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Selective predation by benthivorous fish on stream macroinvertebrates – The role of prey traits and prey abundance

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1   **Selective predation by benthivorous fish on stream macroinvertebrates – the role of prey**

2   **traits and prey abundance**

3

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21   **Short title:** Prey selection by benthivorous fish

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23   **Key words:** *Barbatula barbatula,* feeding behaviour, non-visual foraging, *Gobio gobio*, size

24   selectivity, searching image

25

25   **Abstract**

26   The prey selectivity of fish depends largely on traits of prey and predator. Preferable prey

27   traits might be different for visual predators (such as drift-feeding salmonids) and rather non-

28   visual predators (such as benthic feeders). We evaluated the explanatory power of five prey

29   traits and prey long-term abundance for the prey selection of small benthivorous fish by

30   analysing the macroinvertebrate community and the diet of gudgeon (*Gobio gobio)* and stone

31   loach (*Barbatula barbatula)* in two small submontane streams. Fuzzy Principal Component

32   Analyses, as well as electivity indices, revealed that the fish fed selectively. Prey size and

33   feeding type were the most descriptive variables for the fish diet, followed by mean

34   abundance, whereas microhabitat preference, locomotion mode and current velocity

35   preference were less important. The fish preferred prey that was both small and consistently

36   abundant, grazers and sediment feeders. Larger prey and shredders were avoided. The

37   selection patterns of both fish species differed from those of visual fish predators but strongly

38   resembled each other. Supporting this, in gudgeon which feeds slightly more visually than the

39   strictly nocturnal stone loach, selectivity concerning prey traits as well as prey mean

40   abundance was slightly more pronounced. We analysed also selectivity for prey clusters based

41   on the three most important variables. The observed selectivity patterns concerning these

42   clusters were less pronounced but supported the other results. The maximum (neutral)

43   electivity index was that of gudgeon for small, abundant grazers or sediment feeders,

44   including chironomids.

45   We conclude that prey selection of benthivorous fish that forage mainly non-visually can

46   largely be explained by a small number of prey traits which probably work in combination.

47   The prey preferences of these predators seem to be closely connected to their active foraging

48   mode and to depend partly on the ability to detect prey visually.

49

49 **Introduction**

50   Selectivity in predation largely depends on the traits of both predator and prey

51 (O'Brien, 1979). Since selective predation is one of the strongest mechanisms structuring

52 communities (Sih et al., 1985), knowledge about the influence of prey traits on predator

53 selectivity is a prerequisite for the understanding of community processes. In stream

54 communities, fish often are the top predators. Traits of preferred prey have been analysed for

55 several predatory fish species, but mostly for visual predators. For instance, for drift-feeding

56 salmonids, particularly the drift behaviour and the body size of the prey are important (e.g. de

57 Crespin de Billy and Usseglio-Polatera, 2002; Rader, 1997; Syrjänen et al., 2011). The

58 omnivorous cyprinid *Rutilus arcasii* showed an opportunistic feeding behaviour but also a

59 positive size selectivity (Lobón-Cerviá and Rincón, 1994). To our knowledge, similar studies

60 for benthic, less visual (e.g. olfactory) fish predators are rare, despite the finding that benthic

61 fish may have a stronger predation impact on benthic communities than drift-feeders (Dahl,

62 1998). Because small-bodied benthivorous fish often feed nocturnally and thus non-visually

63 (Culp et al., 1991; Fischer, 2004; Huhta et al., 2000), they might select prey according to

64 other traits than drift-feeders do.

65   In a field experiment in Gauernitzbach and Tännichtgrundbach, two small submontane

66 streams in Central Europe, the benthic fish species gudgeon (*Gobio gobio* L.) and stone loach

67 (*Barbatula barbatula* [L.]) affected the structure of the macroinvertebrate community

68 (Winkelmann et al., 2011; Winkelmann et al., 2014). These two species differ slightly in their

69 habitat preferences and activity rhythms, gudgeon preferring pools with low current velocities

70 but foraging not as strictly nocturnally as stone loach, which in turn uses pools as well as

71 riffles for foraging (Fischer, 2004; Worischka et al., 2012; Zweimüller, 1995). In contrast to

72 gudgeon, stone loach feeds strictly non-visually and locates prey mainly by olfaction and

73 probably also via the lateral line system (Filek, 1960; Street and Hart, 1985). The numerically

74 dominant prey in the diet of both fish species in the above-mentioned streams were larval

75 chironomids, as reported also by other authors (e.g. Magalhaes, 1993; Michel and Oberdorff,

76 1995; Smyly, 1955). Chironomids were also the most abundant group in Gauernitzbach and

77 the second-most abundant (next to gammarids) in Tännichtgrundbach. This suggests a rather

78 opportunistic feeding behaviour of the fish predators. However, their actual predation impact

79 was not only mesohabitat-specific (Worischka et al., 2014; Worischka et al., 2012) but also

80 strongly prey species-specific (Winkelmann et al., 2011; Winkelmann et al., 2007). This

81 provokes the question whether also active prey selection by the fish predators was important

82 in the community-structuring process. For instance, chironomids might be preferred not just

83 due to their availability in high numbers but due to one or more 'preferable' traits. In the same

84 way as trait-based sensitivity against stressors is not independently distributed over

85 macroinvertebrate taxa (Schuwirth et al., 2015), also vulnerability against a certain predator

86 might be determined by correlated traits. Additionally, chironomids might not be the only

87 preferred prey item because they share such traits with other taxa. In order to separate the

88 effects of numerical prey availability and prey traits on predator selectivity, we evaluated the

89 importance of long-term prey abundance patterns as an additional factor during the analysis.

90 Long-term mean abundance – even though not a prey trait - is a variable influencing the

91 general encounter rate of a prey to a predator and thus can enhance the formation of a

92 searching image (Tinbergen, 1960). This is not restricted to visual predation (Atema et al.,

93 1980). The importance of abundance for prey selectivity can be seen in the switching

94 behaviour of predators: They often respond to changes in relative prey abundance by shifting

95 their preference to the most abundant prey and feeding disproportionately on it (Murdoch,

96 1969; Real, 1990). We assume that such short-term responses simply integrate over longer

97 time periods and, together with searching images, could lead to a general preference for

98 constantly abundant prey which goes beyond opportunistic feeding.

99   We investigated the prey selection by gudgeon and stone loach in Gauernitzbach and

100   Tännichtgrundbach over four years in a reach scale field predation experiment. We addressed

101   the following questions: (1) Do the two fish predators feed opportunistically or selectively,

102   and do they show similar prey preferences in spite of different spatial and temporal activity

103   patterns? (2) Can certain prey traits and/or long-term prey abundance explain feeding

104   selectivity of benthic fish? (3) Do the fish predators prefer any groups of prey being defined

105   by shared traits? For this purpose, we quantitatively analysed the macroinvertebrate

106   communities of the streams and the diet composition of the benthic fish, characterizing prey

107   taxa using five traits as well as their long-term mean abundance.

