1   **Invasive crayfish impacts on native fish diet and growth vary with fish life stage.**

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16   *Running title: 'Invasive crayfish impacts on native fish'*

17

18   **Abstract**

19   Assessing the impacts of invasive organisms is a major challenge in ecology. Some widespread

20   invasive species such as crayfish are potential competitors and reciprocal predators of ecologically

21   and recreationally important native fish species. Here, we examine the effects of signal crayfish

22   (*Pacifastacus leniusculus*) on the growth, diet, and trophic position of the chub (*Squalius cephalus*)

23   in four rivers in Britain. Growth rates of 0+ chub were typically lower in sympatric populations

24   with signal crayfish compared with allopatric populations, and this effect could be traced through to

25   2+ chub in one river. However, growth rates of older chub (5+ to 6+) were typically higher in the

26   presence of crayfish. Sympatry with crayfish resulted in lower chub length-at-age and mass-at-age

27   in half of the rivers sampled, with no change detected in the other rivers. Stable isotope analyses

28   (δ^{13}C and δ^{15}N) revealed that both chub and crayfish were omnivorous, feeding at multiple trophic

29   levels and occupying similar trophic positions. We found some evidence that chub trophic position

30   was greater at invaded sites on one river, with no difference detected on a second river. Mixing

31   models suggested crayfish were important food items for both small and large chub at invaded sites.

32   This study provides evidence that invasive species can have both positive and negative effects on

33   different life stages of a native species, with the net impact likely to depend on responses at the

34   population level.

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36   Key Words: chub *Squalius cephalus*; competition; diet shift; invasive species; scalimetry; signal

37   crayfish *Pacifastacus leniusculus*; stable isotopes.

38

39

Introduction

The spread of organisms beyond their natural geographic range is a serious global threat causing both ecological and economic damage (Clavero and García-Berthou 2005; Roy et al. 2012) and rates of invasion show little sign of abating in some systems (e.g. Jackson and Grey 2012). Aquatic ecosystems are particularly vulnerable to the impacts of invasive organisms; the spread of non-native species is often facilitated by human activities and by the rapid dispersal possible in water (Rahel 2007; Strayer and Dudgeon 2010). A number of recent studies have demonstrated that invasive species may increase or decrease the growth rates, and alter the diets of, native organisms through several key mechanisms, including competition, predation, and transmission of pathogens (e.g. Correia 2001; King et al. 2006; Maguire and Grey 2006). Where prey availability is affected, a dietary shift to a different or previously under exploited prey resource may occur in order to maintain foraging efficiency (Syväranta and Jones 2008).

Owing to their omnivory, large body size and potential to dominate benthic biomass, some crayfish have become key invasive species that can affect ecosystem processes, services, and biodiversity, as well as the abundance, distribution, growth, diet and behaviour of native organisms (Lodge et al. 2000). North American signal crayfish (*Pacifastacus leniusculus* D. 1852) were introduced into Europe in the 1970s for aquaculture and represent one of the most widespread non-indigenous crayfish species (Holdich et al. 2009). Research has tended to focus on the interactions between signal and native crayfish (Holdich et al. 2009; Olsson et al. 2009a; Ercoli et al. 2014) and more recently with other invasive crayfish (Jackson et al. 2014); consequently less is known about interactions with fishes. In rivers, signal crayfish may reduce the growth and abundance of small benthic fishes, through interspecific competition and predation (Guan and Wiles 1997; Light 2005), and out-compete fish for refugia (Griffiths et al. 2004); yet other research has reported no effects of invasive crayfish on juvenile fish survival (Stenroth and Nyström 2003). However, there have been relatively few attempts to assess the specific impacts that signal crayfish may have on larger fish

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65 species of ecological and recreational importance (Reynolds 2011; Ruokonen et al. 2012; but see

66 Bašić et al. 2015). Understanding the full-range of crayfish-fish interactions, and both the

67 individual- and population-level consequences are essential if fish populations are to be managed

68 successfully.

69 Our study compared the growth, diet, and trophic position of a native predatory fish when found in

70 allopatry and sympatry with signal crayfish, and tested three hypotheses. Our first hypothesis was

71 that fish growth rates would be lower at invaded sites because signal crayfish have been shown to

72 reduce the availability of many prey taxa, such as aquatic invertebrates (Stenroth and Nyström

73 2003; Crawford et al. 2006), benthic fishes (Guan and Wiles 1997), and macrophytes (Nyström et

74 al. 1996). Such changes in prey availability led to our second hypothesis; that fish diet would

75 change after crayfish invasion by shifting to increased use of prey items typically unavailable to

76 crayfish, such as terrestrial invertebrates. Our third hypothesis was that the impacts of crayfish upon

77 fish would be greater for smaller relative to larger individuals via reciprocal predation as well as

78 competition, with each species consuming particular life stages of the other species. Crayfish

79 predominantly feed on fish eggs and larvae, but will also attack small individuals, whereas fish

80 consumption of crayfish typically increases with fork length and hence is greater for adult fish

81 (Hellawell 1971b; Blake and Hart 1995; García-Berthou 2002; Gladman et al. 2012).

82 We tested our hypotheses using chub (*Squalius cephalus* L.), native to rivers across Europe, and a

83 potential competitor and reciprocal predator of invasive crayfish. Chub are omnivorous, foraging on

84 aquatic and terrestrial invertebrates, macrophytes, detritus, fishes and other small vertebrates

85 (Hellawell 1971b; Mann 1976) and are popular with anglers. Impacts on chub growth and feeding

86 could alter food web structure, energy flow, community composition and the recreational value of

87 lowland rivers. Thus, juvenile chub would experience reduced prey availability and increased

88 predation pressure, whereas larger chub would experience smaller reductions in prey availability

89   (with larger gape increasing prey range) and this would be partially offset by the greater inclusion

90   of signal crayfish in their diet (Nyström et al. 2006).

91

92   **Materials and Methods**

93   We used two complementary study approaches to assess the effects of signal crayfish on the

94   growth, condition, and trophic position of chub in four lowland British rivers (Table 1). For two

95   rivers (Evenlode and Cherwell), we used a before-after approach to compare effects on chub before

96   and after signal crayfish invasion. From a further two rivers (Rother and Chad Brook), we used a

97   space-for-time approach in which chub from sites with established signal crayfish populations were

98   compared with chub from uninvaded sites upstream on those rivers; within each river we selected

99   invaded and uninvaded sites with comparable hydrological conditions (i.e. discharge), physical

100   structure (i.e. channel width, depth), land use, and ecological communities, in order to avoid such

101   differences confounding our ability to detect the effects of crayfish on chub. Signal crayfish were

102   first recorded in 2000 and 1995, in the Evenlode and Cherwell, respectively, and thus archived

103   scales provided by the Environment Agency from chub caught before 2000 (Evenlode) and 1995

104   (Cherwell) were used to obtain pre-invasion growth data, while scales from chub spawned after

105   2000 and 1995 were used to obtain post-invasion data (Environment Agency data, 2008). The

106   Rother was invaded by signal crayfish between 1973-1975 (Environment Agency data 2008).

107   Extensive sampling indicated that the invaded stretch extended from a weir (51°00'15.16"N,

108   00°53'04.96"W) downstream to 51°00'15.07"N, 00°52'54.70"W; immediately upstream of the

109   weir, from 51°00'11.93"N, 00°53'05.04"W to 51°00'09.03"N, 00°53'41.02"W was uninvaded.

