

1 **Ecosystem effects of invasive crayfish increase with crayfish**
2 **density**

3 Running head: **Ecosystem effects of invasive crayfish**

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29 **Abstract**

- 30 1. The nature and extent of effects of increasing densities of non-native species on
31 stream ecosystems remain poorly understood. Non-native crayfish are among the
32 most invasive aquatic species and we hypothesized that, in temperate streams, the
33 extent of trophic modification increases with non-native crayfish density.
- 34 2. Instream flow-through mesocosms in the River Lune, NE England, were used over a
35 47-day period in summer to measure density effects of invasive signal crayfish
36 *Pacifastacus leniusculus* on different ecosystem components (benthic fish,
37 macroinvertebrates, algal biomass and leaf litter breakdown). Effects were measured
38 through three density treatments (low, medium and high; 4, 8, or 12 crayfish with 5
39 bullhead *Cottus perifretum* per 1.5 m²) and two control (crayfish and fish absent;
40 crayfish absent and benthic fish present) groups.
- 41 3. Impacts of crayfish on macroinvertebrates (density, taxonomic composition), fish
42 (growth, diet), algal standing stock and decomposition rates increased with crayfish
43 density. Direct effects of increased crayfish density were more important than indirect
44 trophic cascade effects.
- 45 4. Despite similar invertebrate abundance and richness across enclosures before
46 introducing crayfish and bullhead, they differed significantly from controls at the end of
47 the study, with >80% reduction in macroinvertebrate abundance recorded in the high-
48 density group. Stable isotope ($\delta^{15}\text{N}$, $\delta^{13}\text{C}$) analysis showed that the trophic niche of
49 bullhead, but not signal crayfish, changed when the species were in sympatry.
50 Bullhead in treatment enclosures occupied a lower trophic position in the food web
51 than those from the control group. Bullhead in the high-density group lost 4.2% of body
52 mass over the study period, confirming the existence of resource competition. Leaf
53 litter break down was 59.2% faster, and algal biomass was 91.4% lower in the
54 treatment with the highest crayfish density compared to the control without study
55 animals.
- 56 5. This study indicates that signal crayfish, even at a low density, can strongly alter
57 multiple ecosystem components in streams, and emphasizes the need for minimizing
58 the spread of invasive crayfish within and between streams.

59

60 **Keywords:** Biological invasion, ecosystem processes, enclosure-exclosure, non-native
61 species, stable isotopes, trophic cascade

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64 **1. INTRODUCTION**

65 Biological invasion by non-native species is currently one of the major anthropogenic threats
66 to global biodiversity (Gallardo *et al.*, 2016; Early *et al.*, 2016; Pyšek *et al.*, 2020). Invasive
67 species can affect native species and ecosystems in multiple ways including by alteration of
68 food web structure, by decreasing species richness and by reducing the number of links per
69 species (Gherardi *et al.*, 2009; Galiana *et al.*, 2014). In doing so, they pose a threat to
70 ecosystem integrity and functioning (Olden *et al.*, 2004; Carbonell *et al.*, 2017). However,
71 invasion outcome is difficult to predict (Sahlin *et al.*, 2010) and depends on the properties of
72 invading species and biotic and abiotic components of the recipient ecosystems (Lodge *et al.*,
73 2006). Diverse, less-stressed communities are less vulnerable to invading organisms as they
74 support more niche overlaps or competitive interactions (Maestre *et al.*, 2009). Despite large
75 numbers of studies on invasion biology, for most invaders, specific knowledge of the ecological
76 impacts and their underpinning mechanisms remains limited (Rodriguez, 2006; Jackson *et al.*,
77 2014; van Kuijk *et al.*, 2021) and further investigation is needed, especially in river systems
78 (van Kuijk *et al.*, 2021).

79 Many invaded ecosystems support native species which are ecologically or functionally similar
80 to the invading species (Gallardo *et al.*, 2016). Understanding the interactions between these
81 species is important (Carbonell *et al.*, 2017) because they may have important consequences,
82 including cascading effects through community and ecosystem modification (Walsh,
83 Carpenter & Vander Zanden, 2016). The outcome of interactions between native and non-
84 native species is determined by the degree of niche (resources) overlap between competitors
85 (De Roos *et al.*, 2008) and the weaker competitors decline and face extinction threat (Reitz &
86 Trumble, 2002; Weber & Strauss, 2016). Invasive species often exhibit wider niche
87 characteristics and environmental tolerance than native species (Mack *et al.*, 2000). They can
88 be a threat to native species and ecosystems directly, and/or indirectly, through the trophic
89 cascade whereby a consumer affects non-adjacent trophic levels through alteration of prey
90 abundance and/or behaviour and results in an indirect effect on subsequent trophic levels
91 (Threlkeld, 1988; Ousterhout *et al.*, 2018). In an invaded habitat, invasive species can create
92 new trophic links and can also modify or disrupt existing ones (Carvalho *et al.*, 2016; Jackson
93 *et al.*, 2017). Effects of trophic cascade can be severe on aquatic ecosystems (Carpenter,
94 Kitchell & Hodgson, 1985; Walsh *et al.*, 2016; Ousterhout *et al.*, 2018). Therefore, to
95 understand the degree of competitive interaction between invasive species and other
96 functionally similar native species, it is important to study their resource use.