108

109   **Methods**

110   <u>Study site and experimental design</u>

111   Fish and benthic macroinvertebrates were sampled in Gauernitzbach (2nd order, 4.6 km long)

112   and Tännichtgrundbach (3rd order, 5.6 km long), which are tributaries of the River Elbe in

113   southeast Germany (51°06`46.63``N, 13°32`45.04``E; 51°05`12.43``N, 13°35`55.88``E).

114   Besides being located in close vicinity to each other, the streams have very similar physical

115   and chemical characteristics and benthic community compositions (Schmidt et al., 2009;

116   Winkelmann et al., 2003; Worischka et al., 2012). For a large-scale field experiment on top

117   down food web manipulation (Winkelmann et al., 2014; Worischka et al., 2014), an

118   experimental section of 400 m was separated in each stream, using 5 mm steel mesh. After

119   removing all fish (mainly trout stocked for angling) by backpack electrofishing (EGFI 650,

120   Bretschneider Spezialelektronik, Chemnitz, Germany), the experimental sections were

121   stocked with gudgeon and stone loach obtained from streams or small rivers from the same

122   region. Both species are small-bodied, benthivorous, and inhabit mainly streams and small

123   rivers of the Eurasian temperate zone, including the study streams. Fish density was

124 monitored at least four times a year by electrofishing. Losses due to winter mortality, bird

125 predation and occasional emigration during floods were compensated by restocking the fish

126 sections at least twice a year. Prey selection analysis was performed during two sampling

127 periods (2005-2006, 2009-2010) when fish were present in both streams with average

128 densities $\pm$ *SD* of 0.21 $\pm$ 0.19 ind m^{-2} (gudgeon) and 0.20 $\pm$ 0.17 ind m^{-2} (stone loach). These

129 densities exceeded those before the experiment (when trout as predators and competitors were

130 still present) but are in the same range as natural densities observed in comparable streams

131 (Erös et al., 2003; Santoul et al., 2005). The conditions of the predation experiment are

132 therefore well comparable to those of other, 'strictly natural' stream communities.

133

134 <u>Sampling and processing of the samples</u>

135 Six benthic macroinvertebrate samples from each stream, from three pool and three riffle

136 locations, were collected with a Surber sampler (0.12 m², 500 μm mesh size) every four

137 weeks, throughout the entire study period. The samples were sorted under a dissecting

138 microscope. The invertebrates were identified to the lowest practicable taxonomic level,

139 enumerated and measured to the nearest 0.1 mm. For prey taxa including large individuals

140 (*Ancylus fluviatilis*, *Dugesia gonocephala*, Limnephilidae and *Tipula* sp.) not only length but

141 also thickness (the second-largest dimension) was measured in the benthos samples. The

142 individual dry body masses were calculated using length-mass regressions (Benke et al.,

143 1999; Burgherr and Meyer, 1997; Hellmann et al., 2013; Meyer, 1989). Gudgeon for diet

144 analysis were collected on 21 occasions and stone loach on 22 occasions in both streams,

145 between April and October of the four study years, but always with a time lag of at least 4

146 weeks after stocking. Each sampling was carried out shortly after sunrise when the fish had

147 full guts. Five to ten individuals of each species were caught by electrofishing and killed

148 immediately. After measuring total length to the nearest 1 mm and weighing to the nearest 0.1

149 g, their digestive tracts were removed, cooled between ice packs during transport to the

150 laboratory, and stored at -18 °C. We aimed to synchronise benthos and fish sampling,

151 attempting to complete electrofishing the day after benthos sampling. When this was not

152 possible (i.e. the time lag exceeded 1 day), benthic macroinvertebrate densities were

153 interpolated to the respective fish sampling date by calculating the time-weighted average of

154 the benthic densities observed at the nearest sampling dates before and after the respective

155 fish sampling date. Diet analyses were based on the contents of the stomach (stone loach) or

156 anterior gut (gudgeon). Fish diet samples were processed individually, in the same way as the

157 benthos samples, and all individuals of a fish species and a date were pooled later for

158 calculations. The gape width (G) of the fish was estimated from total length (TL) using linear

159 regression equations. These were derived from previous TL and G measurements of

160 individuals from both streams (unpublished data). The equations are $G = 0.0643\ TL - 0.147$

161 for gudgeon ($R^2 = 0.88$, $p < 0.0001$, $n = 382$) and $G = 0.035\ TL + 0.889$ for stone loach ($R^2 =$

162 0.81, $p < 0.0001$, $n = 153$). Gudgeon in our study had a total length of 100.0 ± 24.2 mm (mean

163 $\pm SD$) and an estimated gape width of 6.4 ± 1.7 mm. The mean total length of stone loach was

164 101.1 ± 30.0 mm with gape width 5.0 ± 0.5 mm.

165

166 Data analysis

167 Six variables describing the macroinvertebrate prey were used in this study (Table 1): five

168 traits which we assumed to be of importance for predator selectivity and, additionally, long-

169 term mean abundance. We chose this limited number of variables for two reasons. First, many

170 traits are inter-correlated in benthic macroinvertebrates (Poff et al., 2006) and we tried to

171 choose relatively independent traits *a priori*. Second, prey traits which are of any importance

172 for visual predators only (such as drift behaviour or crypsis), were excluded. Each of the

173 selected variables had 3 to 5 categories. Four of the traits (feeding type, locomotion mode,

174 microhabitat preference and current velocity preference) were taken from a trait compilation

175 by Tachet et al. (2002); the fifth trait (body size) was parameterized from own data. We

176 omitted some trait categories from the compilation of Tachet et al. (2002), which were not

177 relevant for our study streams, e.g. microhabitat 'macrophytes'. The assignment of each taxon

178 to the categories was achieved using a fuzzy coding procedure (Chevenet et al., 1994). This

179 procedure includes the use of weightings expressing the affinity of a taxon to each of the

180 categories. Following Tachet et al. (2002), we used weightings between 0 and 3 for feeding

181 type and current velocity preference and weightings between 0 and 5 for locomotion mode

182 and microhabitat preference. These weightings were transformed into relative proportions

183 within each trait (between 0 and 1). For chironomids, we weighted their trait categories

184 according to the relative abundances of the three dominant subfamilies Orthocladiinae,

185 Tanypodinae and Chironominae (together forming 97.5% of the chironomids, based on

186 routine emergence trap samplings throughout the study period, C. Hellmann, unpublished

187 data) as recommended by Sheldon and Meffe (1993). Additionally, higher proportions for the

188 feeding type category 'predator' than proposed by Tachet et al. (2002) were employed for

189 *Gammarus* spp., *Hydropsyche* spp. and *Isoperla grammatica*, according to results of a

190 previous study from the same streams (Hellmann et al., 2013). The trait body size was based

191 on body mass data from our macroinvertebrate samples. It was also a convenient proxy for

192 energy content per individual because the energy contents per mg dry mass found in the

193 database collected by Brey et al. (2010) were similar for all prey taxa (20.9 ± 2.3 J mg^{-1}, mean

194 $\pm$ *SD*, $n = 37$), except for molluscs with shells, which were rarely eaten by the fish. Five size

195 categories were defined *a priori* (Table 1). The assignment of a taxon to the size categories

196 was done as follows: We calculated the mean individual dry body mass (geometric mean) of

197 each taxon for each sampling date and stream separately. The obtained values were each

198 assigned to one of the five size categories, and their relative frequencies constituted the

199   weightings for each taxon in the fuzzy coding. Long-term mean abundance was also coded

200   like a trait: We assigned density values (ind m^{-2}) for each taxon at each sampling date and

201   stream to three abundance categories (Table 1) and used the relative frequencies of the

202   categories for each taxon as weightings.