110   Signal crayfish invaded Chad Brook from the confluence with the River Stour after 2000 to a weir

111   at 52°04'43.71"N, 00°42'54.31"E (Environment Agency data 2008). Thus, the river above the weir

112   to 52°04'49.33"N, 00°43'31.43"E was designated as the uninvaded site, while the river below the

113   weir to 52°26'10.99"N, 00°43'46.80"E was classified as the invaded site.

Growth rates

116   Age estimation based on annuli counts from calcified tissues such as scales has been routinely used

117   for chub (Hellawell 1971a; Mann 1976). Scale-derived growth data allow long-term assessment of

118   the effects of perturbations (i.e. growth pre- and post-crayfish invasion). Chub were sampled by

119   angling in the Rother (n = 32) and Chad Brook (n = 36) during June-September in two years: 2008

120   and 2011. Mass (±1 g) and fork length (±1 mm) were determined in the field and three scales were

121   removed from each chub from the flank between the dorsal fin and lateral line. All individuals were

122   returned alive. For the Rivers Evenlode (n = 68) and Cherwell (n = 58), archived scales provided by

123   the Environment Agency from chub caught before 2000 (Evenlode) and 1995 (Cherwell) were used

124   to obtain pre-invasion growth data, while scales from chub spawned after 2000 and 1995 were used

125   to obtain post-invasion data. Scales were examined using a SMZ1000 dissection microscope

126   (Nikon, Japan) and estimates of length-at-age were back calculated using the Fraser-Lee formula,

127   assuming a length of first scale formation of 15.9 mm (Economou et al. 1991).

Stable Isotope Analyses

130   Stable isotope ratios of carbon and nitrogen vary in a conservative, predictable manner between

131   trophic levels and thus changes in those ratios can be an effective technique in assessing dietary

132   shifts of consumers in response to the invasion of an ecosystem by an alien species (Jackson et al.

133   2012). Non-destructive sampling is facilitated where tissue such as scales can be sampled, making

134   stable isotope analysis an ideal investigative tool for aquatic ecosystems with small fish populations

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135   of conservational or recreational value (Perga and Gerdeaux 2003; Grey et al. 2009). We used the

136   baseline-corrected estimates of trophic height (*sensu* Cohen et al. 2003) to compare the trophic

137   position of chub between sites with and without invasive crayfish and mixing models to determine

138   relative contributions from food sources. We combined these complementary methods, assessing

139   growth rates by traditional techniques of scalimetry and then analysing the recent (<2 years)

140   material for stable isotopes sequestered in the scales (Grey et al. 2009). Scale isotope ratios were

141   converted to muscle ratios to facilitate the comparison with crayfish and prey species.

142   We analysed $\delta^{13}C$ and $\delta^{15}N$ of chub scales, crayfish, and putative prey to assess the trophic position

143   of chub and crayfish, their diets, and potential dietary overlap. Qualitative sampling was carried out

144   in May 2008 and June 2011 at invaded and uninvaded sites on the Rother and Chad Brook to collect

145   potential dietary resources. Aquatic invertebrates (min. $n= 5$ individuals pooled per taxa),

146   macrophytes ($n= 5$ leaves pooled from different individual plants of the dominant species present),

147   and small fish ($n \geq 5$ per species) were obtained by kick sampling; terrestrial invertebrates ($n= 5$

148   individuals pooled per species) were obtained by sweeping riparian vegetation with a butterfly net.

149   Detritus (~250 g) was taken from the main channel substrate. Signal crayfish were also collected

150   from invaded sites at Chad Brook ($n= 18$) and the Rother ($n= 19$) by kick-sampling. Carapace

151   length was determined for each individual by measuring from the rostrum tip to carapace posterior.

152   All samples except chub scales were frozen at -20 °C until preparation for stable isotope analysis. A

153   portion of the outer section of each scale, equivalent to the most recent two annuli, was removed for

154   stable isotope analysis. Each sample was macerated in a glass vial and oven dried at 60 °C for 48

155   hours, then pulverised using an agate mortar and pestle, and 0.6 ± 0.05 mg weighed into tin cups.

156   Samples were combusted using an elemental analyser (Flash EA, 1112 series, Thermo-Finnigan)

157   coupled to a continuous flow isotope ratio mass spectrometer (Finnigan MAT DeltaPlus, Thermo-

158   Finnigan).

159 Chub and crayfish stable isotope ratios were derived from scale and muscle, respectively. Both δ^{13}C

160 and δ^{15}N vary between tissue types, but previous studies have shown that there is a dependable

161 relationship between fish muscle and scale (e.g. Grey et al. 2009). Therefore, to better compare

162 chub to their diet and to the crayfish, a conversion factor was derived from the stable isotope ratios

163 for both scale and muscle tissue. Fifteen chub of three age classes (0+, 1+, and 2+; $n = 5$ for each

164 class) from Calverton Fish Farm (Nottingham, UK), were sacrificed; muscle was excised from the

165 left flank above the lateral line, and both scale and muscle samples prepared as above.

166

167 *Statistical analyses, Isotope-Metrics and Mixing Models*

168 Statistical analyses were performed using R version 3.1.2 (R Development Core Team 2015), with

169 significant effects attributed where $p < 0.05$. For both our before-after invasion sites (Evenlode and

170 Cherwell) and our space-for-time sites (Rother and Chad Brook) we tested the effects of site

171 (invaded versus uninvaded) and sampling year (2008 versus 2011; Rother and Chad Brook only) on

172 chub growth rates for each age-class, and (ii) trophic position (baseline-corrected δ^{15}N), using linear

173 models with Gaussian error structures. Site and year were treated as fixed factors. Analysis of

174 covariance (ANCOVA) was used to test for differences in the relationships between (i) fork length

175 and age, (ii) mass and age, and (iii) trophic position and fork length, between invaded and

176 uninvaded sites. Sampling year (2008 or 2011) was also included as a covariate. Normality and

177 equality of variances were ascertained for residuals via Anderson-Darling and Levene's tests,

178 respectively. Linear regressions were plotted through six basal consumers (invertebrates; three

179 terrestrial, three aquatic) for both the Rother (Aquatic: Trichoptera, Amphipoda, Ephemeroptera;

180 Terrestrial: Coleoptera, Hemiptera, Hymenoptera) and Chad Brook (Aquatic: Gastropoda,

181 Amphipoda, Heteroptera; Terrestrial: Coleoptera, Hemiptera, Diptera), and the perpendicular

182 distance from the generated sloping baseline to the chub or crayfish (measured as change along the

183 δ^{15}N axis) gave the trophic height for each individual.

184 Chub diet shifts ontogenetically, with Hellawell (1971b) reporting that $\leq$ 5+ chub consumed greater

185 proportions of terrestrial and aquatic invertebrates (excluding crayfish), and less plant matter than $\geq$

186 6+ chub. The mean length of a 5+ chub, based on data from this study and a meta-analysis of chub

187 length-at-age data (Mann 1976) was 231.7 mm. Thus, to account for potential ontogenetic shifts,

188 chub were classified on fork length as either small ($<$ 232 mm; Rother, uninvaded n= 4, invaded n=

189 5; Chad Brook, uninvaded n= 2, invaded n= 12) or large ($\geq$ 232 mm; Rother, uninvaded n= 10,

190 invaded n= 13; Chad Brook, uninvaded n= 19, invaded n= 3). The baseline regressions described

191 earlier were used to estimate the mean ($\pm$ 95 % CI) trophic height of chub and crayfish populations.