97 The abundance of invasive species affects the process of biological invasion (Bradley *et al.*,
98 2019) and higher interspecific competition may be expected where population density is high

99 (Muñoz & Cavieres, 2008). Density-related impacts of several freshwater invasive species on
100 ecosystem components have been studied (e.g. impacts of - Topmouth Gudgeon
101 *Pseudorasbora parva* on invertebrates and ecosystem processes in an outdoor pond
102 mesocosm, Jackson *et al.*, 2015; crayfish *Faxonius virilis* on water quality and macrophytes
103 in an experimental ditch, Roessink *et al.*, 2017; crayfish *Pacifastacus leniusculus* on brown
104 trout *Salmo trutta* fry, invertebrates and algae, Stenroth and Nyström, 2003). The latter
105 examined the impacts on multiple ecosystem components in flowing water, but did so in a
106 lime-treated stream. Moore *et al.* (2012) also examined density impacts of *P. leniusculus* on
107 invertebrates, algae and leaf litter decomposition but did so using isolated stream pools during
108 summer low flows.

109 Crayfish are one of the most important groups of invasive species in freshwater, widely known
110 for their impacts on fauna and flora (Twardochleb, Olden & Larson, 2013) and therefore,
111 considered a model invasive animal to study (van Kuijk *et al.*, 2021). Their impacts on
112 ecosystem processes (e.g. primary productivity and leaf litter decomposition: Charlebois and
113 Lamberti, 1996; Jackson *et al.*, 2014), and on biota, including amphibians (Axelsson *et al.*,
114 1995; Gamradt & Kats, 1996), fishes (Findlay, Riley & Lucas, 2015; Wood *et al.*, 2017), various
115 bivalves (Machida & Akiyama, 2013; Meira *et al.*, 2019) and other macroinvertebrates
116 (Nyström, Brönmark & Granéli, 1996; Mathers *et al.*, 2016, 2020) have been studied, mostly
117 in laboratory environments. Nevertheless, our understanding is limited with regard to how
118 crayfish density affects multiple ecosystem components in natural habitats. This is also
119 because of unpredictable impacts of omnivorous species like crayfish due to their broad diets,
120 behavioural flexibility and diverse abiotic / biotic factors in invaded habitats (Klose & Cooper,
121 2012). As the density of invasive crayfish in stream habitats can be very high, often exceeding
122 10 crayfish/m² (Guan & Wiles, 1997; Bubb *et al.*, 2009; Chadwick *et al.*, 2021; Galib, Findlay
123 & Lucas, 2021; Galib *et al.*, 2022), such findings may help improve our understanding of their
124 environmental impacts.

125 Mesocosms are valuable experimental methods for determining the effects of invasive species
126 under replicated and semi-controlled conditions (Stewart *et al.*, 2013). Mesocosm studies of
127 invasive crayfish have evaluated impacts of *P. clarkii* on macrophytes, macro-crustaceans and
128 macroinvertebrates (Rodríguez-Pérez, Hilaire & Mesléard, 2016; Sousa *et al.*, 2019); *P.*
129 *leniusculus* on trout, invertebrates and algae (Stenroth & Nyström, 2003) and interaction
130 among different non-native crayfish species (signal crayfish, *P. leniusculus*; virile crayfish, *F.*
131 *virilis*; red swamp crayfish, *P. clarkii*; and Turkish crayfish, *Pontastacus leptodactylus*) and
132 their impacts on benthic invertebrate communities (Jackson *et al.*, 2014) but only Stenroth and
133 Nyström (2003) examined density effects on multiple ecosystem components in a stream
134 setting. In this study, the impact of non-native signal crayfish *Pacifastacus leniusculus* on

135 macroinvertebrates, benthic fish and ecosystem processes (leaf litter decomposition and algal
136 standing crop) were measured through field enclosure experiments.

137 The following hypotheses were tested: (i) signal crayfish exert strong effects on stream
138 communities in terms of ecological processes, community structure, biomass and food webs.
139 It was predicted that these effects on different ecosystem components would be larger with
140 increasing density of signal crayfish; (ii) signal crayfish impact native benthic fish through
141 interference competition, negatively affecting their growth through food web alteration. Thus,
142 density effects on trophic niche and growth of benthic fish were predicted in response to
143 varying densities of signal crayfish.

144 **2. METHODS**

145 **2.1 Study site and focal species**

146 This study was conducted in the River Lune, an upland limestone tributary of the River Tees
147 in Northeast England (54°37'09.6"N 2°03'19.8"W to 54°37'13.0"N 2°03'09.4"W), invaded by
148 signal crayfish in 2013 (Galib *et al.*, 2021). We chose an existing recently invaded site as it
149 was not ethically feasible to carry out such a study in a non-invaded area, due to the risk of
150 escape of non-native crayfish and pathogen spread. The study site comprised a 350-m long,
151 ~10-m wide channel incorporating glide and riffle mesohabitats with natural substrate (mostly
152 cobble, boulder and gravel), ~0.2–0.5-m deep at base flows. The stream is bordered by
153 pasture with a narrow riparian zone of native broadleaved trees, while instream flora is
154 dominated by benthic microalgae, with patches of water moss *Fontinalis* sp. The invertebrate
155 community is typical of British upland rhithral environments, and includes abundant
156 Ephemeroptera, Plecoptera and Trichoptera, while the fish fauna is dominated by salmonids
157 (*Salmo trutta*, *S. salar*) and benthic fishes, particularly bullhead *Cottus perifretum* (formerly
158 known as *C. gobio* in the UK). Like signal crayfish, bullhead use and compete for benthic
159 refuges in streams (Bubb *et al.*, 2009) and so were chosen as a focal species through which
160 to examine effects of crayfish on trophic niche and growth of a potential competitor. Bullhead
161 are principally carnivorous, specialising on benthic invertebrates (Dahl, 1998), whereas signal
162 crayfish are more omnivorous (Stenroth & Nyström, 2003).