203   In total, 42 benthic macroinvertebrate taxa identified in the streams, including the

204   dummy taxon 'others', were assigned to the six variables (i.e. five traits plus mean

205   abundance), forming a 'taxa × traits' array (Table S1 in supporting information). For

206   convenience, we kept the commonly used denotation 'traits' instead of the more general term

207   'variables'. The taxon 'others' received average weightings for all categories. In order to

208   avoid a biased estimation of prey selection, we included only the actual edible prey size

209   spectrum for the fish in the calculations. This was based on a gape width of 4.5 mm, which

210   was estimated as the lower value of $\overline{G}$ − 1 SD of both fish species, (i.e. 4.7 mm for gudgeon

211   and 4.5 mm for stone loach). Thus, prey individuals thicker than 4.5 mm qualified as non-

212   edible for most of the fish; these were therefore excluded from the calculations. For

213   Oligochaeta (except *Eiseniella* sp.) in the benthos samples we used correction factors of 0.2

214   for abundance and 5 for body mass. The correction was necessary because individuals of the

215   dominant subfamily (Naidinae) tend to fragment into roughly five fragments per individual

216   during sampling (personal observations). This leads to abundance being easily overestimated

217   and body mass being underestimated. In the fish diet samples, no corrections were needed

218   because only a few whole individuals and no fragments were found. Terrestrial prey was

219   excluded from the calculations, contributing only 0.6% ± 1.6% (mean ± SD, all samples) to

220   the total numeric abundance in the fish diets.

221   A 'benthos samples × traits' array and a 'diet samples × traits' array were created for each

222   fish species. For the arrays, the fuzzy-coded categories of each of the six variables were

223   weighted with the relative abundances of the taxa in the respective samples, for each sampling

224   date and stream. The different foraging habitat preferences of gudgeon and stone loach

225   (Worischka et al., 2012) were accounted for in the benthos samples. For gudgeon which used

226   almost exclusively pool mesohabitats, macroinvertebrate abundances from pool samples were

227   weighted higher than those from riffle samples (97 resp. 3 %) whereas for stone loach, both

228   mesohabitats were weighted equally. This was done in order to reflect the actual mesohabitat-

229   specific prey availability for each fish species and so to avoid a biased selectivity analysis.

230   We performed a fuzzy principal component analysis (FPCA) on the 'benthos samples × traits'

231   and 'diet samples × traits' arrays (R-package ade4 version 1.5-1; Dray and Dufour, 2007;

232   Thioulouse et al., 1997) to assess the importance of the variables, i.e. traits, for the difference

233   between the corresponding benthos and diet samples (hypothesis 1). FPCA is a robust

234   modification of principal component analysis (Cundari et al., 2002) and was successfully

235   applied to fish diet analysis before (Sanchez-Hernandez, 2014; Sanchez-Hernandez et al.,

236   2011). In order to compare the available prey in the benthos directly to the consumed prey, we

237   combined the two arrays to one joint dataset (one below the other, Legendre and Legendre,

238   2012, p 702) for each fish species.

239   Prey selection of the fish sampled on each date was calculated using the relativized electivity

240   index E^* (Vanderploeg and Scavia, 1979), based on both prey traits and prey taxa:

241     $$E_i^* = \frac{W_i - (1/n)}{W_i + (1/n)}$$ (1)

242   with

243     $$W_i = \frac{r_i / p_i}{\sum_i r_i / p_i}$$ (2)

244   with r_i being the proportion of a prey item i in the diet and p_i its proportion in the

245   environment, and n being the number of different prey items. E^* can have values between -1

246   (complete avoidance) and, theoretically, 1 (complete preference), with $E^* = 0$ indicating

247   neutral selection. Among the large number of available electivity indices, E^* was

248   recommended in the reviews by Lechowicz (1982) and, with minor reservations, by Lazzaro

249   (1987). We chose it for our study because it has the random value 0 (which we regard to be

250   most intuitive), is robust against variation of the number of prey types and amenable to (non-

251   parametrical) statistical testing. For the trait-based calculation of E^* we used the relative

252   abundance data for trait categories from the fuzzy-coded 'benthos samples × traits' and 'fish

253   diet samples × traits' arrays as r_i and p_i. A general challenge in electivity calculation is the

254   occurrence of a taxon in a diet sample but not in the corresponding benthos sample. This

255   happens occasionally with rare taxa and results in a seemingly infinite quotient between the

256   relative abundances in the diet and in the environment. We attempted to solve this problem

257   while maintaining a high taxonomic resolution by replacing the respective zero values (only

258   for taxa actually eaten by the fish in this sample) in the benthos samples by a value of 'half of

259   the minimum detection level' (0.5 individuals per benthos sample or 4.265 ind m^{-2}). The

260   constrained habitat use of gudgeon was, like for the FPCA, incorporated by weighting the

261   macroinvertebrate abundances in the benthos from pools higher. In addition to analysing size

262   selection regarding whole prey taxa, we wanted to get an idea of selection for the same size

263   categories *within* one prey taxon. For this purpose we used the example of *Gammarus* spp.,

264   which was abundant in the benthos with a broad size spectrum and frequently eaten by the

265   fish, calculating E^* for each size category.

266   In order to see whether the fish show any preferences for prey taxa sharing certain

267   combinations of traits or variables, we grouped the prey taxa by the most meaningful

268   variables from the previous analyses. We intended to create a clearer and more realistic

269   classification by omitting those variables we already found to have less influence on the prey

270   selectivity of the fish. For this purpose, we reduced the 'taxa × traits' array to those three

271   variables clearly identified as important in both the FPCA (highest loadings on the first two

272   axes) and the trait-based electivity indices (highest ranges). We performed a hierarchical

273   cluster analysis based directly on the reduced array. The number of clusters was determined

274   manually from the dendrogram by cutting at the height H with the largest difference to the

275   two 'neighbouring' solutions, i.e. at $H = 1.62$. For each of the obtained 8 clusters, E^* was

276   calculated. All statistical analyses and graphics were carried out using the software R (version

277   3.0.2, R Development Core Team, 2013).

278

279   **Results**

280   <u>Multivariate analysis of selective *vs.* opportunistic feeding</u>

281   Gudgeon and stone loach showed similar and pronounced prey selectivity patterns in

282   our study (for a detailed presentation of the diet composition see Table S2 in the supporting

283   information). In the FPCA plots of the combined datasets for benthos and gudgeon diet (Fig.