192 SIAR mixing model fractionation values (Parnell et al. 2010) were derived as follows. A mean Δ^{13}C

193 value (2.1 ‰) was calculated from four controlled feeding studies (*Coregonus nasus*: +2.0 ‰,

194 Hesslein et al. 1993; *Oncorhynchus mykiss*: +1.3 ‰, Rounick and Hicks 1985; *Oncorhynchus*

195 *mykiss*: +1.9 ‰; *Salvelinus fontinalis*: +3.3 ‰ McCutchan Jr et al. 2003). As chub are omnivorous

196 and the fractionation can be dependent on the nitrogen content of food items, a value of +2.3 ‰ was

197 used for Δ^{15}N following McCutchan Jr et al. (2003). These values were added to all source items

198 under the following categories: crayfish, macrophytes, detritus, terrestrial invertebrates, small fish,

199 and aquatic invertebrates. Cannibalism is not thought to be common among chub (Hellawell 1971b;

200 Mann 1976), and as few individuals were large enough to ingest any other within our samples,

201 cannibalism was excluded from the analysis.

202 Although crayfish diet has also been reported to vary ontogenetically (Guan and Wiles 1997;

203 Bondar et al. 2005), analysing crayfish in various size classes had negligible effects on SIAR output

204 and therefore crayfish were analysed as a single group. A Δ^{13}C value of +2.0 ‰ was taken from a

205 feeding experiment using *Procambarus clarkii* (Rudnick and Resh 2005) while +2.3 ‰ was used

206 once more for nitrogen for the same reasons as for chub. As cannibalism in signal crayfish has been

207 reported, crayfish were included as a potential food source (Guan and Wiles 1997; Stenroth and

208 Nyström 2003).

209

210 **Results**

211 Chub from uninvaded sites on all four rivers exhibited decreasing annual growth rate with

212 increasing age (Fig. 1). However, this pattern did not hold for the invaded sections on three of our

213 four rivers, where annual growth rates increased again at ages of 5+ or above (site dependent).

214 Chub growth rates were significantly lower at invaded relative to uninvaded sites for 0+ chub in all

215 rivers (Table 2). Whilst there were indications of lower growth rates in 1+ and 2+ chub in some

216 invaded river sites, growth rates were only significantly lower in the Evenlode. Older fish from

217 invaded sites exhibited accelerated growth rates compared to uninvaded sites in the Rother at ages

218 5+ and 6+, at 6+ in Chad Brook, and at 5+ in the Evenlode (Table 2). Chub growth rates differed

219 significantly between 2008- and 2011-sampled fish in only 3 out of 13 models; 1+ Rother, 3+ and

220 4+ Chad Brook. Significantly greater fork length-at-age at uninvaded relative to invaded sites was

221 found for Chad Brook and Evenlode (Table 3; Fig. 2). Furthermore, Chad Brook chub achieved

222 greater mass-at-age at uninvaded relative to invaded sites (Table 3; Fig. 2).

223 There was a linear relationship between chub scale and muscle $\delta^{13}C$ ($F_{1,13}= 40.17$, $p< 0.001$), and

224 between scale and muscle $\delta^{15}N$ from Calverton fish farm ($F_{1,13}= 60.51$, $p< 0.001$). Muscle tissue

225 was ^{13}C-depleted (mean ± SD: -2.2 ± 0.5 ‰) and ^{15}N-enriched (0.8 ± 0.3 ‰) relative to scale tissue

226 and the corresponding regression equations shown (Supplementary Information) were used for

227 converting scale isotope ratios for further comparisons.

10

228   We found no evidence that chub muscle baseline-corrected $\delta^{15}N$ was related to fork length or

229   sampling year at either the Rother or Chad Brook (Fig. 3; Table 3). However, chub baseline-

230   corrected $\delta^{15}N$ was higher at the invaded site on Chad Brook, but no differences were detected for

231   the Rother (Fig. 3; Table 3). The relative trophic positions of chub and crayfish, as inferred from

232   isotopic bi-plots (Fig. 4), indicate that both species fed on multiple food sources. Large chub from

233   the invaded Rother site had a mean ($\pm$ 95 % CI) trophic height (measured as $\delta^{15}N$) of 5.5 $\pm$ 0.9 ‰,

234   compared with 4.7 $\pm$ 0.7 at the uninvaded site (Table 4). Similarly, small chub at the invaded site

235   had a trophic height of 5.1 $\pm$ 1.5 compared with 4.5 $\pm$ 0.5 at the uninvaded site. The mean trophic

236   heights of large and small chub from the invaded site were 1.1 and 0.8 ‰ higher, respectively, than

237   that of crayfish. However, in Chad Brook both large and small chub were estimated to have similar

238   trophic heights in invaded and uninvaded sites, with crayfish trophic height similar to those of chub

239   (Table 4).

240   SIAR model ouputs indicated that terrestrial invertebrates were the most important prey resource

241   for chub, comprising up to 50 % of chub diet (Fig. 5; Supplementary Information). In contrast,

242   aquatic invertebrates (other than crayfish) constituted < 20 % of chub diet for all sites on both

243   rivers. Furthermore, small chub relied even less on aquatic invertebrates at invaded sites, declining

244   from 13 to 7 % in the Rother and from 17 to 7 % in Chad Brook. At invaded sites signal crayfish

245   were estimated to make a mean contribution of up to 26 % and 19 % of chub diet in the Rother and

246   Chad Brook, respectively. Similar dietary use of crayfish was found for both size classes of chub.

247   For both rivers the contribution of small fish to chub diet was found to be reduced at the invaded

248   sites. Crayfish exhibited a high degree of omnivory in both rivers, with modelled dietary

249   contributions showing wide ranges for all potential food sources (Supplementary Information).

250   Crayfish in Chad Brook showed greater consumption of specific taxa, with aquatic invertebrates

251   and small fish making mean dietary contributions of 42 and 20 %, respectively. Cannibalism among

252   signal crayfish was estimated to make a mean contribution of 12 % to crayfish diet in both rivers.

253

Discussion

255   Many studies have reported on the negative impacts of invasive species, but there have been

256   relatively few on how the influence of an invader may be beneficial to a recipient system (Caldow

257   et al. 2007; Letnic et al. 2009; Tablado et al. 2010). Our study illustrates that there can be both

258   beneficial and detrimental consequences of invaders on a native species, with the life stage of the

259   native species influencing whether the impact was positive or negative. The growth rates of young

260   individuals of a native fish were reduced when in sympatry with a non-native crustacean, yet some

261   older fish exhibited increased growth rates in the presence of the invader. Our isotope mixing

262   models suggests that crayfish were incorporated as an additional dietary component at invaded sites.

263   Therefore, the influence of the invasive crayfish may be perceived as both negative and positive to

264   chub.

265   At all sites where chub existed in sympatry with signal crayfish, the 0+ fish exhibited lower growth

266   rates, and this was maintained in the Evenlode until fish were aged 2+. Signal crayfish can prey

267   directly upon small fish (Guan and Wiles 1997) and consequently small fish may spend more time

268   engaged in predator-avoidance, limiting foraging opportunities (Light 2005) and reducing growth

269   rates in chub (Allouche and Gaudin 2001). Whilst our results were correlative, a consistent pattern

270   of reduced juvenile chub growth was detected in our four datasets: a space-for-time approach in

271   Evenlode and Cherwell, and a before-after invasion approach in Rother and Chad Brook. Chub

272   growth rates will likely have been further influenced by additional, unmeasured variables, as

273   evidenced by the observed inter-annual differences in chub growth rates in 3 of 13 comparisons.