163 **2.2 Experimental design**

164 Twenty five 5-mm mesh enclosures (1.5-m long × 1-m wide × 0.7-m height = 1.5 m² area),
165 each with natural sediment, were affixed to the stream bed (see Method S1 for details) in glide
166 habitat, suitable for crayfish and bullhead. The sediment introduced to the enclosures was
167 carefully sorted to ensure it did not contain fish or crayfish (see Method S1 for details). The
168 mesh size enabled passage through and colonisation by small (and early instars of)

169 invertebrate species, but not crayfish and fish. A period of 27 days enabled post-installation
170 enclosure conditioning and macroinvertebrate colonisation. Subsequently, the experiment
171 was conducted over 47 days, between 20 July and 31 August 2018. Five different
172 experimental groups, comprising two controls (controls, without native fish or signal crayfish;
173 fish controls, native fish only) and three treatments (low-, medium-, and high-density
174 treatments; with varying densities of signal crayfish and a fixed density of native benthic fish),
175 each with five replicates, were employed. All control and treatment groups were replicated in
176 five randomised complete blocks, installed within the study reach. Position of enclosures
177 belonging to different groups was assigned randomly within each block. Five bullhead per
178 enclosure were used in fish controls and treatment groups. Densities of signal crayfish (25 –
179 30 mm carapace length; $p > 0.05$ across treatment groups) were 4, 8 and 12 in low-, medium-
180 and high-density treatment groups respectively (see Method S2 for crayfish and bullhead
181 collection methods).

182 Densities of crayfish used in this experiment reflected the range commonly observed in
183 Northern England (up to 24 crayfish m^{-2} , exceptionally up to 110 crayfish m^{-2} ; Table S1). Equal
184 numbers of male and female crayfish were used per enclosure. Bullhead density reflected the
185 natural density in English rivers (Table S1). Similar sized bullhead (70.4 ± 3.6 mm; 4.4 ± 0.8
186 g; LMM, $p > 0.05$ across groups) were used to avoid any size and biomass-biased results.
187 Fish and crayfish were collected from the River Lune through electrofishing and hand-net
188 searching respectively and individually marked using Visible Implant Elastomer (VIE;
189 Northwest Marine Technology, Inc., USA) (see Method S2).

190 **2.3 Macroinvertebrate sampling**

191 In order to determine macroinvertebrate taxonomic richness, abundance and community
192 structure before introducing fish and crayfish to the enclosures, macroinvertebrate samples
193 were collected from each of the enclosures ($n = 3$ per enclosure) using a 0.1- m^2 Surber
194 sampler 14 days before the start of the experiment. Collection was repeated, by the same
195 method, on the final day of experiment. Invertebrate samples were preserved using 70%
196 ethanol solution and identified to family level under a low power microscope. Functional
197 feeding groups of macroinvertebrates were based on Cummins (2019).

198 **2.4 Recapture and stable isotope analysis**

199 On the final day of the experiment, crayfish and bullhead were collected from each enclosure,
200 counted, identified by VIE marks, and length and mass were remeasured. All crayfish ($n =$
201 120) and a proportion of bullhead (60%, $n = 15$ from each treatment/control) were euthanized,
202 brought back to the laboratory on ice and stored at $-20^{\circ}C$ prior to stable isotope analysis.

203 In order to determine trophic position and niche breadth of signal crayfish at different densities,
204 stable isotope analysis (SIA) of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) in tissues was carried out.
205 We used muscle tissue from abdominal somites for signal crayfish and lateral musculature for
206 bullhead due to their sufficiently fast turnover rate (Bondar *et al.*, 2005; Jackson *et al.*, 2014)
207 and the timescale of our experiment (*cf.* Jackson *et al.*, 2014). Tissue samples were collected
208 through dissections of thawed samples, dried at 60°C in an oven for 24 h then pulverised using
209 an agate mortar and pestle. SIA were performed on 45 crayfish (three per enclosure) and 60
210 bullhead (three per enclosure) in the Stable Isotope Biogeochemistry Laboratory (SIBL),
211 Durham University. Approximately even sex ratio of crayfish was maintained (22 male, 23
212 female) during selection of crayfish for SIA. Three bullhead and five signal crayfish collected
213 from outside the enclosure in the study locality (hereafter free-living group) at the end of
214 experiment were also analysed.

215 Individual benthic macroinvertebrates belonging to different families which were potential prey
216 for bullhead and signal crayfish and represent different dietary guilds in the food web were
217 collected from the study site at the end of the experiment for SIA. Samples of Chironomidae
218 (principally members of the tribe Chironomini), Baetidae, Gammaridae, Heptageniidae,
219 Rhyacophilidae and Hydropsychidae ($n = 5$ for each) were prepared as above.

220 Since crayfish are omnivorous, SIA of several plant materials were included. At the field site,
221 periphyton samples were scraped off rocks with a nylon brush in deionised water, collected
222 and taken to the laboratory for analysis. Samples were centrifuged with deionised water and
223 oven-dried prior to homogenisation (Bondar *et al.*, 2005). Samples of in-stream leaf litter, fallen
224 riparian tree leaves (common alder, *Alnus glutinosa*; common oak, *Quercus robur*) used in the
225 enclosure, and in-stream organic debris were collected, and dried for 72 h at 60°C, followed
226 by homogenisation. Five samples from each group were considered for SIA. Terrestrial
227 invertebrates were not considered as potential dietary items because they did not appear in
228 Surber-sampled invertebrates. Moreover, both signal crayfish and bullhead tend to feed on
229 benthic prey rather than floating or drifting ones (Dahl, 1998).

230 **2.5 Determination of algal biomass and leaf-litter decomposition**

231 At the time of enclosure deployment, a clean 10×10 cm unglazed ceramic tile was added into
232 each enclosure to quantify periphyton standing stock. At the end of the experiment, tiles were
233 removed, biofilms were collected into darkened plastic bottles by gently brushing the tiles with
234 a clean toothbrush in deionised water. Samples were stored on ice, transported to the
235 laboratory and stored at -20°C. Chlorophyll- α concentration from the samples was determined
236 spectrophotometrically (following Jeffrey & Humphrey, 1975; see Method S3).