284   1), the first axis with an eigenvalue of 0.052 explained a major part (78%) of the total inertia

285   (0.067) whereas the second axis contained much less information (eigenvalue = 0.009 or

286   14%). In the FPCA plot of the combined datasets for benthos and stone loach diet (Fig. 2), the

287   eigenvalues of the first two axes (0.039 and 0.014) were slightly lower than for gudgeon but

288   still explained a major part (56% and 20%) of the total inertia (0.068). The plots showed

289   similar characteristics for gudgeon and stone loach, especially concerning the distribution of

290   the variables (Figs. 1d and 2d). Categories of size, feeding type and abundance were most

291   prominent whereas the other variables seemed to have a very low explanatory power. For

292   both fish species, the arrows of the samples largely follow two main directions, corresponding

293   to the categories 'small', 'abundant' and 'grazer' but are directed opposite the categories

294   'medium sized' and 'shredder'.

295   The differences between the corresponding diet and benthos samples (lengths of the

296   arrows) were mostly larger than the differences among samples, indicating selective predation

297   by both fish species. The stone loach diet samples were more widespread than those of

298   gudgeon, indicating a higher variability in food composition. Additionally, the arrows appear

299   mostly shorter than for the benthos-gudgeon comparison, indicating a slightly weaker

300   selectivity in stone loach. The FPCA plots for both fish species have further characteristic

301   patterns in common: The benthos samples showed a seasonal pattern along the first axis and a

302   separation between the two streams along the second axis. In summer and fall, the difference

303   between streams was greater than in spring. The seasonal differences seemed larger than those

304   between the streams. In the fish diet samples, however, these differences were much smaller,

305   indicating that both fish species showed true and similar preferences independent of stream or

306   season. In addition, the FPCA plots indicated no systematic differences between the two

307   sampling periods (2005-2006 and 2009-2010).

308

309   <u>Electivity indices for single prey variables and prey groups</u>

310   Gudgeon showed significant electivity indices, i.e. $E^* \neq 0$, for 18 of the 26 categories,

311   stone loach only for 6 categories (Fig. 3, Table 2, two-sided Wilcoxon tests with Holm

312   correction, $p < 0.05$, $n = 21$ for gudgeon and $n = 22$ for stone loach). However, only few

313   categories were preferred, most strongly 'very small', 'small' and 'sediment feeder' by

314   gudgeon, and 'sediment feeder' and 'microhabitat wood/roots' by stone loach. By far more

315   categories were avoided by the fish, most strongly 'very large', 'large' and 'shredder' by

316   gudgeon, and 'shredder', 'large' and 'medium-sized' by stone loach. Among all prey items

317   within the edible size spectrum, both fish predators generally preferred small prey taxa and

318   avoided large ones. In contrast, we observed an avoidance of the two smallest size classes

319   *within* the taxon *Gammarus* spp. (Fig. 4) and neutral selectivity for the larger ones. For

320   gudgeon, a preference of abundant prey over common and rare prey was visible although not

321   statistically significant (Fig. 3, Table 2). Stone loach, in general, showed a smaller total range

322   of electivity indices.

323   The variables with the highest ranges of electivity indices between the categories were

324   size, feeding type and mean abundance for both fish species (Table 2), and the categories

325   yielding the highest mean E^* values (independent of their significance) belonged mostly to

326   these three variables (Fig. 3). Because the same three variables were also prominent in the

327   FPCA, the division into prey groups by cluster analysis was based only on them. We found

328   eight distinct groups of prey taxa characterised by one or more categories of the three

329   variables (Fig. 5a): very large sediment feeders (group 1, only *Eiseniella tetraedra*), rare

330   small taxa (2, e.g. *Isoperla* sp.), rare shredders (3, e.g. *Capnia bifrons*), highly abundant

331   grazers and sediment feeders (4, e.g. Chironomidae), highly abundant shredders (5, e.g.

332   *Gammarus* spp.), filter feeders (6, e.g. *Hydropsyche* spp.), predators (7, e.g. *Dugesia*

333   *gonocephala*) and medium-sized grazers (8, e.g. *Rhithrogena semicolorata*). Again, we

334   observed very similar selectivity patterns for gudgeon and stone loach, i.e. a negative average

335   selectivity for most of these prey groups (Figs. 5b and 5c, two-sided Wilcoxon tests with

336   Holm correction, $p < 0.05$, $n = 21$ for gudgeon, $n = 22$ for stone loach). Group 4 was selected

337   neutrally by gudgeon (sole positive E^* value) and groups 1, 4, 6 and 8 by stone loach. The

338   electivity indices of the fish predators for each single taxon are given in Table S2 (supporting

339   information).

340

341   **Discussion**

342   Selective *vs.* opportunistic feeding

343   Combining two different approaches (multivariate analyses and electivity indices), we

344   evaluated the prey selection of gudgeon and stone loach as top predators in two small streams

345   and identified the most important of six prey-characterising variables. The results

346   concordantly suggest that the benthivorous fish foraged rather selectively than

347   opportunistically, selecting some trait categories over others. This selectivity was observed

348 during all seasons even with the fish diets showing a dependency of the predators on

349 seasonally changing prey availability. The trait-based approach seems therefore useful to

350 detect and describe prey selection not only for drift-feeding fish (e.g. Rader, 1997) but also of

351 benthic feeders in streams.

352

353 <u>Importance of prey traits and mean abundance for selectivity</u>

354   Two of the five analysed prey traits (size and feeding type), and long-term abundance

355 as additional characterising variable had a strong influence on predator selectivity in our

356 study. First and foremost, gudgeon and stone loach exhibited a strong size selectivíty,

357 preferring very small and small prey species. This was not due to gape limitation because only

358 the edible size spectrum was included in the analyses. Such a feeding behaviour is in contrast

359 to that of mainly visually foraging fish, which under ideal conditions prefer large, i.e.

360 energetically favourable, prey individuals (Allan, 1981; Rincón and Lobón-Cerviá, 1995;

361 Turesson et al., 2002). Even some non-visually foraging fish such as nocturnal planktivores

362 showed a positive selection for larger prey, which was mediated mainly by size-dependent

363 encounter rate (Holzman and Genin, 2005). The apparently paradoxical size selection of

364 gudgeon and stone loach might be explained on the one hand by their preferred foraging

365 mode, i.e. actively searching the stream bottom (Filek, 1960; Worischka et al., 2012), which

366 makes size-dependent activity of the prey less important for encounter rate. On the other

367 hand, we may also take into account that small taxa are usually more abundant than large taxa

368 (Meehan, 2006). A selection of small prey therefore may indirectly select for abundant prey,

369 and *vice versa*. This was observed with gudgeon and stone loach, which showed a relative

370 preference not only for the categories 'very small', 'small' and 'abundant', but also for small-

371 bodied and abundant prey taxa (chirononomids and simuliids, respectively, see Table S1 and

372 S2 in the supporting infornation). Small size classes of one abundant prey taxon, *Gammarus*

373 spp., were rather avoided by both fish species, but they were also less frequent in the benthos

374 than the medium and large size classes during the sampling periods. Therefore, we suspect

375 that the apparent size selectivity was in fact selectivity for abundant prey taxa. We assume

376 that this behaviour could be a number-maximizing feeding tactic, comparable to that found by

377 Rakocinski (1991) for small darter species. In Optimal Foraging Theory (Emlen, 1966;

378 MacArthur and Pianka, 1966), prey size determines energy content and handling time and

379 prey abundance determines the encounter rate. An increasing selectivity for larger (i.e.