274   Such inter-annual differences may reflect between-year variation in environmental conditions such

275   as water temperature and flow speed, which are known to influence the growth rates of cyprinid

276   fishes (Cragg-Hine and Jones 1969). Such variables could have interacted with crayfish presence to

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277 modulate the effects of crayfish on chub, for example by increasing crayfish numbers or activity

278 (Olsson et al. 2009b). Furthermore, changes in crayfish densities could have affected chub growth

279 rates, as crayfish impacts on native species are typically density-dependent (e.g. Flint and Goldman

280 1975). Whilst our study did not account for these additional factors, we were still able to detect

281 effects of crayfish invasion on chub growth rates.

282 Older chub were generally found to exhibit higher growth rates at invaded sites in three of the four

283 rivers studied. The age at which chub from the invaded sites achieved greater growth rates than

284 those of chub from uninvaded sites varied from 5+ to 6+. Increased chub growth rates associated

285 with the presence of signal crayfish may indicate greater predation on crayfish by larger chub, a

286 plausible inference considering the ^{15}N- and ^{13}C-enrichment of larger chub. Thus the outputs from

287 the mixing models were consistent with the pattern expected of a gape-limited predator of crayfish.

288 Although Evenlode chub aged 6+ exhibited slightly higher growth rates in the post-invasion period,

289 the difference was not significant. Overall, the data upheld our first hypothesis, that juvenile chub

290 growth rates would be lower when sympatric with signal crayfish, and older, larger chub would

291 show the opposite trend.

292 This study provides evidence that signal crayfish may alter the size structure of chub populations.

293 Chub length-at-age was reduced at two of the four invaded sites tested; methodology did not appear

294 to influence our results, as reduced length-at-age was detected for sites at which before-after

295 invasion site (Evenlode) and space-for-time (Chad Brook) approaches were used. Furthermore,

296 mass-at-age was reduced at one of the two invaded sites tested, whereas no increases either length-

297 at-age or mass-at-age in response to invasion were detected, probably due to decreased growth rates

298 of young chub in the presence of signal crayfish. Lower 0+ growth has been reported to result in

299 smaller annual growth increments across the lifetime of individual chub (Bolland et al. 2007). In the

300 Rother and Cherwell, older (≥5+) chub from the invaded sites were found to attain greater length-at-

301 age than conspecifics at uninvaded sites, despite younger (≤3+) chub from the same invaded sites

302   exhibiting lower length-at-age values. Based on our complementary stable isotope data, we propose

303   that greater length-at-age in some older chub was the result of consuming invasive crayfish. Our

304   results concur with previous findings that predators can achieve higher post-invasion growth rates

305   and ultimate body size either by direct predation of the invasive species or by indirect effects (King

306   et al. 2006). The changes in chub size-at-age have implications for food web structure and the

307   abundances of prey items as energy requirement and prey availability (due to gape-limitation) are

308   related strongly to fish body size (Wieser 1991).

309   Our second hypothesis, that chub diet would be altered in the presence of crayfish, is confirmed not

310   only through the incorporation of the invasive crayfish into the diet of chub, but also by a reduction

311   in reliance upon aquatic invertebrates by small chub, and reduced reliance on small fish by chub of

312   both size classes, at the invaded sites. A reduction in the contribution of one food source must lead

313   to compensation through greater dependence on another. Hellawell (1971b) reported that where

314   larger chub exhibit reduced consumption of terrestrial invertebrates, there was increased

315   consumption of fish, frogs and native crayfish. It is therefore likely that an invasive crayfish would

316   be increasingly exploited in the same manner. Furthermore, considering the documented negative

317   effects of crayfish on aquatic invertebrates and macrophytes, it seems less likely that these groups

318   should be more heavily relied upon by the chub of the invaded sites. Indeed, the reduced

319   contribution of aquatic invertebrates (other than crayfish) to chub diet at invaded sites is consistent

320   with previous research that found invasive signal crayfish reduced the total numbers of aquatic

321   invertebrate by 60 % (Crawford et al. 2006). We found some evidence that the potential

322   incorporation of crayfish into the diets of larger chub resulted in elevated baseline corrected $\delta^{15}N$

323   values at Chad Brook, but not at the Rother. Chub at invaded sites incorporated high $\delta^{15}N$ crayfish

324   into their diet, which likely raised large chub $\delta^{15}N$ at invaded sites. SIAR output indicated greater

325   consumption of terrestrial invertebrates by chub compared to crayfish. Morphology and behaviour

326   limits crayfish primarily to benthic foraging (Guan and Wiles 1998) and therefore precludes access

327 to surface drifting prey. However, once terrestrial invertebrates sink they become available to

328 crayfish. In contrast, chub utilise the entire water column from benthos to surface when foraging

329 (Hellawell 1971b).

330 The combined growth data and stable isotope evidence does not support our third hypothesis; that

331 changes in growth rate and dietary shifting would be more pronounced in younger chub. In contrast,

332 the increase in growth rates of older chub at the Rother and Chad Brook were greater in magnitude

333 than those differences between the 0+ fish. However, as isotope data were only acquired for ≥3+

334 chub the impact of crayfish invasion on the diet and trophic position of ≤2+ individuals, which

335 exhibited lower growth rates in sympatry with signal crayfish, remain unknown.

336 We have shown that crayfish invasion can have both positive and negative impacts on the diet and

337 growth of a native fish, using signal crayfish and chub as exemplars. We found some evidence that

338 older chub benefit from the inclusion of crayfish in their diet and can achieve higher growth rates.

339 Younger chub suffer decreased growth rates, probably due to increased predator-avoidance and

340 reduced prey availability. Potentially, an individual chub may experience both negative and positive

341 impacts of invasion as it progresses through different life stages. Whether crayfish invasion can be

342 considered beneficial or detrimental to the native fish population as a whole will depend, at least in

343 part, on whether the lower juvenile growth rates translate into lower recruitment and thus reduced

344 population size. Further longer-term studies of the population level consequences of crayfish

345 invasion are required. Several studies have found lower populations in the presence of signal

346 crayfish (Guan and Wiles 1997; Peay et al. 2009), whereas others have not (Stenroth and Nyström

347 2003; Degerman et al. 2007); comprehensive studies of fish populations pre- to post-invasion and

348 assessed relative to the 'natural state' (i.e. with the presence of native crayfish) are required to

349 address this question.

350

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486

Figure legends

488 **Fig. 1:** A comparison of calculated mean (± SE) yearly growth rates of chub sampled from

489 uninvaded (closed symbols) and invaded (open symbols) sites on (a) the Rother, (b) Chad Brook,

490 (c) the Cherwell, and (d) the Evenlode.

491 **Fig. 2:** Chub fork length-at-age at uninvaded (solid circles and line) and invaded (open circles and

492 dashed line) sites on (a) the Rother, (b) Chad Brook, (c) the Cherwell, and (d) the Evenlode. Chub

493 mass-at-age at uninvaded (solid circles and line) and invaded (open circles and dashed line) sites on

494 (e) the Rother and (f) Chad Brook.

495 **Fig. 3:** Baseline corrected chub δ^{15}N as a function of fork length for uninvaded (solid circles) and

496 invaded (open circles) sites on (a) the Rother and (b) Chad Brook.

