2

3 **Characterising the trophic niches of stocked and resident cyprinid fishes: consistency in**

4 **partitioning over time, space and body sizes**

5

6 Tea Bašić

7 J. Robert Britton

8

9 [1]Centre for Conservation Ecology and Environmental Sciences, Faculty of Science and

10 Technology, Bournemouth University, Poole, Dorset, BH12 5BB, UK.

11

12

13 Running title: Stocking cyprinid fishes

14

16

17 Corresponding author:

18 Professor Rob Britton; +44(0)1202965384; rbritton@bournemouth.ac.uk

19

20

Abstract

Hatchery-reared fish are commonly stocked into freshwaters to enhance recreational angling. As these fishes are often of high trophic position and attain relatively large sizes, they potentially interact with functionally similar resident fishes and modify food web structure. Hatchery-reared barbel *Barbus barbus* are frequently stocked to enhance riverine cyprinid fish communities in Europe; these fish can survive for over 20 years and exceed 8 kg. Here, their trophic consequences for resident fish communities were tested using co-habitation studies, mainly involving chub *Squalius cephalus*, a similarly large-bodied, omnivorous and long-lived species. These studies were completed over three spatial scales: pond mesocosms, two streams and three lowland rivers, and used stable isotope analysis. Experiments in mesocosms over 100 days revealed rapid formation of dietary specialisations and discrete trophic niches in juvenile *B. barbus* and *S. cephalus*. This niche partitioning between the species was also apparent in the streams over two years. In the lowland rivers, where fish were mature individuals within established populations, this pattern was also generally apparent in fishes of much larger body sizes. Thus, the stocking of these hatchery-reared fish only incurred minor consequences for the trophic ecology of resident fish, with strong patterns of trophic niche partitioning and diet specialisation. Application of these results to decision-making frameworks should enable managers to make objective decisions on whether cyprinid fish should be stocked into lowland rivers according to ecological risk.

Keywords: *Barbus barbus*; stable isotope analysis; lowland rivers; trophic niche.

Introduction

The release (stocking) of hatchery-reared fish into freshwater fisheries remains a widespread management technique used around the world to enhance recreational angling (Cowx 1994; Hunt *et al*. 2014). It can involve the supplementary stocking of extant species as well as the introduction of non-indigenous species (Antognazza *et al*. 2016). It is often completed in preference to alternative options to enhance fish communities, such as habitat management (Arlinghaus & Mehner 2005). Given their attraction to anglers through their sporting qualities, stocked fish are often species that grow to relatively large sizes and have high trophic positions (Holmlund & Hammer 2004; Fujitani *et al*. 2016), such as apex predators (Eby *et al*. 2006). Correspondingly, stocked fishes can influence the natural functioning of ecosystems through, for example, increasing species richness at higher trophic levels and altering food-web linkages and complexity (Eby *et al*. 2006).

Releases of fish into an ecosystem where the resources are not fully exploited can lead to their exploitation of vacant dietary niches that facilitates their integration into the community by minimising competition with resident fishes (Shea & Chesson 2002; Jackson & Britton 2014; Tran *et al*. 2015). However, as stocking exercises often involve the enhancement of population sizes of existing species to increase angler catch rates (Cowx 1994), it could lead to increased intra- and inter-competition for food resources (Vehanen, Huusko & Hokki 2009). The niche variation hypothesis then predicts populations will become more specialised in their diet (Van Valen 1965), resulting in reductions in trophic niche sizes following stocking (Human & Gordon 1996; Olsson *et al*. 2009). Conversely, increased competition for resources can also result in enlarged population trophic niches that enable species and individuals to maintain their energy requirements by switching to more general diets

3

70   (Svanbäck & Bolnick 2007). These theoretical perspectives can be used as the basis for

71   testing how stocking can impact the trophic ecology of resident species (Tran *et al*. 2015).

72

73   In European rivers, *B. barbus* are stocked regularly in areas covering both their indigenous

74   and non-indigenous ranges (Antognazza *et al*. 2016). In England, riverine populations are

75   regularly enhanced with hatchery-reared fish of between 10 and 25 cm (age 1+ and 2+ years).

76   These either supplement indigenous populations or provide new catch-and-release angling

77   opportunities in the non-indigenous range (Wheeler & Jordan 1990). Should these fish

78   survive the stocking process (Bolland *et al*. 2008, 2009) then they can persist for at least 20

79   years (Britton, Davies & Pegg 2013), providing considerable benefits to catch-and-release

80   recreational angling (Britton & Pegg 2011). Whilst there is some knowledge on the genetic

81   outcomes of *B. barbus* stocking (Antognazza *et al*. 2016), there is little knowledge on their

82   ecological impacts This is despite their omnivory, potential for long life spans and individuals

83   attaining weights in excess of 8 kg (Britton & Pegg 2011; Britton, Davies & Pegg 2013). It is

84   also in contrast to knowledge on the impacts of stocked species of the Salmonidae family,

85   where there is substantial information on their impacts on wild stocks (e.g. Ruzzante *et al*.

86   2004; Larsen *et al*. 2015). These impacts include trophic cascades that result from the

87   increased abundance of species in higher trophic positions in the food web (Eby *et al*. 2006).

88   Unlike cyprinid fish, many stocked salmonids are captured and removed by anglers soon after

89   their stocking, limiting long-term impacts due to short residence times (Baer, Blasel &

90   Diekmann 2007). Where these salmonids do survive in the wild, their relatively short

91   lifespans can limit their persistence, although ecological and genetic consequences can still

92   accrue (Simon & Townsend 2002; Le Cam *et al*. 2015).

93

94 The aim of this study was to thus quantify the ecological consequences of *B. barbus* stocking

95 for resident fishes through determining their trophic interactions and consequences for

96 somatic growth rates. This was completed over three spatial and timescales, and for fish of a

97 range of body sizes. As *B. barbus* can attain large body sizes and their functional traits favour

98 feeding on the benthos, assessments mainly used co-habitation experiments and field studies

99 involving chub *Squalius cephalus*. This is a similarly large-bodied, omnivorous and long-

100 lived species (e.g. Mann 1976) that occurs in sympatry with *B. barbus* in lowland rivers in

101 England. Due to the ecological theory outlined, particularly the niche variation hypothesis

102 (van Valen 1965), it was predicted that following a stocking event, *B. barbus* and *S. cephalus*

103 will have reduced trophic niche sizes as a result of increased diet specialisations, with

104 concomitant decreases in the somatic growth rates of both fishes.

105

106 **Materials and methods**

107

108 *Pond mesocosms*

109 The pond mesocosm experiment tested the outcomes for the trophic niches and somatic

110 growth rates of both fishes between their allopatric and sympatric contexts. Three treatments

111 were used: both species in allopatry (n = 10), and a final treatment where they were present in

112 sympatry (n = 5 + 5), with three replicates per treatment. This enabled testing of their trophic

113 niche size and position in allopatry and thus how being in sympatry affected these trophic

114 metrics. All fish used were juveniles, of starting lengths between 60 and 88 mm and sourced

115 from aquaculture.

116

117 Each mesocosm comprised of an independent enclosure situated within one larger natural

118 pond (30 x 12 m; 1 m depth). The rationale of the use of enclosures was that they provided

uniform habitats across the treatments and replicates in which the fish would be exposed to same prey fauna. As these preys were all located within the larger pond then their stable isotope values would be similar. Thus, any differences in the stable isotope data of the fishes would be the result of their dietary interactions within the treatments, not due to inherent variability in the stable isotope values of their prey. The enclosures comprised of aluminium frames of 1.66 m (length) x 1.05 m (width) x 1.2 m (height) that were enclosed within a net of 7 mm square mesh that prevented fish movements in and out of the enclosure, but allowed the movement of water and invertebrates. The enclosures were located randomly across the larger pond, with spacing of at least 0.5 m between them to ensure they provided enclosed and independent habitats for each replicate and that were identical at the commencement of the experiment. Anti-predator netting (15 mm mesh) was placed over the top of all enclosures. The enclosures were sufficiently heavy that their remained stationary throughout the experimental period without moving and without needing to be tied down. The height of the enclosures meant they settled on the substrate, with macrophytes able to grow within each of them (mainly *Elodea* spp.)

The experiment commenced in May 2014 and ran for 100 days, providing sufficient time for fish dorsal muscle to reach isotopic equilibrium (Jackson *et al*. 2013; Busst & Britton 2016). The mean water temperature during the experiment was 18.2 ± 0.3 ^{o}C, measured using a temperature logger in the centre of the pond that recorded temperature hourly (TinyTag TGP-4017). The enclosures were placed into the pond 7 days prior to the start of the experiment and all fish were measured prior to their release (fork length, nearest mm). On day 100, each enclosure was removed from the pond with the fish removed, euthanized (anaesthetic overdose, MS-222) and placed on ice. At the same time, samples of macro-invertebrates were taken from each enclosure via sorting through the remaining pond substrate and macrophytes.

144   These were mainly Chironomid larvae, but also included *Gammarus pulex*, *Asellus aquaticus*

145   and corixids.