380 energetically more profitable) prey with increasing absolute prey abundance (Werner and

381 Hall, 1974) is likely only as long as handling time is constant and prey is encountered

382 simultaneously, a typical situation for planktivorous fish. For small benthivorous fish species,

383 it is more realistic to assume that handling time is relatively long and increases with prey size,

384 prey is encountered sequentially, and satiation occurs sooner during feeding. Under these

385 conditions, an increasing preference for smaller prey would be the most efficient feeding

386 tactic (Gill, 2003; Hart and Ison, 1991).

387 Thus, the consideration of size and abundance in combination seems to be necessary in

388 prey selectivity analyses. Switching as a short-time response to fluctuations in relative prey

389 abundances seems to be common in fish predators (Hughes and Croy, 1993; Ringler, 1979;

390 Zhao et al., 2006) and probably also occurred in our study system. However, the fuzzy-coded

391 long-term mean abundance categories we used in our analysis represent more information

392 than just the momentary relative abundance, namely whether a prey is regularly encountered

393 by the predator with a high probability. Therefore, we assume that the general preference of

394 gudgeon and, to a lesser extent, also of stone loach for highly abundant prey can be explained

395 only with a combination of at least two mechanisms, a fast-acting one (switching) and a slow

396 one. The latter could be a certain 'inertia' of the searching image (Tinbergen, 1960), as

397 learning processes, for instance the acceptance of novel, rare prey types among known, highly

398   abundant prey types, are often associated with a time delay (Fraser et al., 2013). Another

399   learning process is the recognition of non-profitable patches in heterogeneous environments.

400   Here, predators seem to need much more time to identify such patches regarding prey

401   abundance than regarding prey body mass (Esposito et al., 2010). The search mode of

402   gudgeon and stone loach as benthic feeders is probably strongly patch-related. It is therefore

403   conceivable that the short-term preferences of gudgeon and stone loach have merged over

404   time into a general preference for abundant prey. Supporting this line of thought, Johnson et

405   al. (2007) as well as Uieda and Pinto (2011) indicate the highest electivity indices of fish

406   predators for the (overall) numerically dominant prey in the respective benthic community. In

407   more homogeneous pelagic predator-prey systems, where visual foraging is also more

408   important, prey ingestion more often seems to be proportional to relative prey abundances, or

409   the preferred prey is not the most abundant one (e.g. Storch et al., 2007; Verliin et al., 2011).

410   Macroinvertebrate feeding type was, next to size, the most important trait influencing

411   the prey selectivity of the fish; grazers and sediment feeders were generally preferred in our

412   study. In accordance with our findings, benthic grazers in stream enclosures were subject to a

413   strong top-down influence by benthivorous sculpins, which was partly explained by their

414   body size and partly by their feeding habit and resulting exposition (Rosenfeld, 2000). The

415   feeding modes grazing and sediment feeding are often closely associated in benthic

416   macroinvertebrates, i.e. many taxa use both (see Table S1 in the supporting information). In

417   contrast, the category 'shredder' was, although very common among the benthic

418   macroinvertebrates in the studied streams and also in the fish diet, negatively selected by the

419   fish. This was true even for highly abundant shredders as can be seen from the cluster-based

420   selectivity analysis, underlining the high relevance of prey feeding type for predator

421   selectivity. In a previous field experiment in Gauernitzbach (Winkelmann et al., 2007),

422   gudgeon had a much stronger predation effect on *Gammarus pulex* (an important shredder)

423   than on *Rhithrogena semicolorata* (an important grazer). A special characteristic of

424   macroinvertebrate shredders is that they are able to use their food source as refuge. The

425   effectiveness of this predator avoidance strategy has been shown by Szokoli et al. (in press).

426   The prey traits locomotion mode, microhabitat preference and current velocity

427   preference were of lower importance for prey selection in our study although all three may

428   theoretically influence the encounter rate from the prey side. For actively searching benthic

429   predators like gudgeon and stone loach, the locomotion mode of the prey might be less

430   important than for ambush predators or slow-moving active predators (Muotka et al., 2006;

431   Sih and Moore, 1990). Microhabitat preferences of benthic macroinvertebrates in streams

432   may influence predator encounter rate especially if they include the use of refuges such as

433   crevices (Fairchild and Holomuzki, 2005). This seems to have played a minor role in the

434   studied streams. The positive electivity indices for 'microhabitat wood' may result from a

435   temporary preference for simuliids and other abundant taxa with a high affinity to this

436   microhabitat type. Even though current velocity preferences of the fish were already

437   accounted for in the calculations, the electivity pattern of gudgeon concerning current velocity

438   preference as a prey trait was still stronger than that of stone loach, indicating that typical

439   riffle taxa were avoided by gudgeon also when they occurred in pools.

440

441   Selectivity for prey groups

442   Chironomids, numerically dominating the diet of both fish predators in the studied

443   streams, are small and highly abundant and, mostly, also grazers or sediment feeders. They

444   might share this 'preferable' combination of characteristics, which is equivalent to a trait

445   syndrome *sensu* Poff et al. (2006) with other prey taxa. Instead of prey selection based on

446   single prey variables or taxa, our third hypothesis therefore focussed on selection for prey

447   groups sharing combinations of variables. We found less distinct selectivity patterns than

448   Yamada et al. (2010), who could largely explain age-dependent diet composition of marine

449   seagrass-bed fishes with a model approach combining taxonomic and trait-based prey groups.

450   However, the patterns that we observed are in concordance with the other results of our study,

451   especially concerning selectivity for small and abundant taxa and the contrary influence of the

452   feeding types 'grazer'/'sediment feeder' and 'shredder'.

453

454   <u>Consequences for predator coexistence</u>

455   The prey selectivity differences found between the fish species correspond to

456   differences in their habitat use and foraging mode. For gudgeon, which partly detects prey

457   visually and has a greater affinity to pools (Worischka et al., 2012), we observed generally a

458   more distinct selectivity (positive and negative) than for stone loach. This concerned single

459   prey traits, for instance prey size or current velocity preference, but also prey groups sharing

460   trait combinations. A possible explanation for the lower degree of selectivity in stone loach

461   might be its strictly non-visual and benthic foraging mode (Filek, 1960; Worischka et al.,

462   2012). Compared to drift-feeding fish, much less is known about the preferred prey traits for

463   benthic feeders. The selectivity patterns we found for two benthic predators differed clearly

464   from those of drift-feeders and other visual predators (e.g. Rader, 1997) but resembled each

465   other remarkably, despite the above-mentioned differences. Gudgeon and stone loach even

466   seemed to select a similar spectrum of prey variables in both studied streams whereas the

467   benthic samples from the streams differed regarding these variables. The co-occurrence of

468   two top predators with such similar prey preferences suggests a strong food competition.