237 A mesh pack of 10 mm aperture, filled with 3 g of dried oak leaf-litter, was added to each
238 enclosure to measure breakdown rates (after Woodward et al., 2008). The mesh packs were
239 allowed to condition in the enclosures for 2 weeks prior to the start of the experiment (Bondar
240 *et al.*, 2005). On the final day of the experiment, all leaf litter was removed from each mesh
241 pack and placed into labelled zip-lock bags. In the laboratory, macroinvertebrates were
242 separated from the leaf litter samples which were dried to constant mass at 60°C. Breakdown
243 rate was calculated as percentage dry mass loss per day (61 days in total; 14 days of
244 conditioning, 47 days of experiment). It was assumed that the loss of leaf litter before
245 introducing crayfish/bullhead was comparatively minimal and similar across enclosures.

246 **2.6 Water quality parameters**

247 During the experiment, water level and temperature were recorded every 15 minutes using a
248 logger (Hobo, Onset Computer Corporation, MA, USA). Water depth, water temperature, pH,
249 dissolved oxygen (DO) and flow velocity within each enclosure were recorded weekly,
250 between 10.00 and 12.00 h on each sampling day. Mean water temperature during the study
251 period was $14.6 \pm 1.1^\circ\text{C}$ (range: $11.9 - 17.7^\circ\text{C}$). No high-flow event occurred during the study
252 period and the mean water level was 0.46 ± 0.1 m.

253 **2.7 Data analysis**

254 All analyses were performed in statistical software R (version 3.4.3; R Core Team, 2017).
255 Linear Mixed-Effects Modelling (LMM) was employed to analyse repeated measures
256 macroinvertebrate richness and abundance data using the 'lme4' package (Bates *et al.*, 2015)
257 and 'lmerTest' (Kuznetsova, Brockhoff & Christensen, 2016) packages. During analysis,
258 experimental groups ($C_1 - C_2$ and $T_1 - T_3$), time (before and after) and their interaction (group
259 \times time) were considered fixed effects and replications (enclosure ID), nested within
260 experimental blocks, were considered a random effect. To determine the dissimilarities among
261 macroinvertebrate communities across groups, time, and their interaction, a Permutational
262 Multivariate Analysis of Variance (PERMANOVA) was carried out using the 'vegan' package
263 (Oksanen *et al.*, 2018).

264 Similarity Percentage Analysis (SIMPER), based on decomposition of Bray-Curtis dissimilarity
265 index (Clarke, 1993) was used to determine the average percent dissimilarity over time (before
266 vs. after) and condition (control and treatment groups) and to identify the contribution of
267 macroinvertebrate families, belonging to each experimental group, responsible for average
268 dissimilarity between 'before' and 'after' communities.

269 Body mass change of bullhead between groups was compared using LMM as outlined above.
270 A nested analysis of variance (ANOVA) was used to analyse algal biomass and leaf-litter
271 breakdown to determine the effects of crayfish density by comparing control and treatment
272 groups. Nested ANOVA was performed by defining 'experimental block' as a random effect in
273 the model. Post-hoc comparisons of the mean values of control and treatments groups were
274 obtained using the 'multcomp' package (Hothorn, Bretz & Westfall, 2008). For comparison
275 between groups, the standardised effect size, Hedges' g (Hedges, 1981), was calculated by
276 using the 'effsize' package (Torchiano, 2018). Physico-chemical properties of water were
277 analysed using LMM outlined earlier. Before analysis, data were checked for normality by
278 Shapiro–Wilk test (Peat & Barton, 2005) and necessary transformations (square-root for
279 macroinvertebrate abundance data, McDonald, 2014; and $\log(x + 1)$ for water quality data,
280 Clarke, 1993) were made to meet assumptions for the test.

281 To analyse crayfish and bullhead diet, Stable Isotope Mixing Models (SIMMs), using the
282 "simmr" package (Parnell *et al.*, 2010) were applied. Diet-Tissue Discrimination Factor (DTDF;
283 see Method S4) values were added to the food source isotope values before SIMM analysis
284 (Phillips *et al.*, 2014). Nitrogen and carbon isotopic values of crayfish and bullhead groups
285 were compared using LMMs in which groups were tested as a fixed effect and crayfish sources
286 (i.e. enclosure IDs and free-living) as a random effect. As two isotopes ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) were
287 considered in this study, only up to three prey sources ($n + 1$, where n is the number of isotope
288 analysed) can be used in SIMMs to calculate a unique solution for prey sources (Phillips &
289 Gregg, 2003). Therefore, an *a priori* aggregation approach was used whereby source data
290 (isotopic values) were plotted and similar sources forming clusters were grouped before
291 analysis (Phillips *et al.*, 2014). Potential food sources for bullhead include different families of
292 macroinvertebrates, bullhead eggs, newly-hatched bullhead and signal crayfish (Western,
293 1969; Copp, Warrington & De Bruine, 1994; Dahl, 1998). Only different families of
294 macroinvertebrates were considered during modelling as no smaller signal crayfish and
295 bullhead eggs or larvae were recorded from enclosures during Surber sampling. During
296 modelling of bullhead diet, macroinvertebrate families were assigned to three groups based
297 on their feeding guilds and $\delta^{13}\text{C}$ values after examining pairwise comparisons of
298 macroinvertebrate families (Ben-David *et al.*, 1997b; Ben-David, Flynn & Schell, 1997a;
299 Phillips, Newsome & Gregg, 2005). These groups were as follows: chironomids
300 (Chironomidae, dominated by the tribe Chironomini); grazers and shredders (Gammaridae,
301 Hydropsychidae, Heptageniidae and Baetidae); predatory caddis (Rhyacophilidae). For
302 modelling of signal crayfish diet, leaf litter (with debris) and algae were also considered due to
303 the omnivorous feeding nature. For crayfish, in order to reduce the number of potential prey

304 groups to three all macroinvertebrates were treated as a single group (Phillips *et al.*, 2005;
305 Fry, 2013; Petitet & Bugoni, 2017) (see Method S4).