146

147   In the laboratory, the fish were re-measured and a sample of dorsal muscle was taken for

148   stable isotope analysis. Their growth rates were calculated as incremental length (IL),

149   determined from $(L_{t+1} - L_t) / {}_t$, where L_t = initial starting lengths, L_{t+1} = total end lengths and ${}_t$

150   = number of days. The macro-invertebrate samples were sorted to species, enabling three

151   samples per species to be prepared for stable isotope analysis. There was no requirement to

152   sort the species by size, as they were similar in body sizes. Each of these samples comprised

153   of between 3 and 6 individuals. A random selection of fish dorsal muscle samples (n = 15 to

154   18 per species and treatment; minimum number of samples per replicate = 4) was then also

155   selected for stable isotope analysis. All of these samples were then dried at 60°C for 24

156   hours, ground and weighed, and analysed at the Cornell Isotope Laboratory, New York, USA

157   for their stable isotopes of δ^{13}C and δ^{15}N that were expressed as isotope ratios per mille (‰).

158   For initial analyses, δ^{15}N data were transformed to trophic position (TP), using the equation

159   TPi = $[(\delta^{15}N_i - \delta^{15}N_{base})/3.4]+2$, where TP_i is the trophic position of the individual fish, $\delta^{15}N_i$

160   is the isotopic ratio of that fish, $\delta^{15}N_{base}$ is the isotopic ratio of the primary consumers (macro-

161   invertebrates), 3.4 is the fractionation between trophic levels and 2 is the trophic position of

162   the baseline organism (Post 2002).

163

164   The stable isotope data were initially used in linear mixed models to assess differences

165   between the species, and their allopatric and sympatric treatments. Species were entered into

166   models according to their treatments so, for example, *B. barbus* was present in models as (1)

167   allopatric *B. barbus*, and (2) in sympatry with *S. cephalus*. The dependent (response) variable

168   was δ^{13}C or δ^{15}N and each model was fitted with mesocosm number as a random effect on

169   the intercept. This was to prevent inflation of the residual degrees of freedom that would

170   occur had each individual fish been used as a true replicate (Tran *et al*. 2015). The differences

171   in the stable isotope values by species and treatment were determined using estimated

172   marginal means and linearly independent pairwise comparisons with Bonferroni correction

173   for multiple comparisons. A similar linear mixed model approach was also used to test for

174   differences in the initial fish lengths between the species and their treatments, and to assess

175   differences in IL between treatments per species at the end of the experiment, with the same

176   model structure used.

177

178   The stable isotope data were then used to calculate the trophic niche sizes of both species per

179   treatment using the metric 'standard ellipse area' (SEA_c; the subscript 'c' indicates a small

180   sample size correction). These calculations were completed in the SIAR package (Jackson *et*

181   *al*. 2011) in the R computing program (R Development Core Team 2011). The data from

182   each mesocosm were combined for each treatment, as there were no differences between their

183   isotopic baselines due to the enclosures being placed in the same pond. SEA_c is a bivariate

184   measure of the distribution of individuals in their trophic space, with the models used

185   enclosing 60 % of the data. Thus, SEA_c represented the core dietary niche of that population

186   (hereafter referred to as the trophic niche) (Jackson *et al*. 2011; Jackson *et al*. 2012). Where

187   SEA_c overlapped between the sympatric fishes within a treatment then the area and

188   percentage of *B. barbus* overlap with *S. cephalus* was also calculated to indicate the extent of

189   actual resource sharing. In addition, this overlap was also calculated for each combination of

190   species in their allopatric contexts in order to demonstrate their potential niche overlap and

191   enable comparison with their realised niche overlap in sympatry. These comparisons were

192   possible due to the similarity of the habitats and prey items within the enclosures, the result

193   of their placement within one larger pond.

Streams

195 Assessment of the trophic consequences of stocking hatchery-reared *B. barbus* for resident *S.*

196 *cephalus* and other fishes was completed in two streams connected to the River Great Ouse.

197 These were the Houghton Stream (52.328607, -0.116417; Fig. 1) and the St. Ives Chub

198 Stream (hereafter referred to as the Chub Stream; 52.321542, -0.072521; Fig. 1). The source

199 of both streams was an outflowing connection from the main River Great Ouse. They both

200 then flowed for approximately 1500 m before re-joining the main river. Both streams were 6

201 to 10 m in width with depths to 2 m, and comprised of pool and riffle habitat. The Great Ouse

202 at either end of the streams was canalized with highly regulated flows.

203

204 Given the low probability of recapturing marked fish in these wild situations, growth

205 assessments were not included in this aspect of the study. Thus, the focus was only on

206 assessing the trophic interactions between the resident fishes and stocked *B. barbus*. Whilst *B.*

207 *barbus* is indigenous to the Great Ouse catchment (Antognazza *et al*. 2016), the two streams

208 were located at least 30 km downstream of the reaches where *B. barbus* populations were

209 prevalent. However, their flow regimes, habitats and substrates were all considered suitable

210 for *B. barbus* and so fishery managers were trying to establish populations in these streams

211 that had a resident fish community dominated in biomass by *S. cephalus*. Approximately 500

212 hatchery-reared *B. barbus* (100 to 150 mm; age 1+) were released in December 2013 into

213 each stream. A subsequent release of 1000 fish was also completed in December 2014. The

214 recapture of these fishes was completed using electric fishing, completed in July to August

215 2014 and June to September 2015. Due to the habitat of the streams, a combination of wading

216 and electric fishing from a boat was used, with hand-held equipment used throughout. With

217 the focus being in recapturing stocked fish for stable isotope analysis, fishing was qualitative

218 and so did not utilise stop-nets or incorporate population estimates. All the major stream

219 habitats were sampled. All captured fish were identified to species, measured (fork length,

220 nearest mm) and between 3 and 5 scales removed. They were then released back into the

221 streams. Concomitantly, macro-invertebrate samples were collected using kick sampling.

222

The trophic relationships of the fishes from each sampling occasion were assessed using stable isotope analysis. There were two differences from the methods used for the mesocosm experiment. Firstly, for the fishes, stable isotope data were derived from scales rather than dorsal muscles (Busst, Bašić & Britton 2015; Busst & Britton 2016). As it is only the outer proportion of scales that reflect the recent growth of the fish and thus their recent isotopic values, then in all cases only the very outer edge of the scales were removed and analysed (Grey *et al*. 2009). Secondly, to account for differences in the isotopic baseline between years in the streams, the stable isotope data were corrected for these isotopic differences. This process removes the annual variability in the consumer isotope data caused by the annual variation in their putative food sources, so enabling accurate comparison in their metrics (Olsson *et al*. 2009). The δ^{15}N data were transformed to trophic position (TP) as previously described, while δ^{13}C was corrected according to: δ^{13}Ccorr = δ^{13}C$_i$ - δ^{13}C$_{meaninv}$/CR$_{inv}$, where δ^{13}C$_{corr}$ is the corrected carbon isotope ratio of the individual fish, δ^{13}C$_i$ is the uncorrected isotope ratio of that fish, δ^{13}C$_{meaninv}$ is the mean invertebrate isotope ratio (the 'baseline' invertebrates) and CR$_{inv}$ is the invertebrate carbon range (δ^{13}Cmax - δ^{13}Cmin) (Olsson *et al*. 2009). Standard ellipse area (SEA$_c$) for each species and the extent of *B. barbus* overlap with resident fishes were then calculated as per the mesocosm experiment. Wherever possible, only fishes of similar lengths were compared for their trophic niche sizes and overlap to prevent confounds relating to ontogenetic shifts in fish diet.

Lowland rivers

The trophic niche breadths and overlaps of *B. barbus* and *S. cephalus* were then assessed in lowland rivers to determine whether the patterns observed at smaller spatial scales were apparent in more complex situations. Three rivers were used, two sections of the River Great

247 Ouse, the River Lea and River Avon. The Lea and Great Ouse have indigenous *B. barbus*

248 populations while the Avon population is non-indigenous but established for over 100 years

249 (Antognazza *et al*. 2016). All the rivers have received stockings of hatchery-reared *B. barbus*

250 in the last twenty years, although it could not be determined whether the fish analysed here

251 were of wild or hatchery origin.

252

253 The two sites on the Great Ouse were at Newport Pagnell (Site 1: 52.088232, -0.714125; Fig.

254 2) and Odell (Site 2: 52.209929,-0.584748; Fig. 2). These sites were both approximately 100

255 m in length and up to 20 m wide, and comprised of large pool-riffle habitat. The site on the

256 River Lea was at Batford (51.821735,-0.337205; Fig. 3). The sampled site was approximately

257 100 m in length, with widths up to 12 m. The habitat comprised of smooth flowing glides.

258 Both rivers were sampled by electric fishing from a boat in July 2014. Due to their size,

259 qualitative approaches were used with no stop nets. The data collected were as described for

260 the side channels, although an invertebrate sample was unable to be collected from the River

261 Lea. For the River Avon, fish samples were collected by angling from Ellingham (50.874070,

262 -1.804103; Fig. 4), with an invertebrate baseline collected by kick sampling. In all cases, the

263 sizes of the fishes sampled from these sites were considerably larger than those used

264 experimentally and in the side channels. At all sites, fish lengths were recorded (fork length,

265 nearest mm) and scale samples taken. These scales were then used in the stable isotope

266 analysis, using the methodology already outlined for the streams. The stable isotope metrics

267 of trophic niche size (as SEA_c) and trophic overlap were then compared between the *B.*

268 *barbus* and *S. cephalus* within each site. This meant there was no requirement to correct the

269 data and so all the stable isotope analyses were completed as per the mesocosm experiment.