469   However, competition seems to be weakened by the different spatial and temporal activity

470   patterns (gudgeon being temporally flexible and stone loach spatially) which might have

471   facilitated resource partitioning (Worischka et al., 2012). Such competition-minimizing

472   mechanisms have been observed also in other studies (Copp, 1992; Greenberg, 1991;

473   Sanchez-Hernandez et al., 2011). But even without a strong resource partitioning, the shared

474   use of a highly abundant main food resource, i.e. chironomids, probably allows the two

475   predator species to coexist. Therefore, the observed prey selectivity patterns are most

476   probably advantageous for both fish predators.

477

478   **Acknowledgements**

490

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669

670

670 **Tables**

671 **Table 1** Variables and their categories characterising prey, as used in calculations and plots.

Trait abbr.	Trait (explanation)	Categories abbr.	Categories (explanation)
abu	**mean abundance** (*log10* of mean abundance (ind m^{-2}))	a1 a2 a3	low (≤ 1) medium (1 … ≤ 2) high (2 … ≤ 4)
fty	**feeding type**	ff fg fp fse fsh	filter feeder grazer predator sediment feeder shredder
loc	**locomotion mode**	lc ld lse lsw	crawling digging sessile swimming
mha	**microhabitat**	ma mg ml mm ms mw	algae/macrophytes gravel/sand/silt leaf litter mud stones wood, roots
size	**size** (*log10* of body mass (mg dry))	s1 s2 s3 s4 s5	very small ($\leq$ -2) small (-2 …$\leq$ -1) medium (-1 …$\leq$ 0) large (0 …$\leq$ 1) very large (1 …$\leq$ 2)
vel	**current velocity preference**	v1 v2 v3	zero (0 ms^{-1}) low (< 0.25ms^{-1}) high (> 0.25 ms^{-1})

672

672 **Table 2** Range of the electivity indices E^* of gudgeon and stone loach for the single

673 categories within each variable (mean of all sampling dates and streams ± SD) and results of

674 Wilcoxon test with Holm correction, p_{adj} values < 0.05 (broad) indicate that E^* was

675 significantly different from 0 (see also Fig. 3).

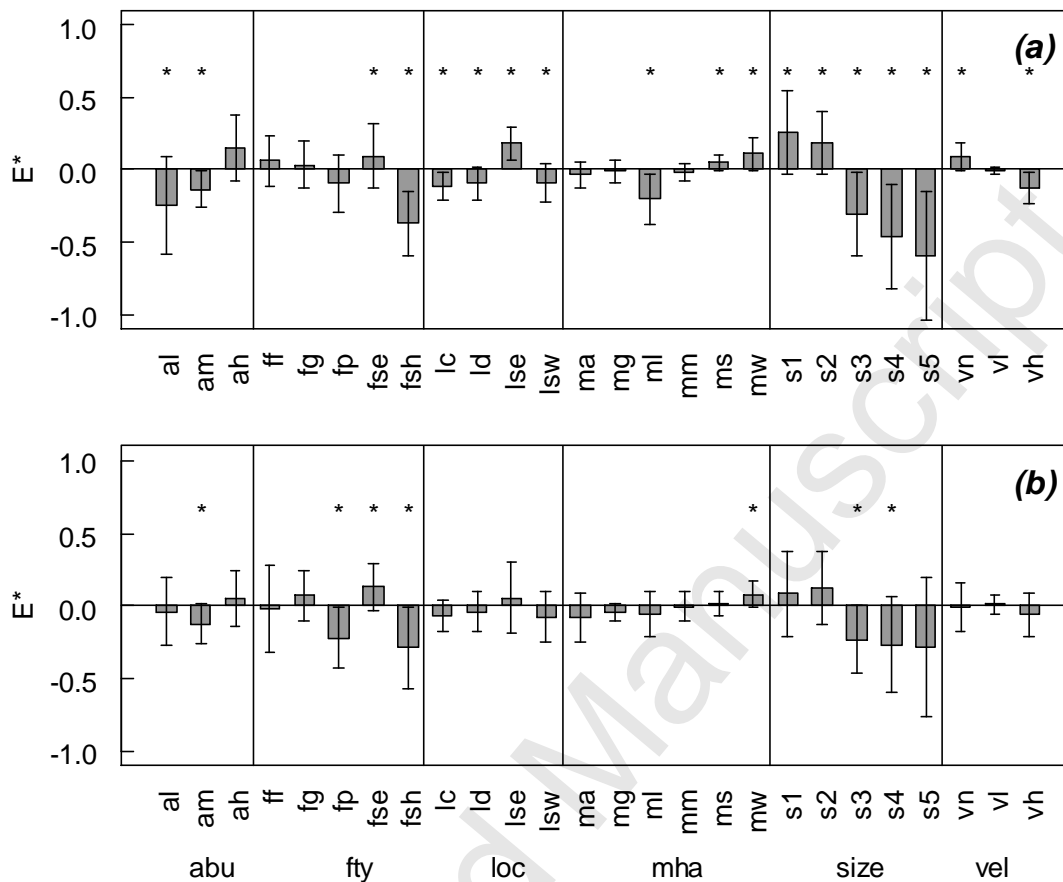| Variable	Gudgeon			Stone loach		
Category	Range of E^*	V	p_{adj}	Range of E^*	V	p_{adj}
abu	0.57 ± 0.32			0.39 ± 0.25		
a1		36	**0.043**		114	1.000
a2		8	**0.001**		8	**0.001**
a3		189	0.072		159	1.000
fty	0.61 ± 0.24			0.67 ± 0.25		
ff		167	0.456		130	1.000
fg		167	0.456		175	0.596
fp		58	0.322		17	**0.005**
fse		207	**0.011**		32	**0.046**
fsh		7	**0.000**		19	**0.007**
loc	0.35 ± 0.18			0.38 ± 0.23		
lc		4	**0.000**		48	0.316
lc		27	**0.015**		80	1.000
lse		228	**0.000**		162	1.000
lsw		38	**0.050**		59	0.703
mha	0.37 ± 0.16			0.34 ± 0.14		
ma		68	0.456		63	0.912
ml		15	**0.003**		82	1.000
mm		80	0.687		105	1.000
msa		101	0.711		37	0.093
mst		210	**0.007**		156	1.000
mw		209	**0.008**		206	**0.019**
size	1.03 ± 0.44			0.85 ± 0.34		
s1		201	**0.020**		165	1.000
s2		207	**0.011**		176	0.596
s3		17	**0.004**		17	**0.005**
s4		11	**0.001**		31	**0.045**
s5		12	**0.006**		55	0.596
vel	0.24 ± 0.14			0.25 ± 0.19		
v1		203	**0.016**		109	1.000
v2		88	0.711		156	1.000
v3		10	**0.001**		80	1.000

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678   **Figures and legends**

679

680   **Figure 1** FPCA of the samples of benthos and gudgeon diet. *(a) – (c)* Plots of the samples,

681   arrows connect the benthos sample (arrow origin) with the respective diet sample (arrow

682   head) for each date and stream. All three plots represent the same dataset, only grouped by *(a)*

683   stream with G = Gauernitzbach and T = Tännichtgrundbach, *(b)* season with 1 = spring, 2 =

684   summer, 3 = fall, and *(c)* year with 05 = 2005 etc. *(d)* Plot of the variables and the

685   eigenvalues of the axes for this FPCA. Bottom right: scaling of the axes for all four plots.