306 The C:N ratios of animal tissue samples (crayfish, 3.94 ± 0.09 ; bullhead, 4.10 ± 0.17 ;
307 macroinvertebrates, 6.87 ± 2.38) exceeded 3.5, indicating that the amount of lipid present in
308 tissues may negatively affect $\delta^{13}\text{C}$ values, but not $\delta^{15}\text{N}$ values (Logan & Lutcavage, 2008;
309 Skinner, Martin & Moore, 2016). Tissue-specific lipid correction models were applied to correct
310 $\delta^{13}\text{C}$: for muscle, $\delta^{13}\text{C}_{\text{lipid-free}} = \delta^{13}\text{C}_{\text{bulk}} - 5.16 + 4.527 \ln(\text{CN ratio})$; for macroinvertebrates,
311 $\delta^{13}\text{C}_{\text{lipid-free}} = \delta^{13}\text{C}_{\text{bulk}} - 2.056 + 1.907 \ln(\text{CN ratio})$ (Logan *et al.*, 2008).

312

313 **3. RESULTS**

314 None of the physico-chemical properties differed between experimental groups during the
315 study period (Table S2). Therefore, it is assumed that there were no impacts of water quality
316 on the study results.

317 **3.1 Macroinvertebrates**

318 Before introducing study animals, mean (\pm SD) taxonomic richness of macroinvertebrate
319 varied from 11.8 ± 2.9 (in high-density group) to 12.4 ± 3.3 (in medium-density group) families
320 ($p > 0.05$ across groups; Table S3, Figure 1). Mean macroinvertebrate abundance ranged
321 between 176.6 ± 54.9 per 0.3 m^2 Surber area (in fish control group) and 187.4 ± 54.1 (in
322 medium-density group) and did not vary across groups. At the end of the experiment these
323 values varied from 3.8 (high-density) to 12.4 (controls) for family richness and 35.8 (high-
324 density) to 180.4 (controls) individuals per 0.3 m^2 Surber for abundance. Strong effects of
325 groups, time and their interaction were recorded for taxonomic richness ($F = 29.3 - 113.5$, all
326 $p < 0.001$), abundance ($F = 58.1 - 413.1$, all $p < 0.001$) and community structure ($F = 1.8 -$
327 10.6 , all $p < 0.003$) (Table 1; Table S4; Figure 2). Crayfish density effects were evident across
328 most groups (Table S3). However, at the end of study there was no difference between the
329 two control groups for family richness and abundance (Table S4). Neither was there any
330 difference in family richness between the fish control and low crayfish density treatment, nor
331 the low density - medium density groups (all $p > 0.05$; Table S4).

332 At the end of the study a dramatic decrease in the abundance of macroinvertebrate families
333 was recorded in treatment groups (Table 2). SIMPER results showed that no
334 macroinvertebrate families differed between before and after situations in control groups (all
335 $p > 0.05$; Table 2). The common shredder group Gammaridae declined significantly in
336 abundance over time in the presence of crayfish (all $p < 0.05$; Table 2).

337 Comparing macroinvertebrate community differences by abundance of families at the end of
338 the study, these were similar in both control groups (controls and fish controls) and no
339 difference was recorded in the relative abundance of any of the families between the two
340 controls (Table S5). In the low-density group, when compared to fish controls, the abundance
341 of six families were significantly lower (Table S6) whereas this figure was eight and 15 families
342 for medium-density and high-density groups respectively (Tables S7, S8). Similar differences
343 of macroinvertebrate families were found for crayfish treatment groups when compared to fish
344 controls in which the abundance of four, five and 11 families decreased significantly in low-,
345 medium- and high-density groups respectively (Tables S9 – S11).

346 **3.2 Bullhead growth**

347 Despite no difference in initial body mass of bullhead across treatment groups (LMM: $F = 0.74$,
348 $p = 0.529$) they varied at the end of the study ($F = 3.86$, $p = 0.012$). Bullhead growth rate
349 decreased with increasing crayfish density (linear regression: $t = -2.8$, $p < 0.001$; Figure 1).
350 Bullhead in the high-density group lost mass (negative growth) by 0.18 ± 0.36 g. At the end of
351 the experiment, one bullhead was missing from each of two high-density enclosures.

352 **3.3 Stable isotope analysis**

353 Separation between the isotopic niche spaces of signal crayfish and bullhead ($7.11 \pm 0.46\text{‰}$
354 and $8.93 \pm 0.27\text{‰}$ respectively; Figure 3) was evident, where bullhead occupied a higher
355 trophic position than crayfish. For crayfish there were no differences in isotopic values
356 between males and females (t -tests: both $p \geq 0.440$) and no difference across treatment
357 groups (both $p \geq 0.679$). Nonetheless, both isotope values in bullhead differed significantly
358 among groups (LLMs: $\delta^{15}\text{N}$ $F = 7.15$, $p < 0.001$; $\delta^{13}\text{C}$ $F = 8.75$, $p < 0.001$). Significantly lower
359 $\delta^{15}\text{N}$ values occurred in bullhead from the highest crayfish density compared to other groups
360 (Table S12). Higher $\delta^{15}\text{N}$ values occurred in bullhead from fish controls (mean \pm SD, $9.12 \pm$
361 0.16‰) and the free-living group ($9.12 \pm 0.17\text{‰}$) than from crayfish treatment groups (high–
362 low densities; $8.68 - 8.97\text{‰}$) (Figure 3). For $\delta^{13}\text{C}$, bullhead from both fish controls and the
363 free-living source differed from those in all treatment groups (all $p < 0.05$; Table S12).