270

271   **Results**

272

273   *Pond mesocosm experiment*

274   There were no significant differences in the starting length ranges of the fish (LMEM, *P* =

275   0.09; Table 1). At the conclusion of the experiment, 95 % of the fish that were introduced

276   into the enclosures were recovered. The maximum number of fish missing from a mesocosm

277   was one and it was assumed that these individuals had died during the experiment. The

278   LMEM testing for differences in the final lengths of these fishes revealed that the overall

279   model was significant (*P* < 0.01). The pairwise comparisons indicated that the significant

280   differences were only between *B. barbus* and *S. cephalus*, irrespective of the treatment (*P* =

281   0.02 in allopatry and *P* < 0.01 in sympatry). There were no significant differences in the final

282   lengths of each species between their allopatric and sympatric contexts (*P* > 0.10) (Table 1).

283   When converted to IL, the 95 % confidence range for *B. barbus* in allopatry was 0.98 to 1.10

284   mm d^{-1} and in sympatry 0.98 to 1.09 mm d^{-1}. For *S. cephalus*, this was 1.01 to 1.17 mm d^{-1} in

285   allopatry and 1.02 to 1.17 mm d^{-1} in sympatry. Thus, there were no significant differences in

286   growth rate between the treatments in each species.

287

288   The influence of species and treatment on the stable isotope data was significant for both δ^{13}C

289   and δ^{15}N (*P* < 0.01 in all cases; Table 2). For δ^{13}C, significant differences between the

290   species were evident between their allopatric contexts and when they were in sympatry (*P* <

291   0.01, Table 1, 3); *S. cephalus* was depleted in δ^{13}C compared to *B. barbus*. For δ^{15}N, when

292   analysed as trophic position, there was a significant difference between the species in

293   allopatry (P < 0.01). There was no significant difference in TP between the species in

294   sympatry (P > 0.10; Table 1, 3). Regarding SEAc, both species had larger trophic niches in

295   allopatry than in sympatry, with no overlap between them in both contexts (Table 1; Fig. 2).

296   Additionally, *B. barbus* had a considerably larger trophic niche than *S. cephalus* in both

297   allopatry and sympatry (Table 1).

298

299   *Streams*

300   Across the surveys of the two streams, three fish species were studied, *B. barbus*, *S. cephalus*

301   and dace *Leuciscus leuciscus* (Table 3). Whilst the fish were considerably larger than used in

302   the mesocosm experiments, mean lengths per species were all between 151 and 217 mm

303   (Table 3). Sample sizes tended to be small, especially for *B. barbus*, where only 10 stocked

304   fish were captured in subsequent sampling in the Houghton Stream and 19 in the Chub

305   Stream (Table 3). Although there was some temporal variability in the stable isotope data in

306   each stream, there was a general pattern of minimal trophic overlap between stocked *B.*

307   *barbus* and the resident *S. cephalus* and *L. leuciscus* (< 1 %) with this particularly apparent in

308   samples collected in 2015 (Table 3; Fig. 3, 4).

309

310   *Lowland rivers*

311   The fish sampled across the three rivers tended to be the largest used in the study, with some

312   *B. barbus* present in samples > 600 mm (Table 4). In the River Lea, two size classes of *B.*

313   *barbus* and *S. cephalus* were present and so were analysed and tested separately. As with the

314   second pond mesocosm experiment and the side channels, the extent of the trophic overlap of

315   *B. barbus* with other cyprinid species was minimal (Table 4; Fig. 5, 6). This was the case for

316   both size classes of fish in the River Lea, although there was some shift in this pattern

317   between the size classes (Fig. 5). In the fish of lengths 186 to 237, the *B. barbus* stable

318   isotopes were nitrogen enriched by approximately 3 ‰ compared to *S. cephalus*, but had

319   similar values of δ^{13}C (Table 4; Fig. 5). By contrast, for the fish of above 400 mm, the *B.*

320   *barbus* has enriched δ^{13}C and δ^{15}N compared to *S. cephalus* (Table 4; Fig. 5).

Discussion

Experimental and field evidence suggested that there was substantial partitioning in the trophic niches of sympatric *B. barbus* and *S. cephalus*, with no evidence for resource sharing. This pattern was apparent over a 100 day period in the mesocosm enclosures, with this an important result as it was from an experiment completed in relatively controlled conditions. In the field studies, where there is greater inherent complexity and stochasiticity in the systems that result in more difficulty in deciphering ecological patterns and thus where more caution is needed in interpretation, the trophic niche partitioning was also apparent. This was the case in the two year post-stocking period in the two streams and in the larger fishes sampled in the lowland rivers. Moreover, where there were data available for other fishes in the community, such as *L. leuciscus*, this pattern of *B. barbus* having a very discrete trophic niche was still evident.

The outputs of the allopatric treatment in the mesocosm experiment suggested that *B. barbus* rapidly established a trophic niche that was divergent from allopatric *S. cephalus*, suggesting that there would be no sharing of food resources when the species were in sympatry. When the species were in sympatry, their actual trophic niches did remain separated. However, their niche breadths were reduced in sympatry, indicating some individual specialisation (Araújo, Bolnick & Layman 2011). This result was consistent with both the prediction and the niche variation hypothesis that predicts populations become less generalized in more competitive environments (Van Valen 1965; Human & Gordon, 1996; Olsson *et al*. 2009). Similar patterns of trophic niche divergence and partitioning have been detected when non-native fishes that have been introduced into similar environments. For example, the trophic niche divergence between the small, invasive fish topmouth gudgeon *Pseudorasbora parva* with

346 extant species, including carp *Cyprinus carpio*, facilitates their co-existence (Jackson &

347 Britton 2013; Tran *et al*. 2015). These trophic niche outputs were also important in the

348 context of the growth rates of the fishes. In the mesocosm experiment, the growth rates of

349 both fishes were similar between their allopatric and sympatric treatments, despite their

350 reduced trophic niche sizes. This suggests that when the fishes have access to food resources

351 that are not limiting, their trophic niche partitioning and specializations maintains their

352 energetic requirements to enable their growth rates to be similar between the allopatric and

353 sympatric treatments. This was contrary to the prediction that increased trophic specialisation

354 would result in decreased growth rates. This was also an important result given the difficulty

355 of measuring differences in growth rates in more wild situations, such as the field sites, where

356 there tends to be a wide range of abiotic factors that cause temporal and individual variability

357 in fish growth rates (Beardsley & Britton 2012; Liu, Chen & Britton 2015).

358

359 Introduced and stocked salmonid fishes often cause detrimental impacts for native salmonids.

360 Predation by introduced lake trout (*Salvelinus namaycush*) can limit the distribution of bull

361 trout (*Salvelinus confluentus*) (Donald & Alger 1993) and cause population declines of

362 cutthroat trout (*Onchorhynchus clarki*) (Ruzycki, Beauchamp & Yule 2003). Their stocking

363 can cause trophic cascades (Tronstad *et al*. 2010) that influence predator–prey interactions in

364 surrounding terrestrial ecosystems (Middleton *et al*. 2013). For *B. barbus*, however, there was

365 minimal evidence to suggest that their ecological interactions resulted in any substantial

366 alteration in the trophic ecology of *S. cephalus*. It is acknowledged that the approach used

367 within this study were relatively simple, focusing primarily on the trophic interactions of *B.*

368 *barbus* with *S. cephalus*. This was to ensure that the inter-specific comparisons were being

369 made for functionally similar fishes that grew to relatively similar body sizes and that live for

370 similar long life spans (Britton 2007). This could, however, have resulted in some over-

371 simplification of the outcomes of their stocking into more complex fish communities.

372 However, there is also no evidence of *B. barbus* sharing a trophic niche space with fishes

373 such as *L. leuciscus*, roach *Rutilus rutilus* and graying *Thymallus thymallus*, both here and

374 from other studies (e.g. Bašić & Britton 2015).

375

376 The design of the experimental and field studies meant that regular assessment of the trophic

377 niches of the fishes in each system was not possible. Logistical constraints limited the

378 number of treatments that could be included within the mesocosm experiment. This meant

379 that fish numbers, i.e. density, was maintained across the experimental treatments. This was

380 important to ensure that comparisons could be made in trophic niche sizes between species

381 and the allopatric and sympatric contexts, as the numbers of fish involved were consistent.

382 However, the partitioning of trophic niches between species can be related to competition for

383 food resources and predation (Nilsson 1967) and thus patterns can change as the population

384 abundances of the species increase (Spurgeon *et al.* 2014). Although our patterns of

385 partitioning were strong in the mesocosms and were detected in the field studies, it is

386 acknowledged that the incorporation of more complexity into the experimental designs, such

387 as including treatments that increased fish abundance or also used fish of contrasting body

388 sizes, might have provided greater insights. Moreover, the focus here was on the trophic

389 relationships of the fishes, yet the impacts of stocked and invasive fishes can include other

390 ecological issues, including habitat disturbances (Gozlan *et al.* 2010). Indeed, *B. barbus* act

391 as 'zoogeomorphic agents' in rivers, as their foraging activities reduce bed material stability,

392 increase bedload transport, and impact micro-topographic roughness and sediment structure

393 (Pledger *et al.* 2014, 2015). Thus, their release into rivers where populations are not currently

394 present could have considerable effects on the substrate. By extension, their foraging

395 activities could also impact aspects of the macro-invertebrate communities, although again

396   this was unable to be tested here. In addition, whilst stable isotope data can provide a

397   powerful tool to determine trophic interactions, they are only a proxy for this. Studies that

398   compare the diet of fishes across methods such as stable isotope analysis and stomach

399   contents analysis often show some differences in their results (e.g. Hamidan *et al*. 2015).

400   Consequently, studies that rely solely on stable isotope analysis should be evaluated with

401   some caution (Locke *et al*. 2013).

402

403   The design of fish stocking strategies needs to consider the survival and establishment of the

404   fishes, and their ecological and genetic interactions with extant populations. Knowledge on

405   these aspects and interactions has been well documented for stocked salmonid fishes (e.g.