686   Trait abbreviations see Table 1.

687

688   **Figure 2** FPCA of the samples of benthos and stone loach diet. *(a) – (c)* Plots of the samples,

689   arrows connect the benthos sample (arrow origin) with the respective diet sample (arrow

690   head) for each date and stream. All three plots represent the same dataset, only grouped by *(a)*

691   stream with G = Gauernitzbach and T = Tännichtgrundbach, *(b)* season with 1 = spring, 2 =

692   summer, 3 = fall, and *(c)* year with 05 = 2005 etc. *(d)* Plot of the variables and the

693   eigenvalues of the axes for this FPCA. Bottom right: scaling of the axes for all four plots.

694   Trait abbreviations see Table 1.

695

Figure 3 Electivity indices *E** of gudgeon *(a)* and stone loach *(b)* for the categories of the variables mean abundance (abu), feeding type (fty), microhabitat preference (mha), loco-motion type (loc), size, and current velocity preference (vel). Black lines = median, black squares = mean, boxes = quartiles, whiskers = range. Trait and trait category abbreviations see Table 1. Asterisks indicate *E** values significantly different from 0 (*p*-values see Table 2).
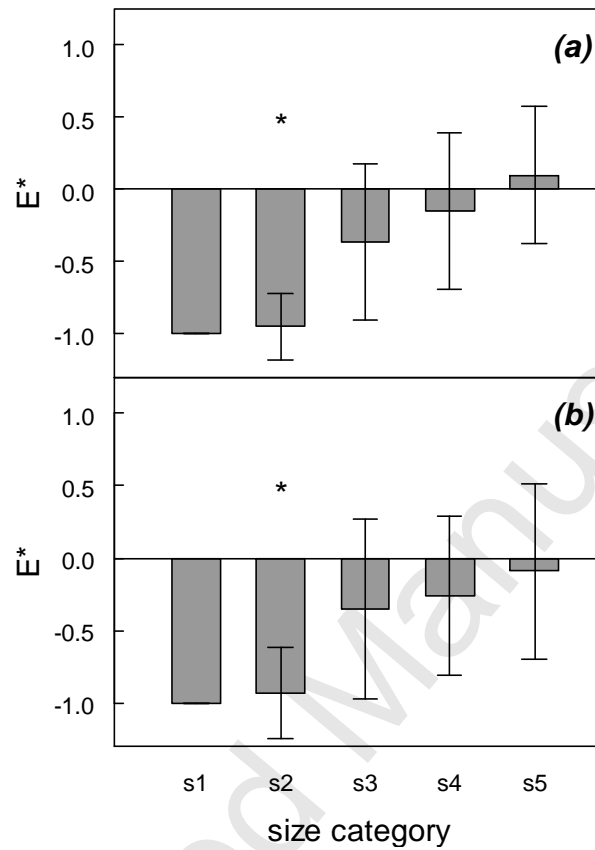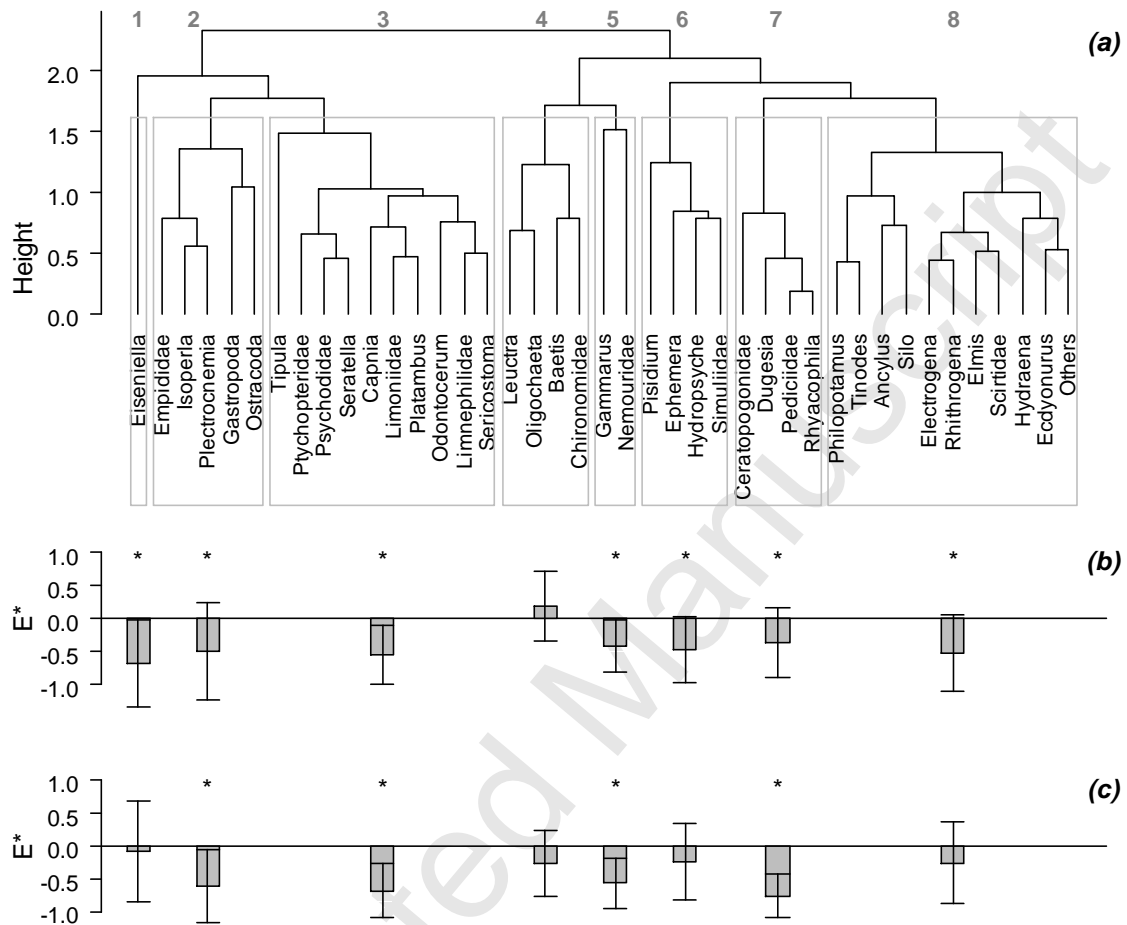
701