497 **Fig. 4:** Isotope bi-plots indicating the mean (± standard error) for chub, crayfish, and the putative

498 prey of both species, for (a) the Rother and (b) Chad Brook. For the Rother small fish were 1+

499 cyprinids, *Phoxinus phoxinus*, *Cottus gobio*, and *Barbatula barbatula*, aquatic invertebrates were

500 Trichoptera, Gammarids, and Ephemeroptera, and terrestrial invertebrates were Formicidae,

501 Arachnidae, Hemiptera, Diptera, and Coleoptera. For Chad Brook small fish were *Phoxinus*

502 *phoxinus*, *Cottus gobio*, *Barbatula barbatula*, and *Gasterosteus aculeatus*, aquatic invertebrates

503 were Gammarids, *Calopteryx sp.*, Heteroptera, Limnaea, and Trichoptera, and terrestrial

504 invertebrates Formicidae, Arachnidae, Diptera, Coleoptera, and Gastropoda.

505 **Fig. 5:** The mean (± 95 % CI) percentage of small and large chub diets comprised by each food

506 source at uninvaded and invaded sites, as indicated by the SIAR mixing model. Normal

507 distributions of isotope data were confirmed by visual inspection of the data.
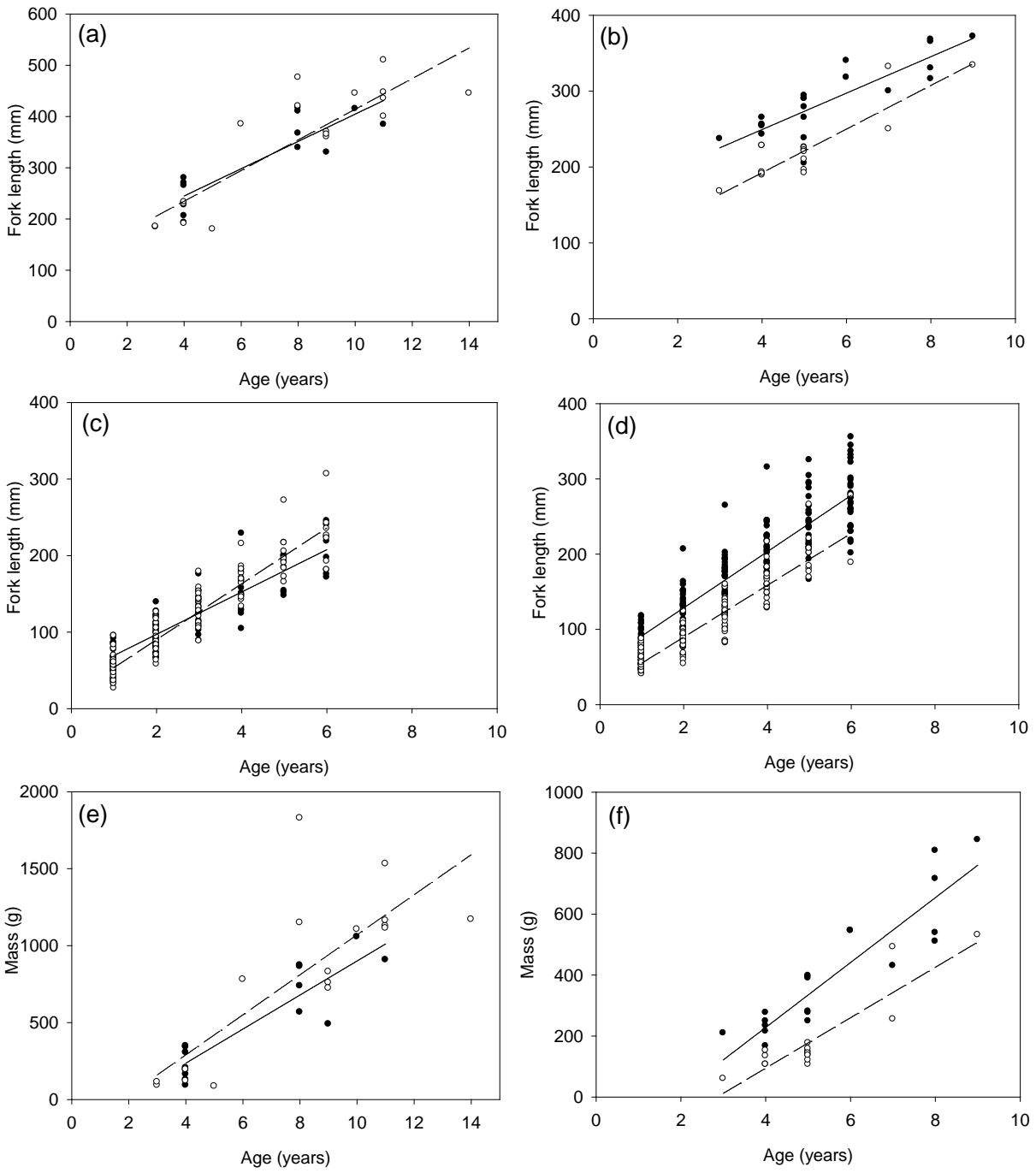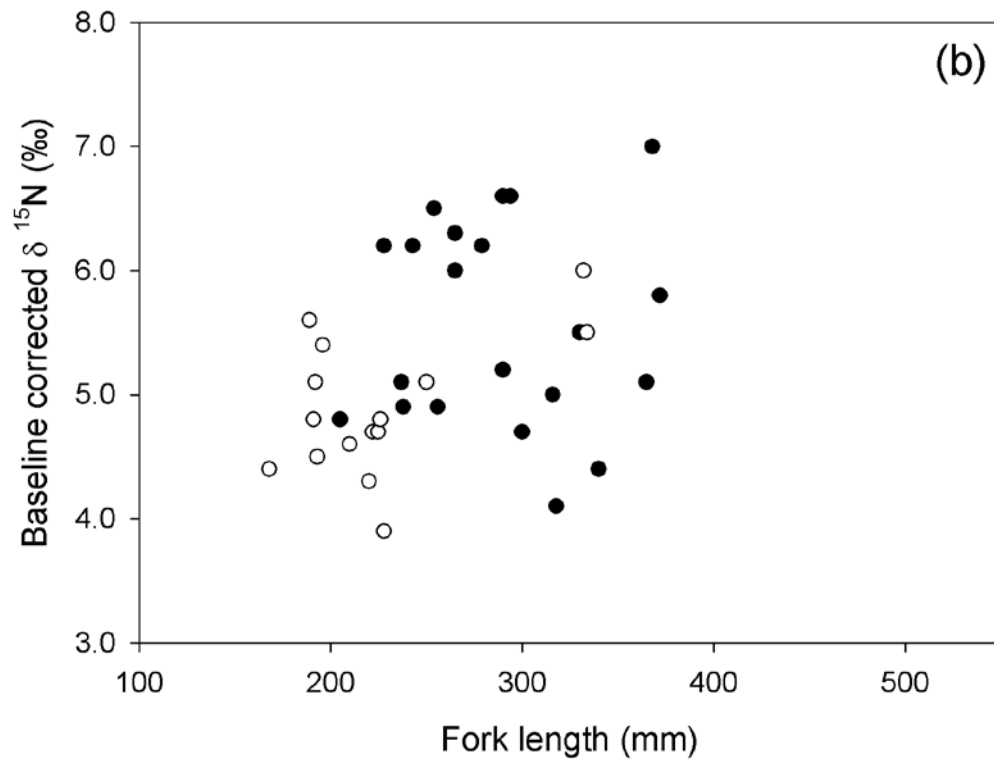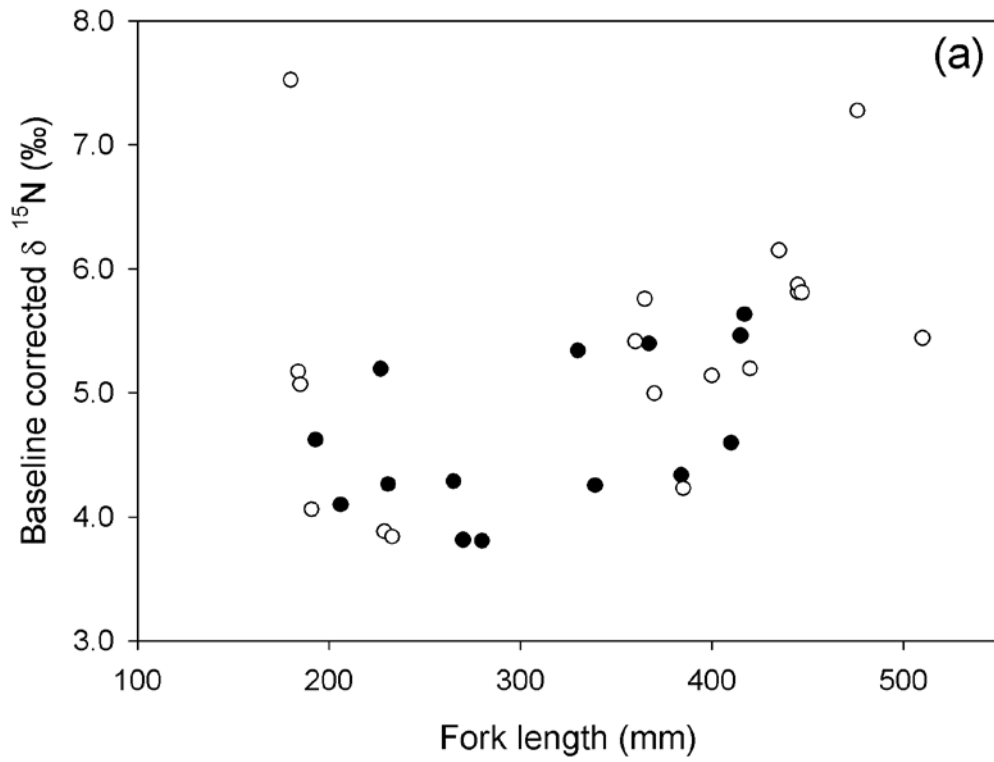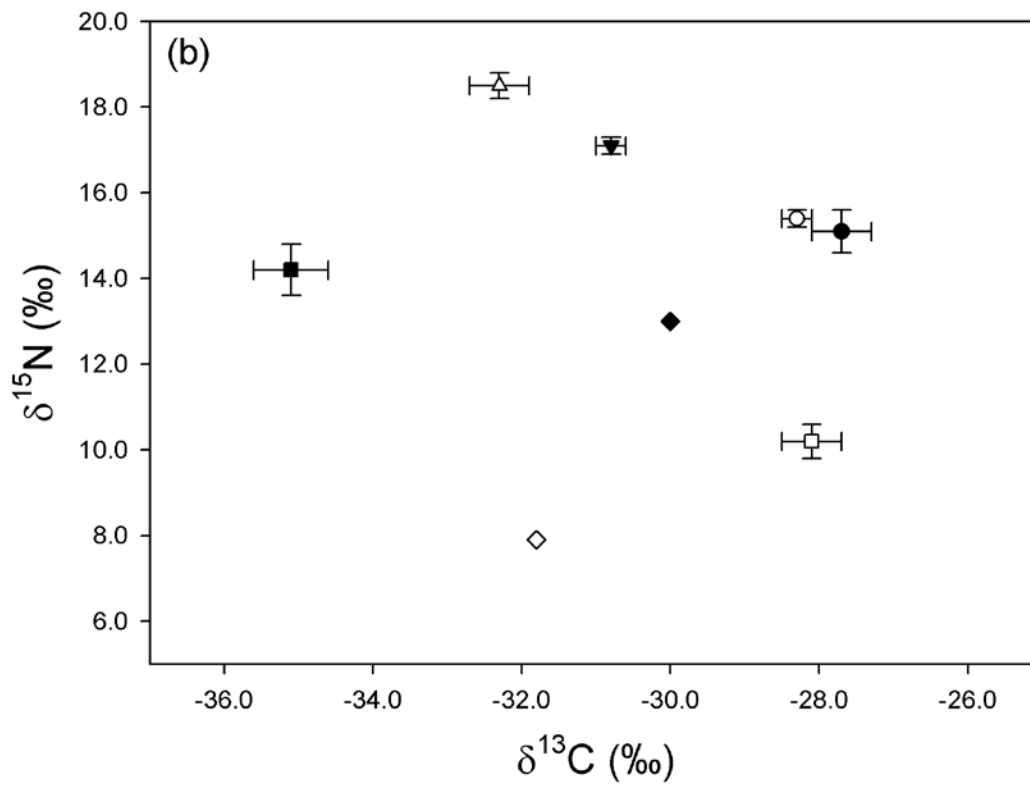
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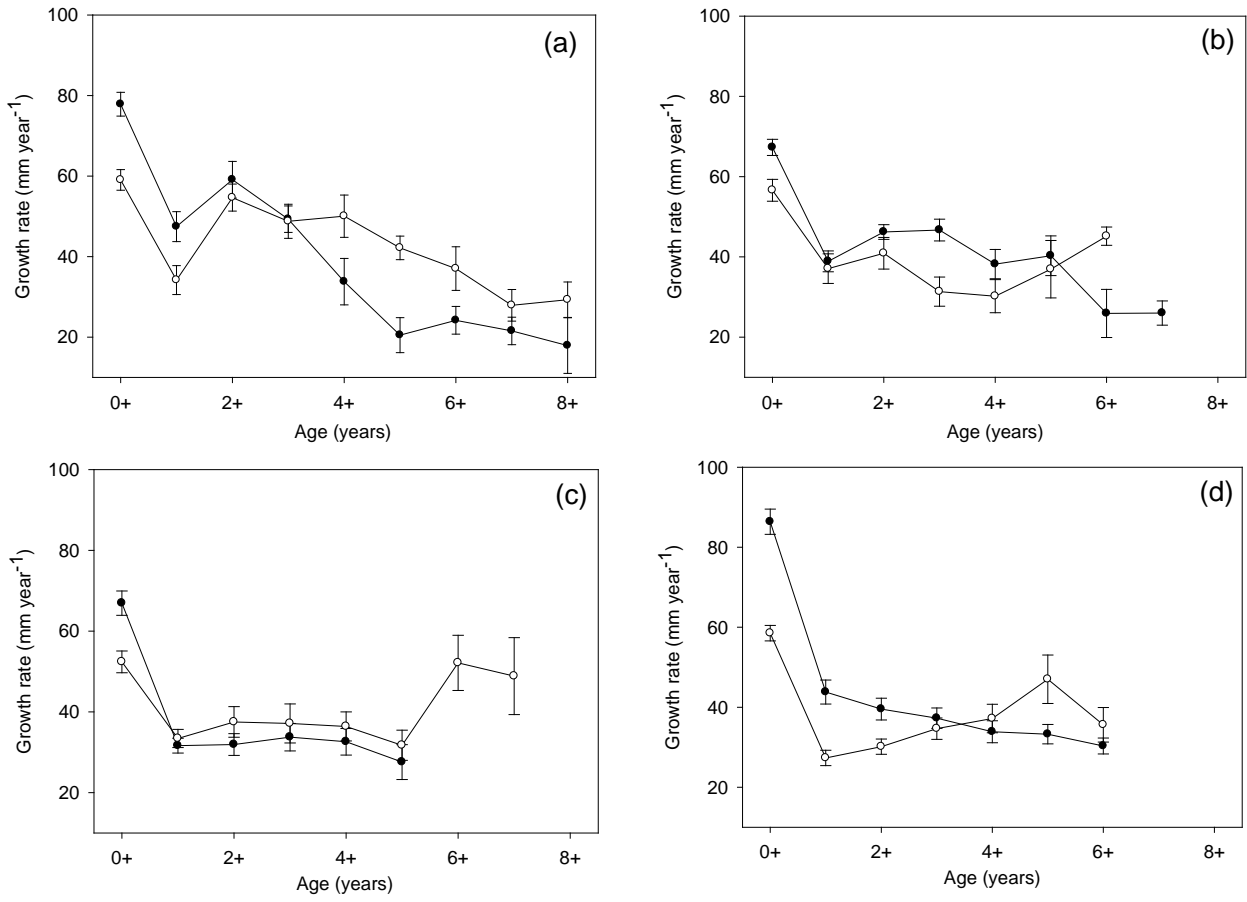
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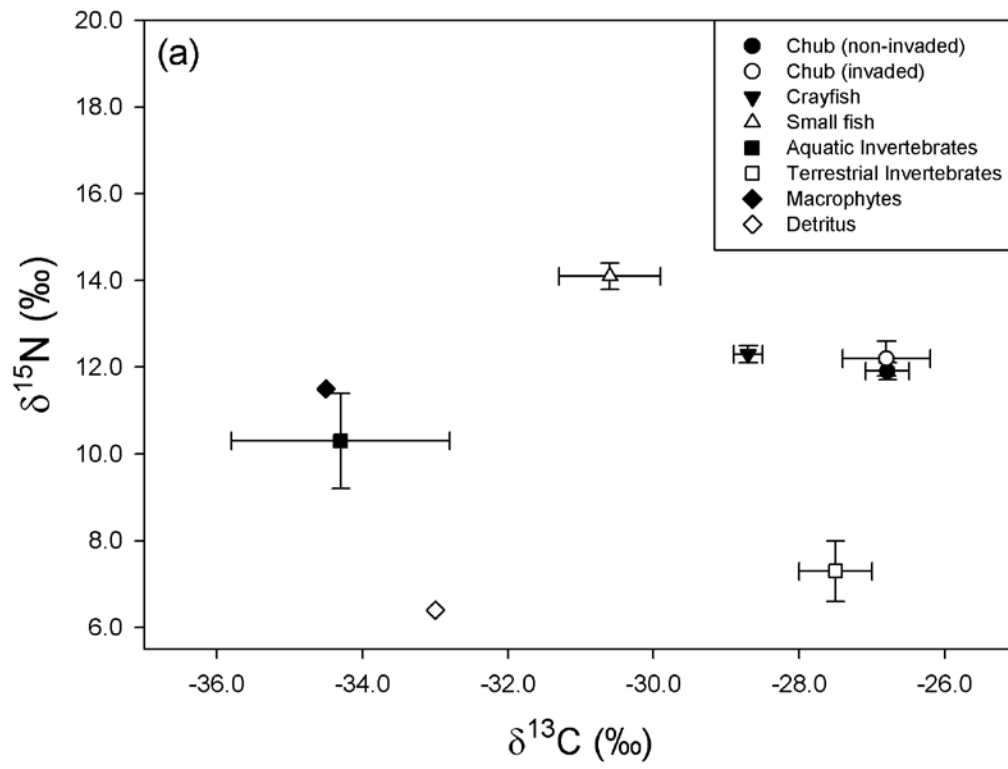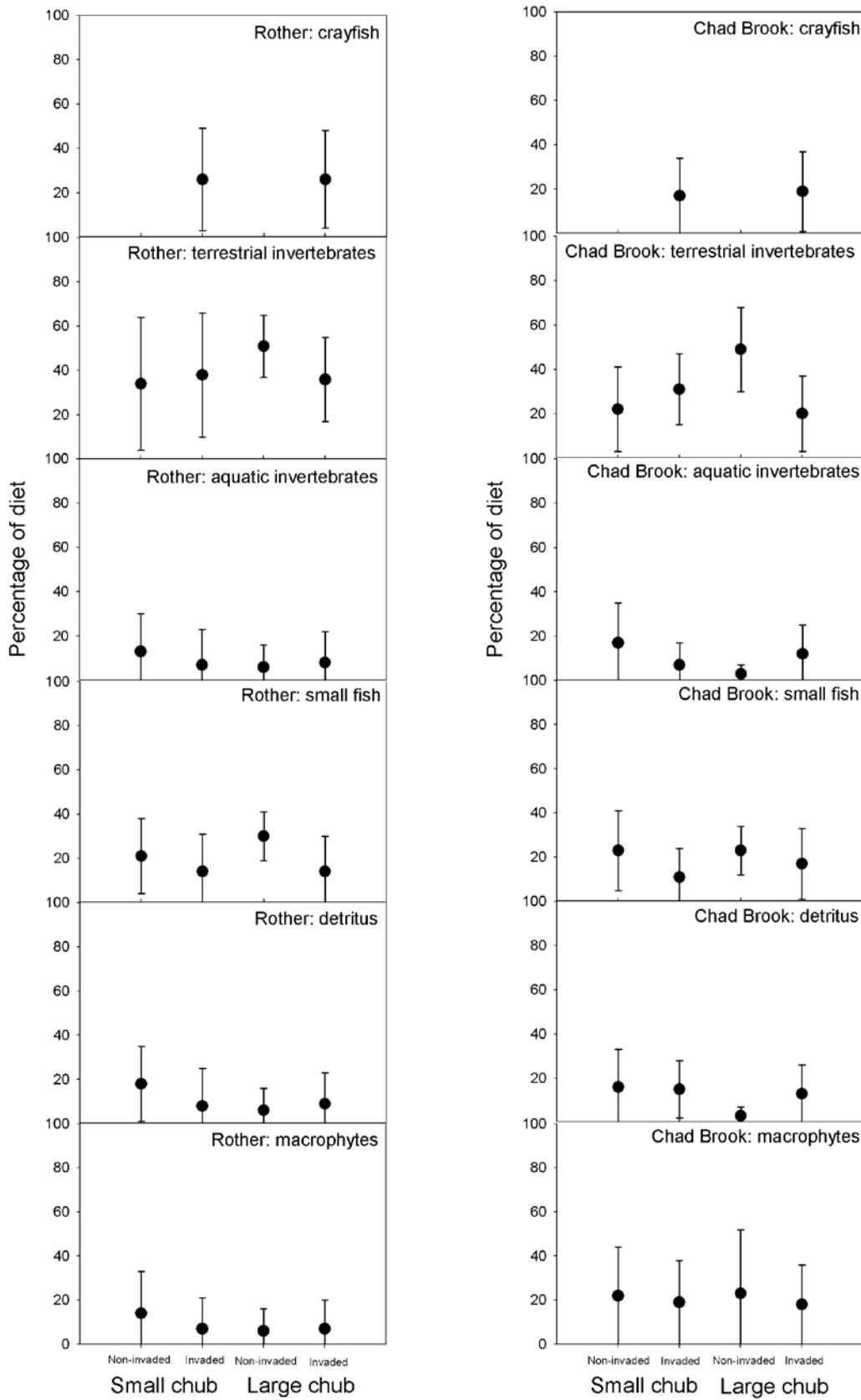
527 **Tables**