406   Simon & Townsend 2002). For fishes from other families, however, there remain

407   considerable knowledge gaps, especially in European lowland rivers. Here, our results

408   suggested that *B. barbus* occupied a trophic niche that was distinct from the other cyprinid

409   fishes analysed. Although this has the caveat around the limitations of the study as outlined

410   above, these results suggest that *B. barbus* stocking can result in relatively minor ecological

411   consequences. This is important, as their stocking can provide considerable recreational and

412   socio-economic benefits (Britton & Pegg 2011). Notwithstanding, Antognazza *et al*. (2016)

413   did reveal that, genetically, the stocking of *B. barbus* between different river basins does

414   impact their genetic integrity. In combination, this suggests that in designing fisheries

415   management strategies for lowland rivers where communities are dominated by cyprinid

416   fishes, a wide range of abiotic, ecological and genetic issues need to be considered. There

417   should be identification of the current constraints on the fish community (Cowx 1994), and

418   whether habitat restoration and rehabilitation are more appropriate management tools than

419   stocking (Pretty *et al*. 2003). Should stocking be demonstrated to be a viable management

420 option, then our work on *B. barbus* indicates that both ecological and genetic considerations

421 must be applied to the decision of why, when and how to stock the fishes.

422

423 **References**

424

425 Antognazza, C.M., Andreou, D., Zaccara, S. & Britton, J.R. (2016) Loss of genetic integrity

426   and biological invasions result from stocking and introductions of *Barbus barbus*: insights

427   from rivers in England. *Ecology and Evolution*. doi: 10.1002/ece3.1906.

428 Araújo, M.S., Bolnick, D.I. & Layman, C.A. (2011) The ecological causes of individual

429   specialisation. *Ecology Letters*, **14**, 948-958.

430 Arlinghaus, R. & Mehner, T. (2005) Determinants of management preferences of recreational

431   anglers in Germany: Habitat management versus fish stocking. *Limnologica*, **35**, 2-17.

432 Baer, J., Blasel, K. & Diekmann, M. (2007) Benefits of repeated stocking with adult,

433   hatchery-reared brown trout, *Salmo trutta*, to recreational fisheries? *Fisheries*

434   *Management and Ecology*, **14**, 51-59.

435 Bašić, T. & Britton, J.R. (2015) Utility of fish scales from stock assessment surveys in stable

436   isotope analysis for initial assessments of trophic relationships in riverine fish

437   communities. *Journal of Applied Ichthyology*, **31**, 296-300.

438 Beardsley, H. & Britton, J.R. (2012) Contribution of temperature and nutrient loading to

439   growth rate variation of three cyprinid fishes in a lowland river. *Aquatic Ecology*, **46**, 143-

440   152.

441 Bolland, J.D., Cowx, I.G. & Lucas, M.C. (2008) Movements and habitat use of wild and

442   stocked juvenile chub, *Leuciscus cephalus* (L.), in a small lowland river. *Fisheries*

443   *Management and Ecology*, **15**, 401–407

444 Bolland, J.D., Cowx, I.G. & Lucas, M.C. (2009) Dispersal and survival of stocked cyprinids

445 in a small English river: comparison with wild fishes using a multi-method approach.

446 *Journal of Fish Biology*, **74**, 2313-2328.

447 Britton, J.R. & Pegg, J. (2011) Ecology of European Barbel *Barbus Barbus*: Implications for

448 River, Fishery, and Conservation Management. *Reviews in Fisheries Science*, **19**, 321-

449 330.

450 Britton, J.R. (2007) Reference data for evaluating the growth of common riverine fishes in

451 the UK. *Journal of Applied Ichthyology*, **23**, 555-560.

452 Britton, J.R., Davies, G.D. & Pegg, J. (2013) Spatial variation in the somatic growth rates of

453 European barbel *Barbus barbus*: a UK perspective. *Ecology of Freshwater Fish*, **22**, 21-

454 29.

455 Busst, G. & Britton, J.R. (2016) High variability in stable isotope diet-tissue discrimination

456 factors of two omnivorous freshwater fishes in controlled *ex situ* conditions. *Journal of*

457 *Experimental Biology.* doi: 10.1242/jeb.137380.

458 Busst, G., Bašić, T. & Britton, J.R. (2015) Stable isotope signatures and trophic-step

459 fractionation factors of fish tissues collected as non-lethal surrogates of dorsal muscle.

460 *Rapid Communications in Mass Spectrometry*, **29**, 1535-1544.

461 Cowx, I. G. (1994) Stocking strategies. *Fisheries Management and Ecology*, **1**, 15-30.

462 Donald, D.B. & Alger, D.J. (1993) Geographic distribution, species displacement, and niche

463 overlap for lake trout and bull trout in mountain lakes. *Canadian Journal of Zoology*, **71**,

464 238–247.

465 Eby, L.A., Roach, W.J., Crowder, L.B. & Stanford, J.A. (2006) Effects of stocking-up

466 freshwater food webs. *Trends in Ecology and Evolution*, **21**, 576-84.

467 Fujitani, M. L., McFall, A., Randler, C. & Arlinghaus, R. (2016) Efficacy of lecture-based

468 environmental education for biodiversity conservation: a robust controlled field

469   experiment with recreational anglers engaged in self-organized fish stocking. *Journal of*

470   *Applied Ecology*, **53**, 25–33.

471   Gozlan, R.E., Britton, J.R., Cowx, I. & Copp, G.H. (2010) Current knowledge on non- native

472   freshwater fish introductions. *Journal of Fish Biology,* **76**, 751-786.

473   Grey, J., Graham, C., Britton, J.R. & Harrod, C. (2009) Stable isotope analysis of archive

474   roach (*Rutilus rutilus*) scales for retrospective study of shallow lake responses to nutrient

475   reduction. *Freshwater Biology*, **54**, 1663–1670.

476   Holmlund, C.M. & Hammer, M. (2004). Effects of Fish Stocking on Ecosystem Services: An

477   Overview and Case Study Using the Stockholm Archipelago Effects of Fish Stocking on

478   Ecosystem Services. *Environmental Management*, **33**, 799-820.

479   Human, K.G. & Gordon, D.M. (1996). Exploitation and Interference Competition between

480   the invasive Argentine Ant, *Linepithema humile*, and Native Ant Species. *Oecologia*, **105**,

481   405-412.

482   Hunt, T.L., Giri, K., Brown, P., Ingram, B.A., Jones, P. L., Laurenson, L.J. & Wallis, A.M.

483   (2014) Consequences of fish stocking density in a recreational fishery. *Canadian Journal*

484   *of Fisheries and Aquatic Sciences*, **71**, 1554-1560.

485   Jackson, A.L., Inger, R., Parnell, A.C. & Bearhop, S. (2011) Comparing isotopic niche

486   widths among and within communities: SIBER - Stable Isotope Bayesian Ellipses in R.

487   *Journal of Animal Ecology*, **80**, 595-602.

488   Jackson, M.C. & Britton, J.R. (2013) Variation in the trophic overlap of invasive

489   *Pseudorasbora parva* and sympatric cyprinid fishes. *Ecology of Freshwater fish*, **22**, 654-

490   657.

491   Jackson, M.C. & Britton, J.R. (2014) Divergence in the trophic niche of sympatric freshwater

492   invaders. *Biological invasions*, **16**, 1095-1103.

493   Jackson, M.C., Allen, R., Pegg, J. & Britton, J.R. (2013) Do trophic subsidies affect the

494   outcome of introductions of a non-native freshwater fish? *Freshwater Biology*, **58**, 2144-

495   2153.

496   Jackson, M.C., Donohue, I., Jackson, A.L., Britton, J.R., Harper, D.M. & Grey, J. (2012)

497   Population-level metrics of trophic structure based on stable isotopes and their application

498   to invasion ecology. *PLoS ONE* **7**, e31757. doi: 10.1371/journal.pone.0031757.

499   Larsen, M.H., Jörgen, I.J., Joacim, N., Søren, T.T. & Kim, A. (2015) Reduced rearing density

500   increases post-release migration success of Atlantic salmon (*Salmo salar*) smolts.

501   *Canadian Journal of Fisheries and Aquatic Sciences*. doi: 10.1139/cjfas-2014-0563.

502   Le Cam, S., Perrier, C., Besnard, A. L., Bernatchez, L. & Evanno, G. (2015) Genetic and

503   phenotypic changes in an Atlantic salmon population supplemented with non-local

504   individuals: a longitudinal study over 21 years. *Proceedings of the Royal Society of*

505   *London B: Biological Sciences*, **282**, 20142765. doi: 10.1098/rspb.2014.1664.

506   Liu, C., Chen, Y. & Britton, J.R. (2015) Influences of environmental and chemical

507   parameters on the spatial growth patterns of four riverine cyprinid fishes. *Knowledge and*

508   *Management of Aquatic Ecosystems*, **416**, 12. doi: 10.1051/kmae/2015008.

509   Locke, S.A., Bulté, G., Forbes, M.R. & Marcogliese, D.J. (2013) Estimating diet in individual

510   pumpkinseed sunfish *Lepomis gibbosus* using stomach contents, stable isotopes and

511   parasites. *Journal of Fish Biology*, **82**, 522-537.

512   Mann, R.H.K. (1976) Observations on the age, growth, reproduction and food of the chub

513   *Squalius cephalus* (L.) in the River Stour, Dorset. *Journal of Fish Biology*, **8**, 265-288.