364 Mixing models indicated that there was negligible change in consumption of different prey
365 items across treatment groups for crayfish (all $p > 0.05$) whereas significant changes were
366 evident for bullhead (Table 3, Figure 4). Macroinvertebrates dominated crayfish diets, with
367 mean contributions of $75.9 - 78.1\%$ of the total diet amount followed by leaf litter / debris (17.4
368 $- 18.9\%$) and algae ($4.4 - 5.2\%$; Table 3). For bullhead, consumption of predatory caddis
369 declined significantly with increasing crayfish density. In the fish control group predatory
370 invertebrates comprised about 50% of bullhead diet whereas it reduced to about 40% in the

371 high-density group ($p < 0.05$). Consumption of chironomid larvae increased significantly, from
372 37.9% in the control with fish to 44.9 – 49% in the low–high density groups ($p < 0.05$; Table 3,
373 Figure 4).

374 **3.4 Algal standing stock and leaf-litter decomposition**

375 The highest chlorophyll- α level (reflective of algal biomass) at the end of the study was
376 recorded in crayfish-free controls (mean \pm SD: 13.1 ± 2.7 mg ml⁻¹) whereas the lowest
377 chlorophyll- α level (1.1 ± 0.4 mg ml⁻¹) was recorded in the high-density group. The highest
378 and lowest rates of daily loss in leaf litter were recorded in the high-density (0.042 ± 0.005 g)
379 and control (0.026 ± 0.004 g) groups respectively (Figure 5). Differences in algal biomass and
380 leaf litter breakdown between groups were evident (both $p < 0.001$). Post-hoc tests confirmed
381 differences between control and treatment groups (Figure 5, Table S13) but not between the
382 two control groups. Small to large effect sizes were found between all control and treatment
383 comparisons, indicating strong influences of crayfish on algal biomass and leaf litter
384 breakdown (Table S13).

385

386 **4. DISCUSSION**

387 This study reveals the pervasive density-related impacts of signal crayfish on stream ecology,
388 comprising negative effects on macroinvertebrates, native benthic fish and algal biomass but
389 increased leaf litter breakdown, at densities of crayfish and fish commonly found in invaded
390 rivers. These impacts are likely to be sufficient to disrupt food webs in invaded rivers, not least
391 because of the high densities that invasive crayfish can occur in temperate watercourses,
392 including upland streams (Chadwick *et al.*, 2021). Our study results differed for native fish and
393 algal biomass results from the only other study of the effects of density of invasive crayfish
394 on multiple ecosystem components in natural habitat by Stenroth and Nyström (2003) and
395 reveals new insights into the impact mechanisms. The changes recorded in this study were
396 not because of water quality parameters but due to signal crayfish, as no significant variation
397 was recorded in any of the water quality parameters across enclosure groups.

398 **4.1 Impacts on macroinvertebrates**

399 Signal crayfish are well known for their effects on macroinvertebrate communities (Nyström *et*
400 *al.*, 1996; Mathers *et al.*, 2016, 2020; Galib *et al.*, 2021). This was evident in this study where
401 decreasing trends of macroinvertebrate abundance and taxonomic richness were recorded in
402 relation to increasing crayfish density. The highest macroinvertebrate richness and abundance
403 were recorded in the control group without signal crayfish and bullhead, and did not change

404 over the course of the experiment. This indicates that the changes in macroinvertebrate
405 taxonomic richness and abundance treatment groups were a result of crayfish and bullhead.
406 Although bullhead are important invertebrate predators (Dahl, 1998) in streams, in our
407 experiment they caused no change in invertebrate abundance, taxonomic richness and only
408 marginal change in community structure. Their foraging, at the density considered in this study
409 (five individuals per 1.5 m²), seems not to have been sufficient to drive changes, as although
410 both richness and abundance reduced to some extent in fish controls, these changes were
411 not significantly different from control group. The 5-mm mesh size will have facilitated
412 macroinvertebrate drift (at least of smaller instars and taxa) into, and continuous colonisation
413 of, the enclosures and offset the impacts of bullhead predation.

414 Heptageniidae contributed the highest proportion (~15%) to the overall difference between
415 before and after communities in treatment enclosures, followed by Chironomidae,
416 Gammaridae, Simuliidae and Baetidae. Gammaridae were significantly reduced in crayfish
417 treatment groups suggesting a strong effect on the shredder community. This reduction in
418 abundance of Gammaridae may be due to both predation and bioturbation by crayfish (Usio
419 & Townsend, 2004). The abundance of some large invertebrates (e.g. Rhyacophilidae and
420 Hydropsychidae) declined in treatment groups. Crayfishes (e.g. *P. clarkii*; Klose and Cooper
421 2012; signal crayfish, Stenroth and Nyström, 2003) prefer to eat larger invertebrates, so it is
422 possible that in our study these taxa experienced higher predation pressure than other
423 macroinvertebrates. Rhyacophilidae and Hydropsychidae are also slow-moving, soft-bodied
424 taxa which may be susceptible to crayfish predation.