702 **Figure 4** Electivity indices E^* of (a) gudgeon and (b) stone loach for size classes of

703 *Gammarus* spp. in the streams Gauernitzbach and Tännichtgrundbach (mean ± SD of all

704 sampling occasions). Size classes correspond to size categories in Table 1. Asterisks mark

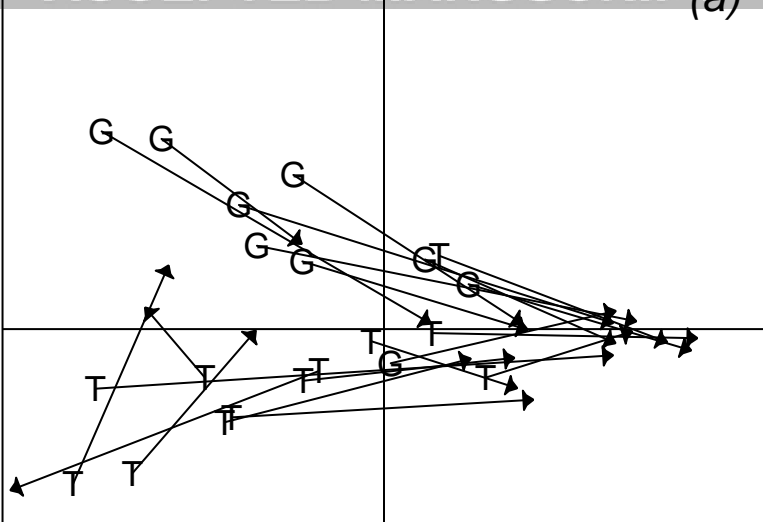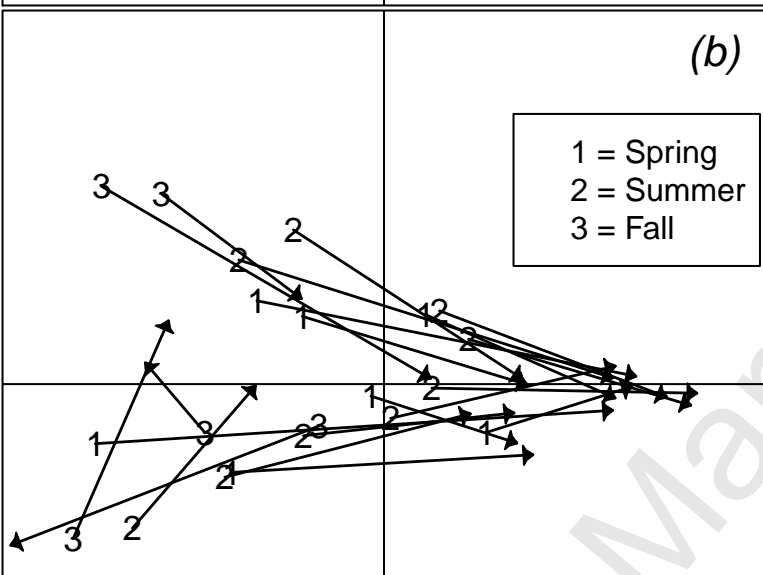705 significant differences from 0 (s1 not tested).

706

707

708   **Figure 5** Hierarchical cluster analysis (a) of the 'taxa × traits' array (grey numbers and

709   rectangles mark the prey groups) and electivity indices E^* of gudgeon (b) and stone loach (c)

710   for each prey group (mean ± SD of all sampling occasions, gudgeon n = 21, stone loach n =

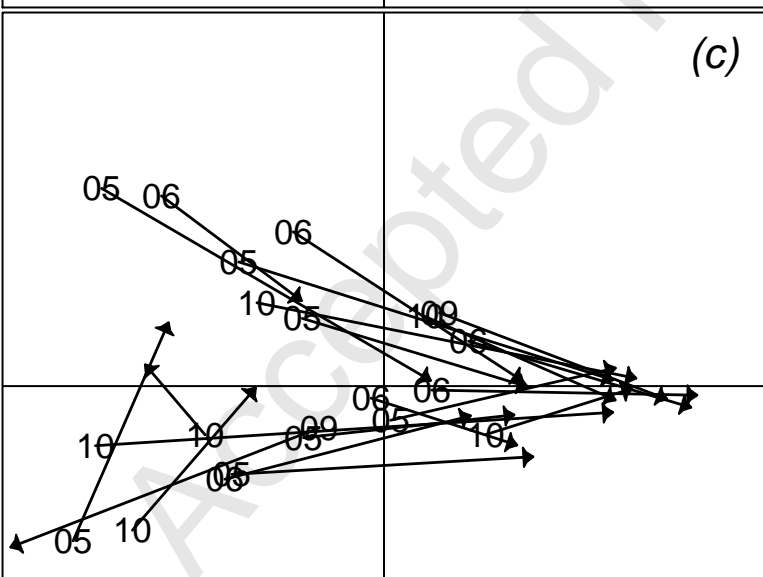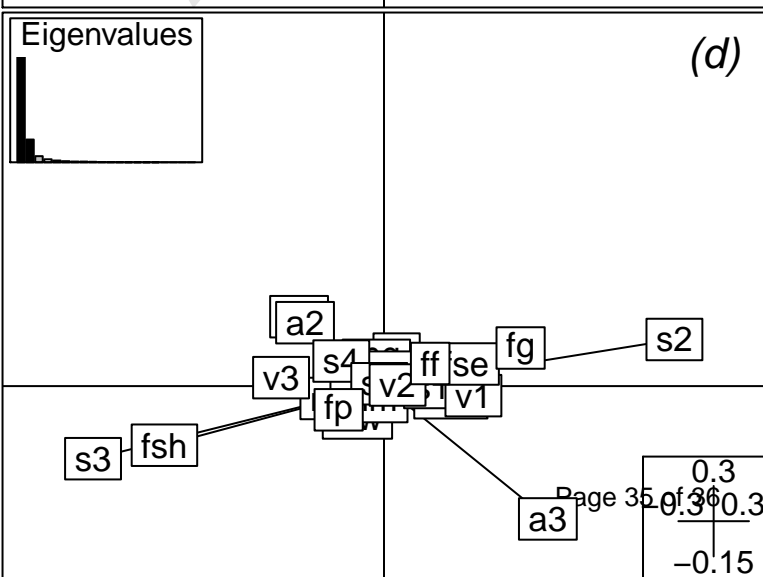711   22). E^* values significantly different from 0 are marked with asterisks.

712

Figure 1

(a)

(b)

1 = Spring
2 = Summer
3 = Fall

(c)

(d)

Eigenvalues

a2
s4
v3
ff
se
fg
s2
v2
v1
fp
s3
fsh
a3

0.3
-0.3 0.3
-0.15

Figure 2

(a)

(b)

1 = Spring
2 = Summer
3 = Fall

(c)

(d)

Eigenvalues