514   Middleton, A.D., Morridon, T.A., Fortin, J.K., Robbins, C.T., Proffitt, K.M., White, P.J.,

515   McWhirter, D.E., Koel, T.M., Brimeyer, D.G., Fairbanks, W.S. & Kauffman, M.J. (2013)

516   Grizzly bear predation links the loss of native trout to the demography of migratory elk in

517   Yellowstone. *Proceedings of the Royal Society of London B: Biological Sciences,* **280**,

518   20130870.

519 Nilsson, N. 1967. Interactive segregation between fish species. In: Gerking, S.D., ed. The

520   Biological Basis of Freshwater Fish Production. Oxford: Blackwell Scientific

521   Publications, pp. 259 - 313.

522 Olsson, K., Stenroth, P., Nyström, P.E.R. & Granéli, W. (2009) Invasions and niche width:

523   does niche width of an introduced crayfish differ from a native crayfish? *Freshwater*

524   *Biology*, **54**, 1731-1740.

525 Open Map - Local [SHAPE geospatial data], Scale 1:10000, Tiles: tl, su, Updated: 10 March

526   2015, Ordnance Survey (GB), Using: EDINA Digimap Ordnance Survey Service,

527   <http://digimap.edina.ac.uk>, Downloaded: 2016-05-18 11:42:49.057.

528 Pledger, A.G., Rice, S.P. & Millett, J. (2014) Reduced bed material stability and increased

529   bedload transport caused by foraging fish: a flume study with juvenile Barbel (*Barbus*

530   *barbus*). *Earth Surface Processes and Landforms*, **39**, 1500-1513.

531 Pledger, A.G., Rice, S.P. & Millett, J. (2016) Bed disturbance via foraging fish increases

532   bedload transport during subsequent high flows and is controlled by fish size and species.

533   *Geomorphology*, **253**, 83-93.

534 Post, D.M. (2002) Using stable isotopes to estimate trophic position: Models, methods, and

535   assumptions. *Ecology*, **83**, 703-718.

536 Pretty, J.L., Harrison, S.S.C., Shepherd, D.J., Smith, C., Hildrew, A.G. & Hey, R.D. (2003)

537   River rehabilitation and fish populations: assessing the benefit of instream structures.

538   *Journal of applied ecology*, **40**, 251-265.

539 R Development Core Team (2011) R: A language and environment for statistical computing,

540   R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL

541   http://www.R-project.org/.

542   Ruzycki, J.R., Beauchamp, D.A. & Yule, D.L. (2003) Effects of introduced lake trout on

543   native cutthroat trout in Yellowstone lake. *Ecological Applications*, **13**, 23–37.

544   Ruzzante, D.E., Hansen, M.M., Meldrup, D. & Ebert, K.M. (2004) Stocking impact and

545   migration pattern in an anadromous brown trout (*Salmo trutta*) complex: where have all

546   the stocked spawning sea trout gone? *Molecular Ecology*, **13**, 1433-1445.

547   Shea, K. & Chesson, P. (2002) Opinion: Community ecology theory as a framework for

548   biological invasions. *Trends in Ecology and Evolution*, **17**, 170-176.

549   Simon, K.S. & Townsend, C.R. (2003) Impacts of freshwater invaders at different levels of

550   ecological organisation, with emphasis on salmonids and ecosystem consequences.

551   *Freshwater biology*, **48**, 982-994.

552   Spurgeon, J.J., Paukert, C.P., Healy, B.D., Kelley, C.A. & Whiting, D.P. (2015) Can

553   translocated native fishes retain their trophic niche when confronted with a resident

554   invasive? *Ecology of Freshwater Fish*, *24*, 456-466.

555   Svanbäck, R. & Bolnick, D.I. (2007) Intraspecific Competition Drives Increased Resource

556   Use Diversity within a Natural Population. *Proceedings of the Royal Society of London B:*

557   *Biological Sciences*, **274**, 839- 844.

558   Tran, T.N.Q., Jackson, M.C., Sheath, D., Verreycken, H. J. & Britton, J.R. (2015) Patterns of

559   trophic niche divergence between invasive and native fishes in wild communities are

560   predictable from mesocosm studies. *Journal of Animal Ecology*, **84**, 1071-1080.

561   Tronstad, L.M., Hall, R.O. Jr, Koel, T.M. & Gerow, K.G. (2010) Introduced lake trout

562   produced four-level trophic cascade in Yellowstone lake. *Transaction of the American*

563   *Fisheries Society*, **139**, 1536–1550.

564   Van Valen, L. (1965) Morphological Variation and Width of Ecological Niche. *The*

565   *American Naturalist*, **99**, 377-390.

566   Vehanen, T., Huusko, A. & Hokki, R. (2009) Competition between hatchery-raised and wild

567   brown trout *Salmo trutta* in enclosures – do hatchery releases have negative effects on

568   wild populations? *Ecology of Freshwater Fish*, **18**, 261–268.

569   Wheeler, A. & Jordan, D.R. (1990) The status of the barbel, *Barbus barbus* (L.) (Teleostei,

570   Cyprinidae), in the United Kingdom. *Journal of fish biology*, **37**, 393-399.

571   Table 1. Number of fishes analysed, the mean starting fork lengths, the mean incremental lengths (IL), mean δ^{13}C, mean δ^{15}N,

572   trophic position (TP) and trophic niche size (as standard ellipse area corrected for small sample size, SEA$_c$) of *B. barbus* and

573   *S. cephalus* at the conclusion of the mesocosm experiment and the extent to which *B. barbus* trophic niche overlapped (%)

574   with *S. cephalus*. Error around the mean represents standard error.

575

Species	Treatment	n	Mean starting length (mm)	Mean IL (mm d^{-1})	Mean δ^{13}C (‰)	Mean δ^{15}N (‰)	Mean TP	SEA$_C$ (‰^{2})	Overlap (%)
B. barbus	Allopatry	18	77.6 ± 0.96	0.34 ± 0.03	-28.2 ± 0.20	11.2 ± 0.05	2.79 ± 0.02	0.56	
	Sympatry	15	77.5 ± 1.31	0.41 ± 0.03	-29.1 ± 0.11	10.8 ± 0.05	2.68 ± 0.02	0.31	0
S. cephalus	Allopatry	17	73.9 ± 1.22	0.45 ± 0.05	-30.3 ± 0.19	10.7 ± 0.05	2.66 ± 0.02	0.54	
	Sympatry	15	76.1 ± 1.60	0.50 ± 0.01	-30.7 ± 0.14	10.8 ± 0.03	2.68 ± 0.01	0.21	0

576   Table 2. Outputs and significance of the final linear mixed models testing the

577   differences in mean δ^{13}C and trophic position (TP) between the species across the

578   mesocosm experiment, where mesocosm was the random effect on the intercept. Mean

579   differences are from estimated marginal means (* = difference significant at $P < 0.05$).

580

Final model structure (and result):

δ^{13}C ~ species x experimental treatment (AIC = 141.8; log likelihood = -64.9; $P < 0.01$)

Trophic position ~ species x experimental treatment (AIC = - 178.9; log likelihood = 95.4; $P < 0.01$)

Pairwise comparison		Mean difference in δ^{13}C	Mean difference in TP
Allopatric	Allopatric *S. cephalus*	2.12 ± 0.36, $P < 0.01$*	0.13 ± 0.03, $P < 0.01$*
B. barbus	Sympatric with *S. cephalus*	0.85 ± 0.36, $P > 0.1$	0.11 ± 0.03, $P = 0.01$*
Allopatric	Sympatric with *B. barbus*	0.36 ± 0.36, $P > 0.1$	0.02 ± 0.03, $P > 0.1$
S. cephalus			
B. barbus in sympatry with *S. cephalus*		1.63 ± 0.23, $P < 0.01$*	0.004 ± 0.02, $P > 0.1$

581

582

583 Table 3. Date of sampling, species, sample sizes, mean fork lengths, mean δ^{13}C and mean

584 δ^{15}N of fish and their trophic niche size (SEAc*; values obtained from data corrected for

585 baseline variations across treatments.) and the extent to which *B. barbus* trophic niche

586 overlaps (%) with other fish species in the community (*S. cephalus* and *L. leuciscus*), at (A)

587 Chub stream and (B) Houghton stream. Error around the mean is standard error.

(A)

Date	Species	n	Mean length (mm)	Mean δ^{13}C (‰)	Mean δ^{15}N (‰)	SEAc (‰^{2})*	Overlap (%)
June	*B. barbus*	7	209.9 ± 9.9	-27.1 ± 0.3	16.2 ± 0.2	0.06	
201	*S.cephalus*	7	217.4 ± 5.7	-26.4 ± 0.3	14.7 ± 0.3	0.11	< 0.01
4	*L. leuciscus*	7	203.1 ± 2.6	-28.1 ± 0.4	17.0 ± 0.3	0.24	0.40
June	*B. barbus*	8	151.1 ± 6.5	-22.3 ± 0.9	13.3 ± 0.8	1.66	
201	*S.cephalus*	8	153.6 ± 8.0	-26.4 ± 0.4	16.6 ± 0.4	0.90	0
5	*L. leuciscus*	8	152.6 ± 9.6	-27.9 ± 0.2	17.1 ± 0.3	0.44	0
Sept	*B. barbus*	4	212 ± 20.9	-27.5 ± 0.1	18.6 ± 0.4	0.16	
201	*S. cephalus*	6	209.2 ±15.3	-26.9 ± 0.1	17.8 ± 0.5	0.30	0
5	*L. leuciscus*	6	184.8 ± 6.6	-28.2 ± 0.1	18.4 ± 0.3	0.31	0

(B)

Date	Species	n	Mean length (mm)	Mean δ^{13}C (‰)	Mean δ^{15}N (‰)	SEAc (‰^{2})*	Overlap (%)
June	*B. barbus*	4	185.3 ± 9.2	-28.2 ± 0.4	17.1 ± 0.5	0.12	
201	*S. cephalus*	6	194.8 ± 6.2	-27.3 ± 1.0	16.0 ± 0.8	1.07	0.58
4	*L. leuciscus*	6	191.7 ± 3.9	-28.7 ± 0.1	17.9 ± 0.1	0.05	0.17
June	*B. barbus*	6	159.0 ± 8.8	-22.8 ± 0.3	13.4 ± 0.4	0.77	
201	*S.cephalus*	5	198.4 ±23.7	-27.5 ± 0.2	17.7 ± 0.3	0.28	0
5	*L. leuciscus*	6	161.7 ±15.1	-28.4 ± 0.5	17.8 ± 0.1	0.20	0

588 Table 4. Species, sample sizes, mean fork lengths, mean δ^{13}C and mean δ^{15}N of sampled fish,

589 their trophic niche breadth (SEA$_c$) and the extent to which *B. barbus* trophic niche overlaps

590 (%) with other sampled fishes (*S. cephalus* and *L. leuciscus*). Error around the mean is

591 standard error.