425 **4.2 Bullhead–signal crayfish interactions**

426 In this study signal crayfish altered bullhead's assimilation of foods without affecting their own
427 trophic position, as SIMMs showed that there was almost no change in crayfish diet across
428 groups, even at the highest density. Bullhead growth was negatively affected by crayfish
429 density. Moreover, bullhead from the high-density group occupied a lower trophic niche and
430 with higher carbon isotope compared to other groups. Taken together, these results indicate
431 a high level of competition, potentially both exploitative and interference (Carpenter, 2005),
432 between signal crayfish and bullhead in which the latter was competitively inferior. This is a
433 similar situation to that for the benthic fish Paiute sculpin *Cottus beldingi*, when sympatric to
434 invasive signal crayfish (Light, 2005). Nonetheless, Stenroth and Nyström (2003) found that
435 native fish (brown trout, *S. trutta fry*) remained unaffected by crayfish in their density effects
436 experiment in a natural stream, which may be due to differences in their behaviour and ecology
437 (brown trout is a relatively fast swimming midwater drift feeder, that uses benthic structure
438 much less frequently than do sculpins).

439 Outputs of SIMMs revealed that bullhead in treatment enclosures consumed less large prey
440 occupying a higher trophic level (e.g. predatory caddis larvae), and more chironomids, than
441 those from control enclosures. This shift from prey occupying a higher trophic level (e.g.
442 Rhyacophilidae) to prey occupying a lower trophic niche (e.g. Chironomidae) resulted in
443 bullhead from the medium- and high-density crayfish treatments occupying lower trophic
444 positions than other groups, especially when compared to the fish control group. A similar
445 explanation may be applied to higher $\delta^{13}\text{C}$ values recorded in bullhead from the high signal
446 crayfish density group. A study with the midwater fish (chub *Squalius cephalus*), common in
447 lowland rivers, showed that young-of-year (YoY, age 0+) chub at signal crayfish invaded sites
448 exhibited a lower growth rate compared to allopatric populations (Wood *et al.*, 2017). By
449 contrast, adult chub from crayfish-invaded sites grew faster than those from uninvaded sites,
450 interpreted as being due to crayfish becoming a key part of the diet of larger chub. Unlike
451 chub, bullhead attain a smaller adult size and can only predate the smallest (mostly YoY)
452 crayfish. In this study all bullhead in crayfish treatment enclosures had a reduced or negative
453 growth over time. This difference in fish growth reflects their differing habitat niches; chub is a
454 moderately fast swimmer in midwater whereas bullhead are slow-moving bottom dwellers and,
455 like crayfish, depend on benthic refuges within the habitat (Freyhof & Kottelat, 2007). It is
456 known from laboratory and field studies that signal crayfish exclude bullhead and other small
457 benthic fishes from refuges, potentially increasing susceptibility of the latter to predators,
458 resulting in reduced small benthic fish abundance where signal crayfish are abundant (Guan
459 & Wiles, 1997; Bubb *et al.*, 2009). Signal crayfish are also known to impact the recruitment of
460 young bullhead (Galib *et al.*, 2021). This study's findings support the hypothesis that the
461 energy assimilation impacts of signal crayfish are greater on species occupying a similar
462 niche, such as bullhead, and provide another mechanism by which impacts to small benthic
463 fishes occur.

464 **4.3 Impact on ecosystem processes**

465 Strong effects on leaf litter breakdown and algal standing stock were recorded in this study.
466 Compared to both control groups, loss of leaf litter was higher in enclosures containing signal
467 crayfish. As abundance of key shredders like *Gammarus* was greatly reduced in the high-
468 density crayfish treatment it would be expected that this would lead to trophic cascade in the
469 system, resulting in reduced leaf litter processing (and resultant loss) in the high-density group.
470 But, the opposite results were recorded, which may be due to direct feeding on leaf litter by
471 invasive crayfish (Dunoyer *et al.*, 2014; Doherty-Bone *et al.*, 2018). Increased cumulative leaf
472 litter removal by signal crayfish could be expected at higher crayfish densities, as in riverine
473 habitats signal crayfish extensively feed on leaf litter and detritus, which can contribute 67.5%

474 of crayfish diet (Mason, 1975; Stenroth & Nyström, 2003). Contribution of leaf litter to the
475 overall diet of crayfish was much less in our study, perhaps due to the limited availability of
476 leaf litter in the enclosure as the mesh enclosure restricted coarse leaf litter from entering.
477 SIMM outcomes suggested a stable proportional consumption of leaf litter by crayfish in
478 enclosures with increasing density, so an increasing density of crayfish would process
479 available leaf litter at a higher rate. This assumes crayfish could directly access leaf litter from
480 the experimental packs, and although they could not enter the packs, it seems likely they could
481 access it using their maxillipeds and chelipeds. Crayfish are active shredders and can play an
482 important role in processing leaf litter in freshwater ecosystems (Usio & Townsend, 2001).
483 This shredder role is expected to be beneficial for collector-gatherer macroinvertebrates
484 including Chironomidae and Oligochaeta (Huryñ & Wallace, 1987). Nonetheless, low densities
485 of invasive crayfish (*Faxonius meeki meeki*) can reduce the biomass of benthic chironomids
486 (Ludlam, Banks & Magoulick, 2015). It is thus complex to predict the impacts of invasive
487 crayfish on ecosystem components as their role depends on multiple associated factors (Klose
488 & Cooper, 2012). For example, in a study conducted in outdoor fibreglass tanks, signal and
489 red-swamp crayfish in sympatry increased the rate of leaf litter decomposition, but it decreased
490 in tanks with virile and Turkish crayfish (Jackson *et al.*, 2014). Our study accords with Jackson
491 *et al.* (2014) as signal crayfish increased leaf litter breakdown in both studies. Bullhead can
492 slow down the organic decomposition process by preying on shredder macroinvertebrates like
493 Chironomidae and Baetidae (Woodward *et al.*, 2008) but this was not the case in the current
494 study at the bullhead densities used. It is likely that defecation by crayfish would have an
495 influence on microbial processing of leaf litter, as it would alter the availability and distribution
496 of nutrients at the stream bed and this deserves study in the future. At densities at which signal
497 crayfish occur in English upland streams, the direct effects of signal crayfish as an active
498 shredder may be much higher and are likely to be enough to substantially accelerate organic
499 matter decomposition.

