlabelled and labelled food, respectively), long-term integrating tissue  
725 of one Type A generalist population ( $GA_{long}$ ), short-term integrating tissue of three GA  
726 populations feeding synchronously within the populations ( $GA_{syn}$ , A-C), and a short-term  
727 integrating tissue of one GA population feeding asynchronously ( $GA_{as}$ ). Data for  $SP_{unlabelled}$   
728 (N=21),  $SP_{labelled}$  (N=15) and  $GA_{long}$  (N=15), were taken from the experiment 1;  $GA_{short}$  A  
729 (n=20),  $GA_{short}$  B (n=19),  $GA_{short}$  C (n=20) and  $GA_{asyn}$  (n=59) were taken from experiment 2.

730

731 **Fig. 5:** Predicted variance of  $\delta^{13}C$  values among *Daphnia* individuals as a function of the  
732 dietary correlation time (*thick solid line*). To illustrate typical experimental uncertainties, the  
733 *thin solid line* adds variance due to non-trophic variability, and the *shaded area* represents  
734 the expected statistical errors (1 s.e.) in the case that 100 individuals are separately  
735 analysed. The *dashed line* gives the variance corresponding to an assumed standard  
736 deviation in  $\delta^{13}C$  among diet items of 2.2 ‰ (see Vuorio et al. 2006).

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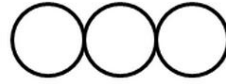
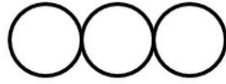
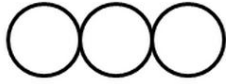
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739 Figure 1  
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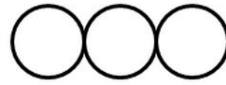
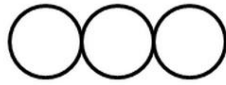
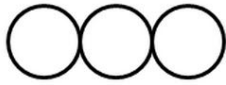
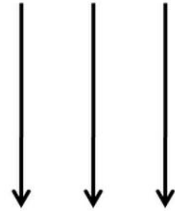
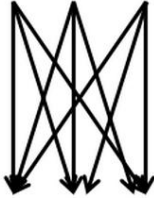
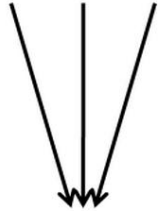
Specialist  
Population (SP)

Type A Generalist  
Population (GA)

Type B Generalist  
Population (GB)