592

Site	Species	n	Mean length (mm)	Mean δ^{13}C (‰)	Mean δ^{15}N (‰)	SEAc (‰^{2})	Overlap (%)
Site 1, Great Ouse	*B. barbus*	7	162.6 ± 44.9	-29.1 ± 0.2	20 ± 0.5	2.54	
	S.cephalus	6	290.2 ± 70.4	-26.5 ± 0.3	20.3 ± 0.8	4.85	0
	L. leuciscus	5	138.4 ± 19.8	-27.0 ± 0.6	18.0 ± 0.8	3.60	< 0.01
Site 2, Great Ouse	*B. barbus*	6	252.5 ± 8.4	-27.6 ± 0.2	17.0 ± 0.2	0.79	
	S. cephalus	6	346.0 ± 39.6	-25.6 ± 0.2	16.9 ± 0.7	2.32	0
	L. leuciscus	6	167.7 ± 1.9	-26.0 ± 0.3	15.0 ± 0.5	3.16	0
Lea (> 400 mm)	*B. barbus*	10	415.1 ± 3.9	-24.3 ± 0.1	16.3 ± 0.5	2.21	
	S. cephalus	9	415.3 ± 3.8	-25.7 ± 0.1	14.2 ± 0.4	3.87	< 0.01
Lea (< 250 mm)	*B. barbus*	10	225.5 ± 4.6	-27.0 ± 0.3	19.4 ± 0.3	1.29	
	S. cephalus	10	213.9 ± 4.2	-27.0 ± 0.3	16.4 ± 0.4	1.02	0
Avon	*B. barbus*	6	586.7 ±13.8	-25.8 ± 0.4	11.2 ± 0.4	3.87	
	S. cephalus	6	531.7 ± 7.0	-22.9 ± 0.6	11.9 ± 0.3	3.38	0

593   **Figure captions**

594

595   Figure 1. Location of the streams used in the Barbus barbus stocking field experiment.

596   Inset: Approximate locations of the streams in Britain. Main map: location of the

597   Houghton stream and Chun stream in relation to the main River Great Ouse and where

598   S1 and S2 represent the stocking locations (OS Open Map – Local 2015).

599

600   Figure 2. Stable isotope bi-plots for the mesocosm experiment, where (○) *B. barbus*

601   individuals, (Δ) *S. cephalus* individuals and (●) mean (± SE) values of putative macro-

602   invertebrate food resources. Solid lines enclose the standard ellipse areas for each species,

603   where black: *B. barbus*, dark grey: *S. cephalus*. Top: species in allopatry; Bottom: species in

604   sympatry.

605

606   Figure 3. Stable isotope bi-plots for the Chub stream where (○) *B. Barbus* individuals, (Δ) *S.*

607   *cephalus* individuals and (+) *L. leuciscus* individuals. Solid lines enclose the standard ellipse

608   areas for each species, where black: *B. barbus*, dark grey: *S. cephalus*, light grey: *L.*

609   *leuciscus*. Note the different scales on the axes. Top: June/August 2014; Middle: June 2015;

610   Bottom: September 2015.

611

612   Figure 4. Stable isotope bi-plots for the Houghton stream where (○) *B. Barbus*

613   individuals, (Δ) *S. cephalus* individuals and (+) *L. leuciscus* individuals. Solid lines

614   enclose the standard ellipse areas for each species, where black: *B. barbus*, dark grey: *S.*

615   *cephalus*, light grey: *L. leuciscus*. Note the different scales on the axes. Top:

616   June/August 2014; Bottom: June 2015.

617

618   Figure 5. Stable isotope bi-plots for the River Lea where (○) *B. Barbus* individuals, (Δ)

619   *S. cephalus* individuals. Solid lines enclose the standard ellipse areas for each species,

620   where black: *B. barbus*, dark grey: *S. cephalus*. Note differences in scales on all axes.

621   Top: all fish between 186 and 237 mm; Bottom: all fish between 400 and 435 mm.

622

623   Figure 6. Stable isotope bi-plots for the Site 1 (Top) and 2 (Middle) on the Great Ouse,

624   and the River Avon (Bottom), where (○) *B. Barbus* individuals, (Δ) *S. cephalus*

625   individuals and (+) *L. leuciscus* individuals with mean (± SE) values of putative food

626   sources: macroinvertebrates (●) and signal crayfish (■). Solid lines enclose the standard

627   ellipse areas for each species, where black: *B. barbus*, dark grey: *S. cephalus*, light