Parameter	Evenlode	Cherwell	Chad Brook	Rother
Catchment area (km^2)	430.0	943.0	47.4	346.0
Length (km)	39.5	64.4	14.4	52.0
Mean annual discharge (m^3 s^{-1})	3.8	5.5	0.3	2.3
Dominant land use	Arable and pastoral agriculture	Arable and pastoral agriculture	Arable agriculture	Arable and pastoral agriculture
Year crayfish invasion first detected	2000	1995	2000	1975
Study approach used	Space-for-time	Space-for-time	Before-after	Before-after
Scalimetry used?	Yes	Yes	Yes	Yes
Stable isotope analysis used?	No	No	Yes	Yes
No. chub (non-invaded site)	28	24	21	14
No. chub (invaded site)	40	34	15	18

528

529 Table 1: A summary of key characteristics associated with each of our four study rivers

530 (Environment Agency data, 2008).

531

532

Age class	Factor	Rother	Chad Brook	Cherwell	Evenlode
0+	Site	**$F_{1,30}=11.44; p=0.002$**	**$F_{1,34}=13.94; p=0.001$**	**$F_{1,56}=12.63; p=0.001$**	**$F_{1,66}=62.95; p<0.001$**
	Year	$F_{1,30}=2.84; p=0.103$	$F_{1,34}=2.95; p=0.095$	-	-
1+	Site	$F_{1,30}=1.63; p=0.212$	$F_{1,34}=1.27; p=0.268$	$F_{1,49}=0.36; p=0.552$	**$F_{1,64}=23.43; p<0.001$**
	Year	**$F_{1,30}=5.98; p=0.021$**	$F_{1,34}=2.14; p=0.153$	-	-
2+	Site	$F_{1,30}=1.49; p=0.232$	$F_{1,34}=0.68; p=0.417$	$F_{1,35}=1.42; p=0.241$	**$F_{1,62}=8.51; p=0.005$**
	Year	$F_{1,30}=0.79; p=0.380$	$F_{1,34}=0.34; p=0.564$	-	-
3+	Site	$F_{1,28}=0.06; p=0.803$	$F_{1,32}=3.42; p=0.074$	$F_{1,24}=0.34; p=0.565$	$F_{1,51}=0.50; p=0.483$
	Year	$F_{1,28}=0.27; p=0.610$	**$F_{1,32}=8.63; p=0.006$**	-	-
4+	Site	$F_{1,18}=3.75; p=0.071$	$F_{1,23}=0.42; p=0.524$	$F_{1,15}=0.55; p=0.470$	$F_{1,41}=0.53; p=0.473$
	Year	$F_{1,18}=2.95; p=0.582$	**$F_{1,23}=5.05; p=0.035$**	-	-
5+	Site	**$F_{1,17}=13.96; p=0.002$**	$F_{1,9}=0.13; p=0.725$	$F_{1,13}=0.54; p=0.476$	**$F_{1,35}=6.31; p=0.017$**
	Year	$F_{1,17}=1.32; p=0.269$	-	-	-
6+	Site	**$F_{1,16}=4.76; p=0.047$**	**$F_{1,6}=18.37; p=0.005$**	-	$F_{1,33}=1.49; p=0.230$
	Year	$F_{1,16}=1.66; p=0.218$	-	-	-
7+	Site	$F_{1,16}=0.13; p=0.722$	-	-	-
	Year	$F_{1,16}=0.17; p=0.691$	-	-	-
8+	Site	$F_{1,10}=1.42; p=0.264$	-	-	-
	Year	$F_{1,10}=0.11; p=0.751$	-	-	-

533

534 **Table 2:** The effects of site (invaded versus uninvaded) and year (2008 versus 2011) on chub

535 growth rates, as indicated by linear models. Significant effects are in bold.

536

Model	River	Term	a (± SE)	Test statistic	p	d.f.	R^2adj
$L = aA + aS + aY$	Rother	Full model	-	32.43	<0.001	31	75.3 %
		A	31.06 (± 3.68)	8.44	<0.001	-	-
		S	8.67 (± 20.48)	0.42	0.675	-	-
		Y	-8.80 (± 8.40)	-1.05	0.303	-	-
$L = aA + aS + aY$	Chad Brook	Full model	-	55.80	<0.001	35	82.5 %
		A	28.63 (± 3.16)	9.06	<0.001	-	-
		S	-39.60 (± 10.90)	-3.63	<0.001	-	-
		Y	6.76 (± 4.38)	4.38	0.133	-	-
$L = aA + aS$	Cherwell	Full model	-	507.30	<0.001	203	83.3 %
		A	32.57 (± 1.02)	31.83	<0.001	-	-
		S	-0.71 (± 3.19)	0.22	0.824	-	-
$L = aA + aS$	Evenlode	Full model	-	975.90	<0.001	299	86.7 %
		A	36.48 (± 1.01)	36.09	<0.001	-	-
		S	-41.16 (± 3.32)	-12.40	<0.001	-	-
$M = aA + aS + aY$	Rother	Full model	-	23.22	<0.001	31	68.4 %
		A	135.43 (± 19.46)	6.96	<0.001	-	-
		S	158.98 (± 108.27)	1.47	0.153	-	-
		Y	-46.39 (± 44.39)	-1.05	0.305	-	-
$M = aA + aS + aY$	Chad Brook	Full model	-	55.60	<0.001	35	82.4 %
		A	96.35 (± 11.49)	8.38	<0.001	-	-
		S	-171.11 (± 39.64)	-4.32	<0.001	-	-
		Y	-4.08 (± 15.94)	-0.26	0.800	-	-
$N = aL + aS + aY$	Rother	Full model	-	3.73	0.023	31	20.9 %
		L	0.002 (± 0.002)	0.72	0.479	-	-
		S	-0.457 (± 0.354)	-1.29	0.208	-	-
		Y	0.138 (± 0.188)	0.73	0.469	-	-
$N = aL + aS + aY$	Chad Brook	Full model	-	3.60	0.024	35	18.2 %
		L	0.005 (±0.003)	1.60	0.119	-	-
		S	0.634 (± 0.298)	2.13	0.041	-	-
		Y	-0.199 (± 0.136)	-1.47	0.151	-	-

538

Table 3: The effects of chub age (A), site (S; invaded versus uninvaded) and year (Y; 2008 versus 2011; Rother and Chad Brook only) on chub fork length (L), and mass (M). We also tested the effects of chub fork length, site, and year on chub baseline corrected $\delta^{15}N$ (N) for the Rother and Chad Brook. Test statistics were F and t for full model and individual terms respectively.

543

River	Group	Baseline corrected δ^{15}N (‰)	
		Mean	± 95 % CI
Rother			
Uninvaded	Small chub	4.54	0.48
	Large chub	4.69	0.44
Invaded	Small Chub	5.14	1.27
	Large chub	5.46	0.47
	Crayfish	4.35	0.30
Chad Brook			
Uninvaded	Small chub	5.53	1.36
	Large chub	5.58	0.38
Invaded	Small Chub	4.74	0.26
	Large chub	5.52	0.48
	Crayfish	5.48	0.30

545

546 **Table 4:** The trophic position of chub and crayfish, as measured by the perpendicular distance from

547 a linear regression fitted to six basal resources (sloping isotope baseline) to the consumer δ^{15}N

548 value.