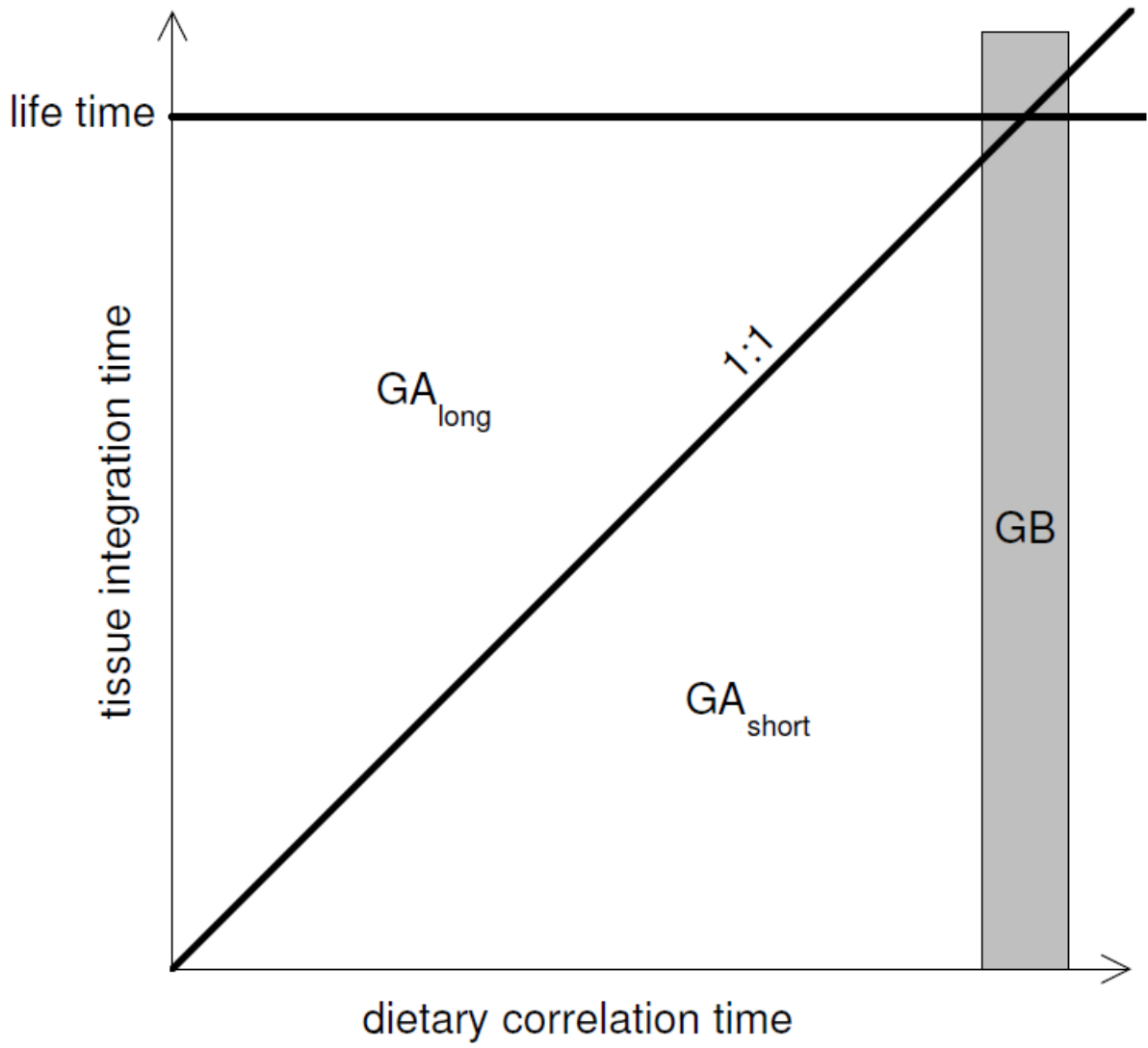
Consumers



Prey types

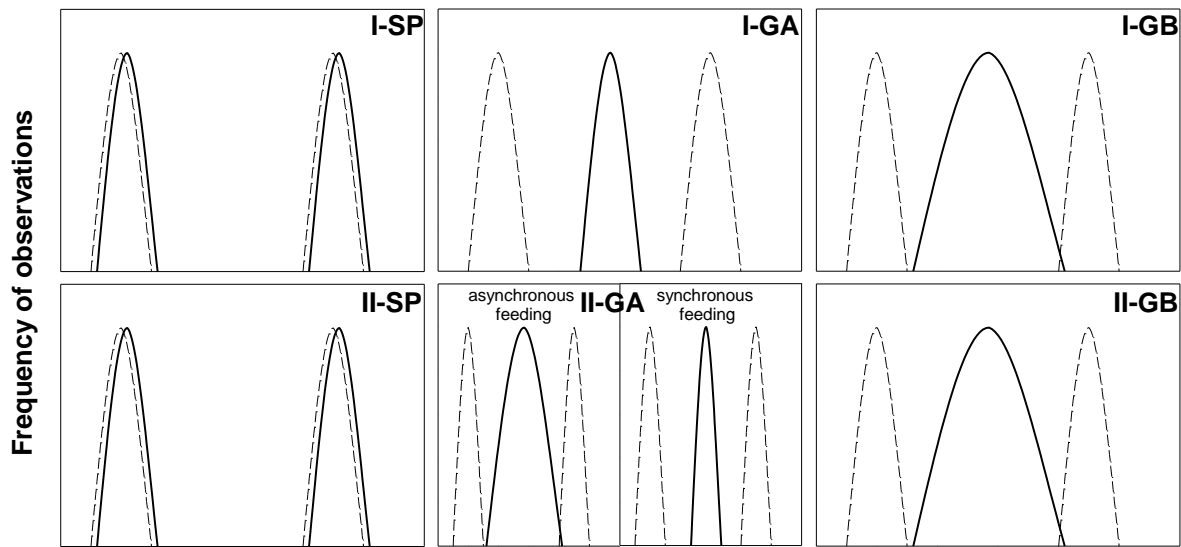
741  
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743 Figure 2  
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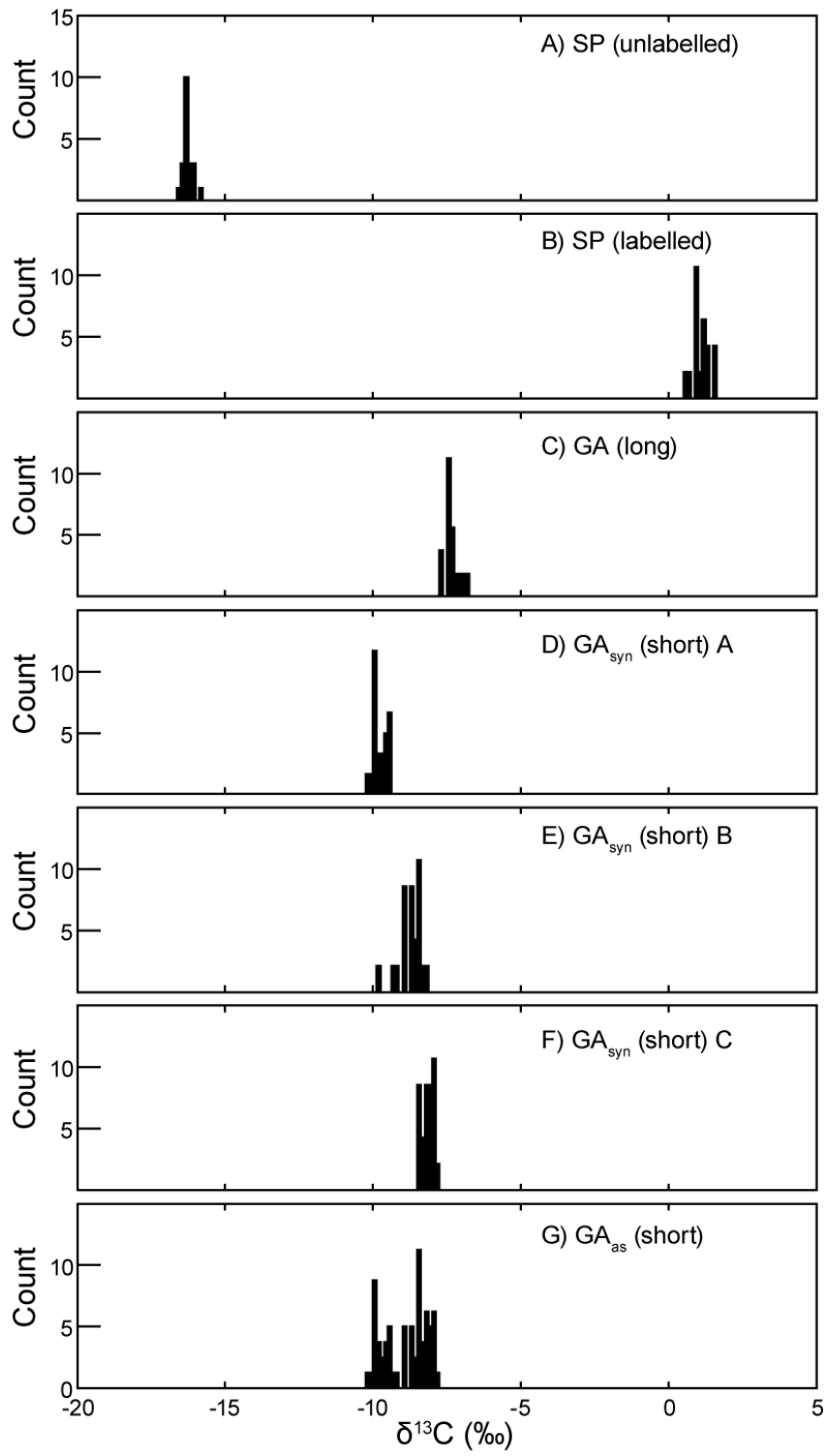
747 Figure 3



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750 Figure 4  
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755 Figure 5  
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