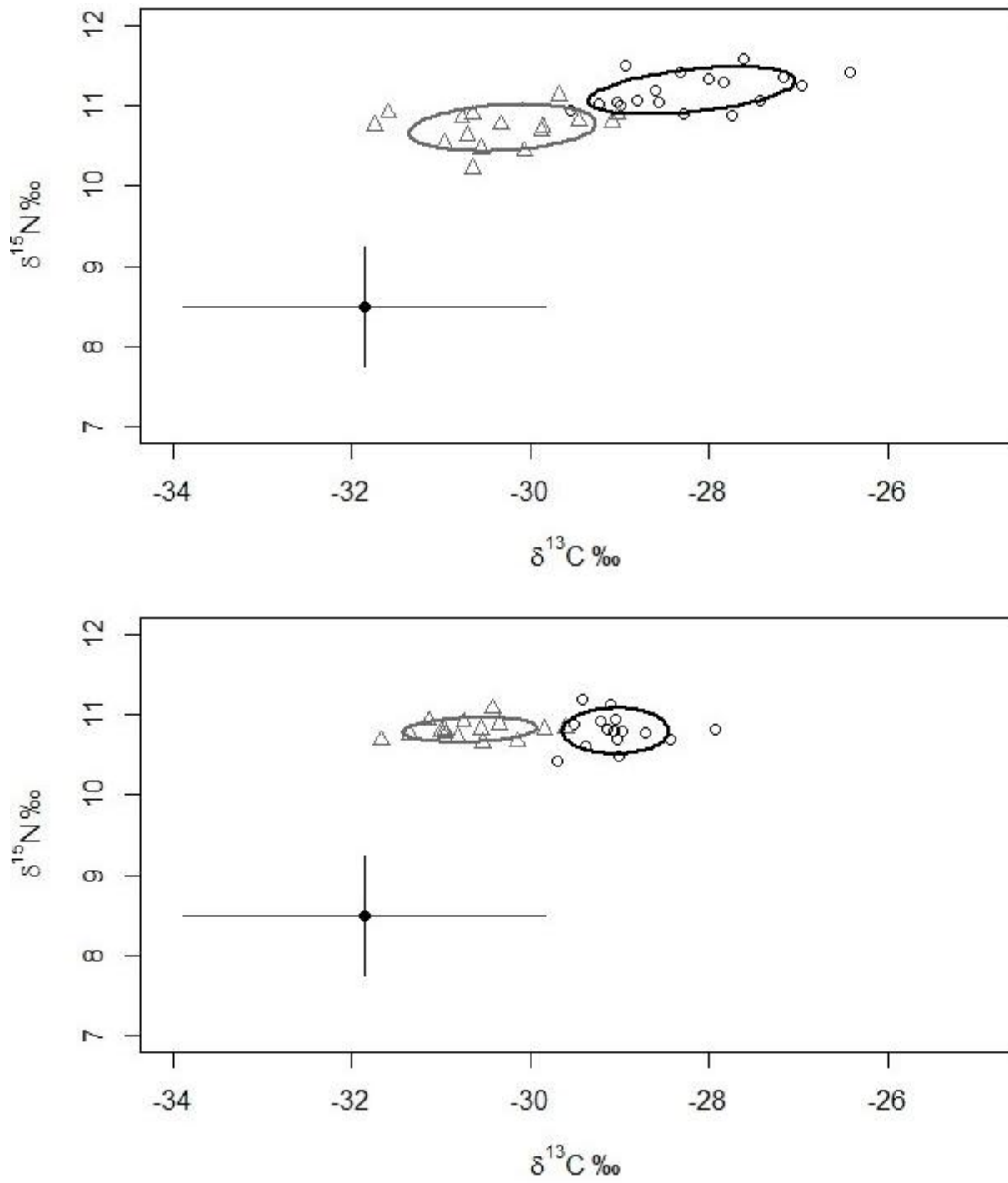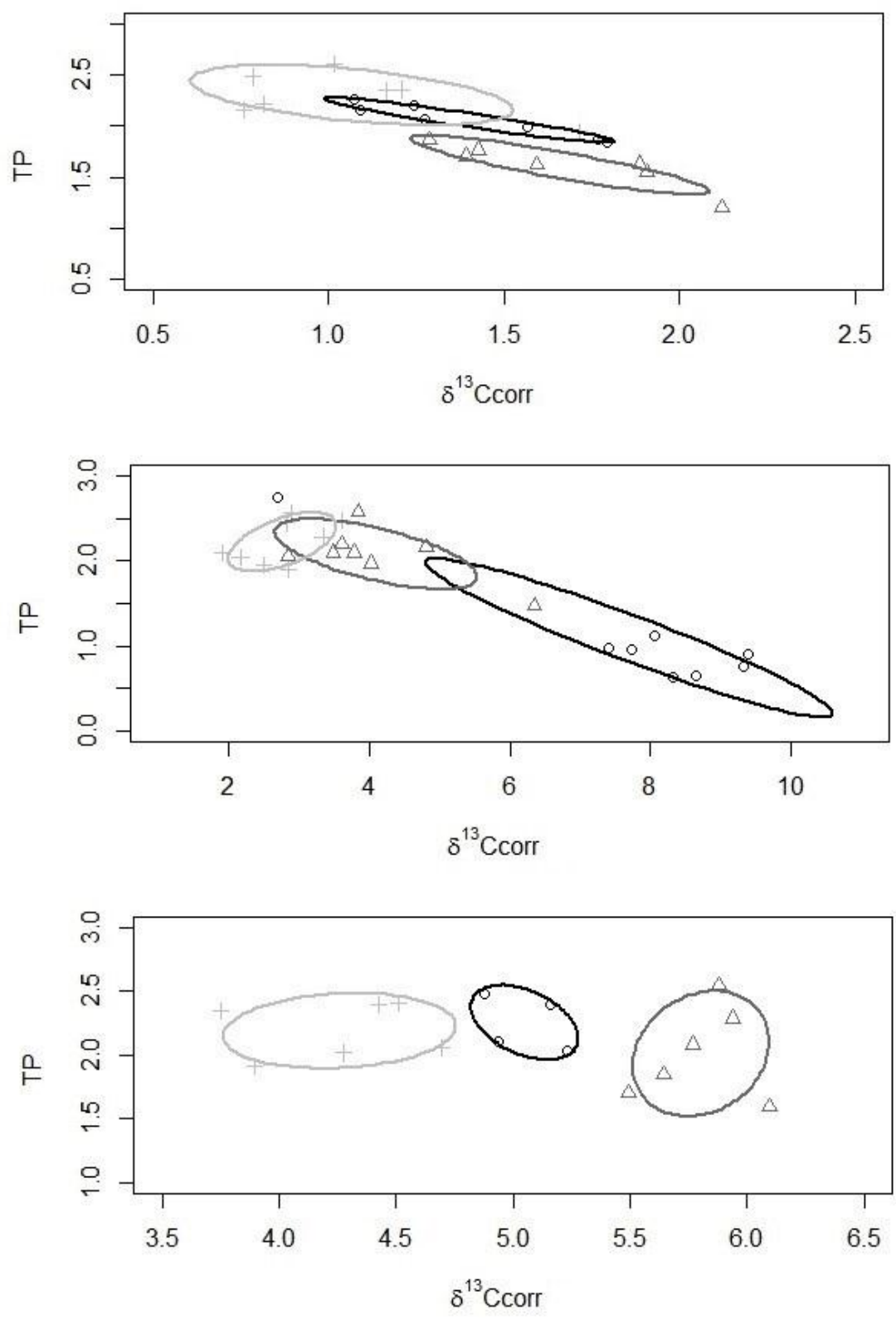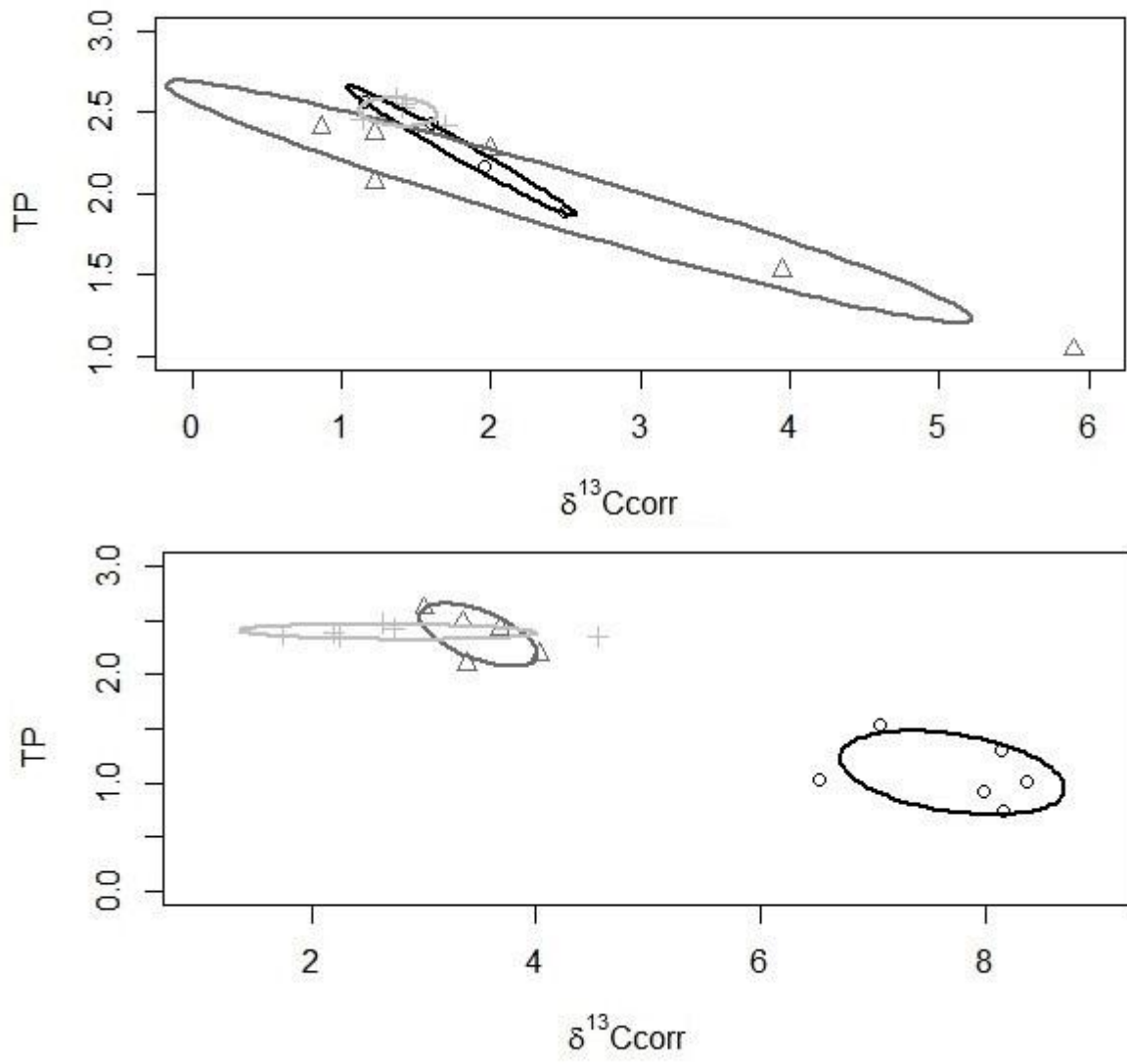628   grey: *L. leuciscus*. Note the different scales on the axes
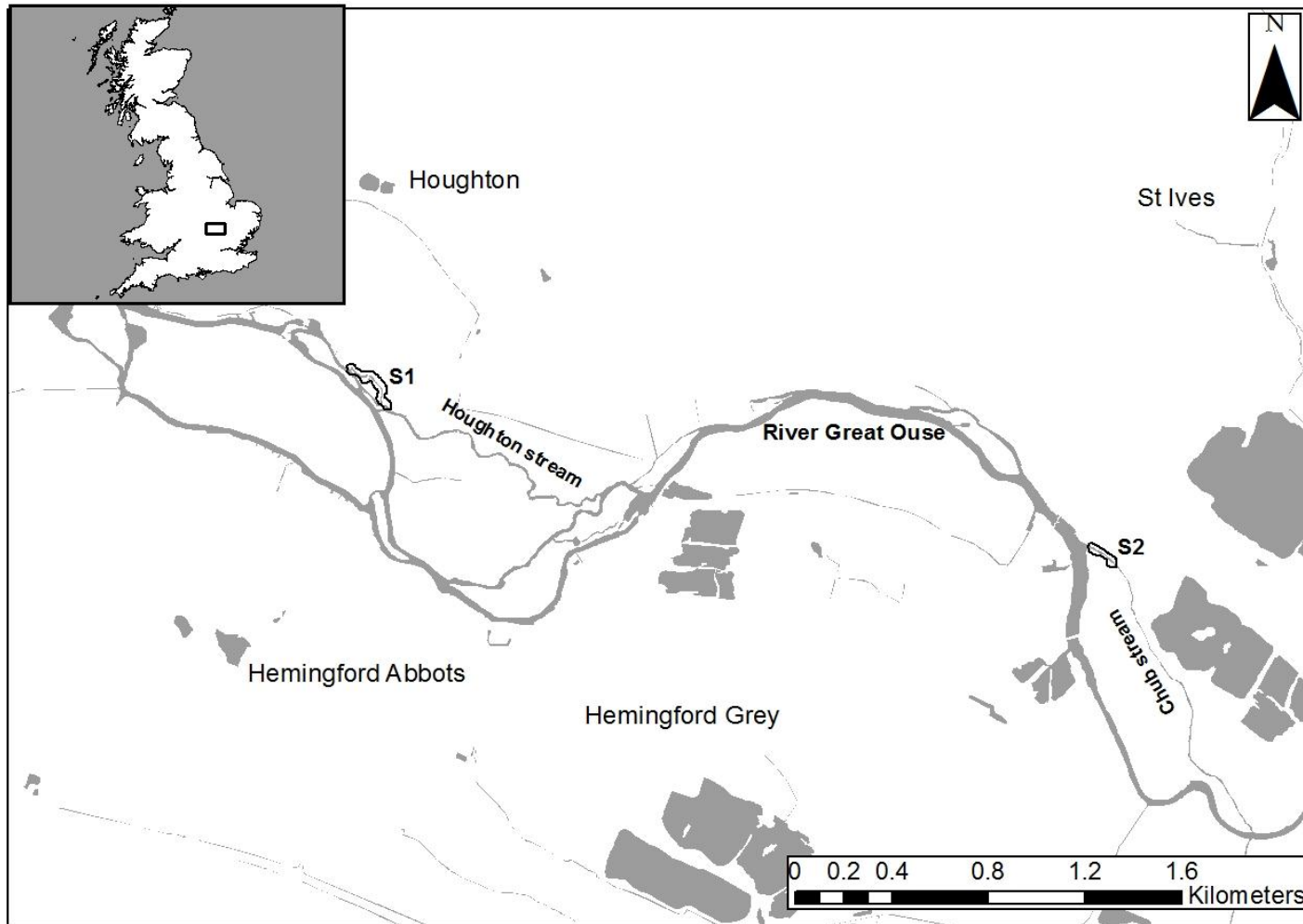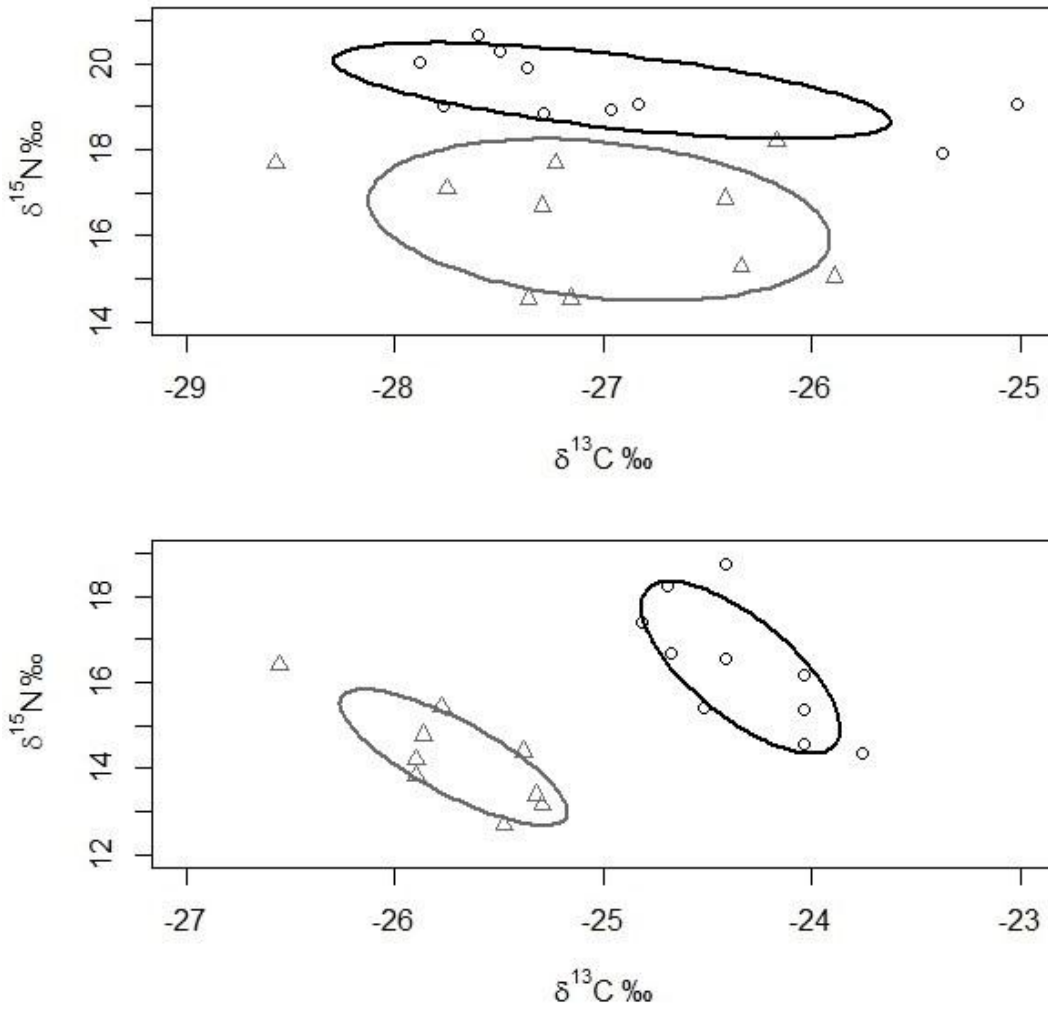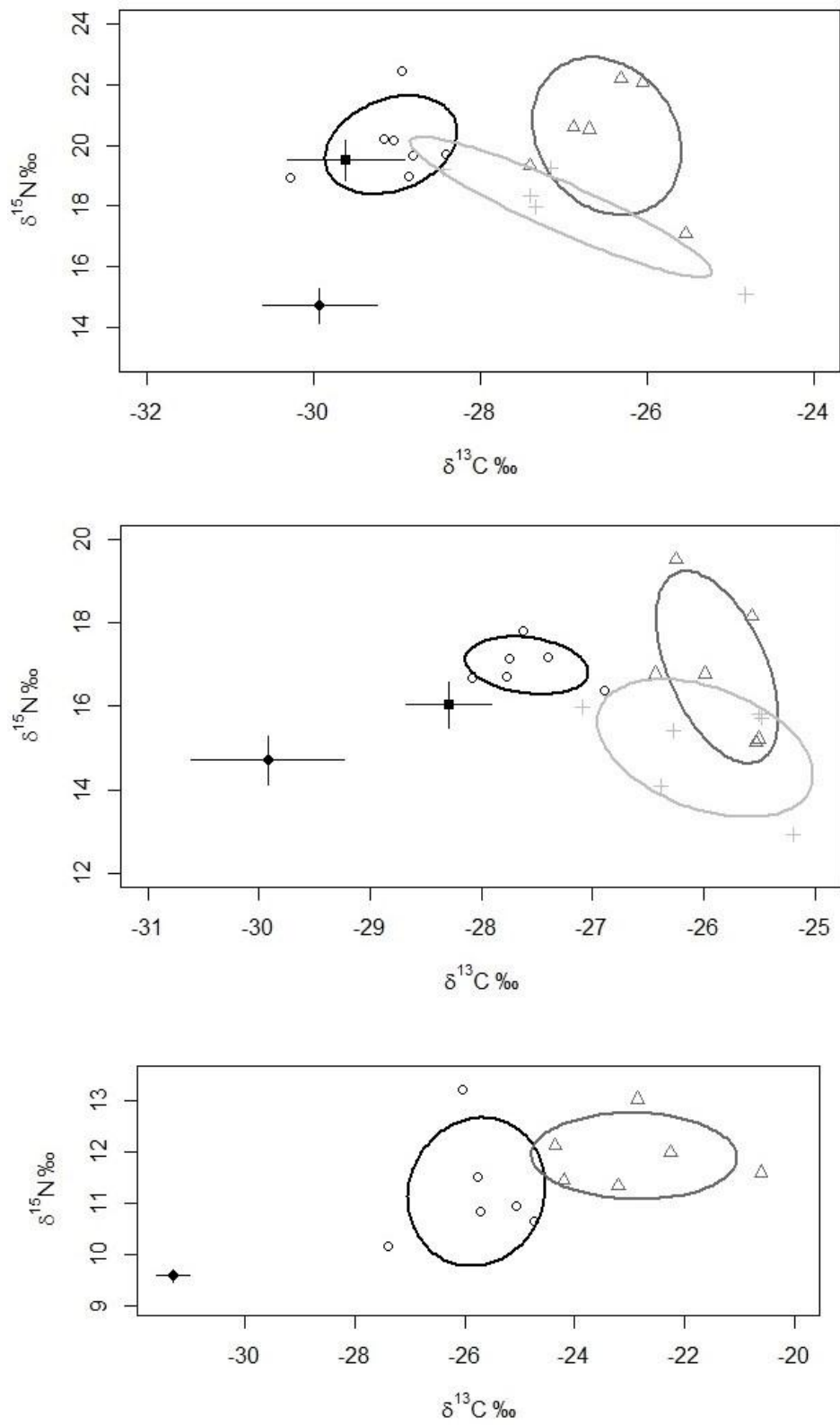
629

630   Figure 1.

631

Figure 2.

Figure 3.

Figure 4.

Figure 5.

Figure 6.