500 According to SIMMs, macroinvertebrates constituted the major portion of signal crayfish diet
501 in our study, by contrast with the findings of Bondar *et al.* (2005). One possible explanation
502 may be restricted access to detritus matter originating from outside the enclosures which might
503 have prevented signal crayfish from consuming a greater amount (Ludlam *et al.*, 2015).
504 However, Whitley and Rabeni (1997) reported that 30 – 50% of crayfish production is
505 derived from direct consumption of animal matter which agrees with our study results. No
506 crayfish sex effects on isotopic signatures were found, suggesting that there is no difference
507 in foraging and diet between the sexes (Usio *et al.*, 2009; Ercoli *et al.*, 2021).

508 For algal standing stock, chlorophyll- α levels were lower in crayfish treatments than controls
509 with and without bullhead and exhibited a density-dependent pattern. Algae provided a

510 relatively constant but small contribution to crayfish diet across treatment groups. Therefore,
511 a direct crayfish grazing effect causing lower algal biomass can be expected in stream
512 environments with a high density of crayfish, as revealed in this study and elsewhere (Keller
513 and Ruman, 1998), though not in all cases, including the only other study of density effects of
514 stream-dwelling invasive crayfish (Stenroth & Nyström, 2003). However, signal crayfish also
515 reduce the abundance of slow-moving herbivore taxa in streams (Mathers *et al.*, 2016; Galib
516 *et al.*, 2021), as happened in this study. This could partially release algae from grazing
517 pressure via a trophic cascade and lead to increased algal biomass. Similar impacts of
518 bullhead on grazing macroinvertebrates could be expected (but note, bullhead also fed
519 extensively on invertebrate predator taxa) and this might also increase algal biomass through
520 decreasing grazer pressure (Dahl, 1998). In our study the abundance of grazers and algal
521 standing stock were negatively affected in enclosures with signal crayfish, indicating a broad
522 spectrum of impacts over multiple components of the ecosystem. Lower algal standing stock
523 in treatment enclosures could also partly be explained by bioturbation due to crayfish activities
524 such as burrowing, inundating biofilms with sediment (Harvey *et al.*, 2014), thereby limiting
525 algal standing stock (Doherty-Bone *et al.*, 2019). Crayfish in streams with coarse sediment
526 rarely burrow as they use cobbles and boulders for refuges (Galib *et al.*, 2021), and burrowing
527 was not observed in our study. In addition, we did not see any obvious silt deposition on
528 substrates within the enclosures. However, winnowing of fine sediment during crayfish feeding
529 and movement often occurs (Harvey *et al.*, 2014) and bioturbation produced through crawling
530 on the surface of the sediments may affect algal biomass adversely (Usio & Townsend, 2004).
531 Therefore, we believe that both direct grazing effects of signal crayfish and bioturbation were
532 more important than indirect trophic cascade effects in treatments with high crayfish density,
533 and plant biomass can be reduced even at a low crayfish density (Momot, 1995; Ludlam *et*
534 *al.*, 2015). It has been shown that signal crayfish does not undergo ontogenetic dietary niche
535 shifts in streams (Bondar *et al.*, 2005) and there is no effect of body size or seasons on isotopic
536 signature values (France, 1996; Stenroth *et al.*, 2006). Therefore, it is likely that the results of
537 this study would be representative of signal crayfish of a wide range of sizes in streams of the
538 type studied, while acknowledging that the mesocosms used are not true representations of
539 the stream environment.

540

541 **5. Conclusions**

542 In conclusion, this study demonstrates the density-driven, but multi-faceted, trophic impacts
543 of one of the most invasive temperate crayfish species on upland stream habitats and reveals
544 underlying mechanisms of such impacts. It also indicates that, at a higher density, direct

545 effects of signal crayfish on the ecosystems exceeded potential trophic cascading effects, and
546 were enough to modify existing trophic links in invaded habitat. Superior competitive ability of
547 signal crayfish to bullhead occupying a similar physical habitat niche indicates that, in
548 established invasive crayfish populations where the density is high, native competitors such
549 as benthic fishes may decline or be extirpated as reported in some upland streams (Galib *et*
550 *al.*, 2021). For these reasons we urge that the spread of invasive crayfish, within and between
551 catchments, is controlled as fully as possible through biosecurity, barriers and other
552 techniques (Peay *et al.*, 2019; Jones *et al.*, 2021; Mozsár *et al.*, 2021). However, study of
553 signal crayfish impacts in uninvaded stream habitat is also recommended in order to determine
554 whether the response of ecosystem components differs from our study. Studies on ecosystem
555 effects of invasive crayfishes in other rivers with finer sediments are also encouraged.

556

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567

568 **Data availability statement**

569 Data from this research are available from the corresponding authors upon reasonable
570 request.

571

572 **Conflict of interest**

573 We declare we have no competing interests.

574

575 **Authors' contributions**

576 Shams Galib: Conceptualization, Methodology, Investigation, Data curation, Formal analysis,
577 Stable-isotope analysis, Writing-Original draft preparation, Visualization, Writing-Reviewing
578 and Editing. Jingrui Sun: Investigation, Writing-Reviewing and Editing. Darren Gröcke: Stable
579 isotope analysis, Writing-Reviewing and Editing. Martyn Lucas: Conceptualization,
580 Methodology, Investigation, Supervision, Formal analysis, Writing-Reviewing and Editing.

581

582

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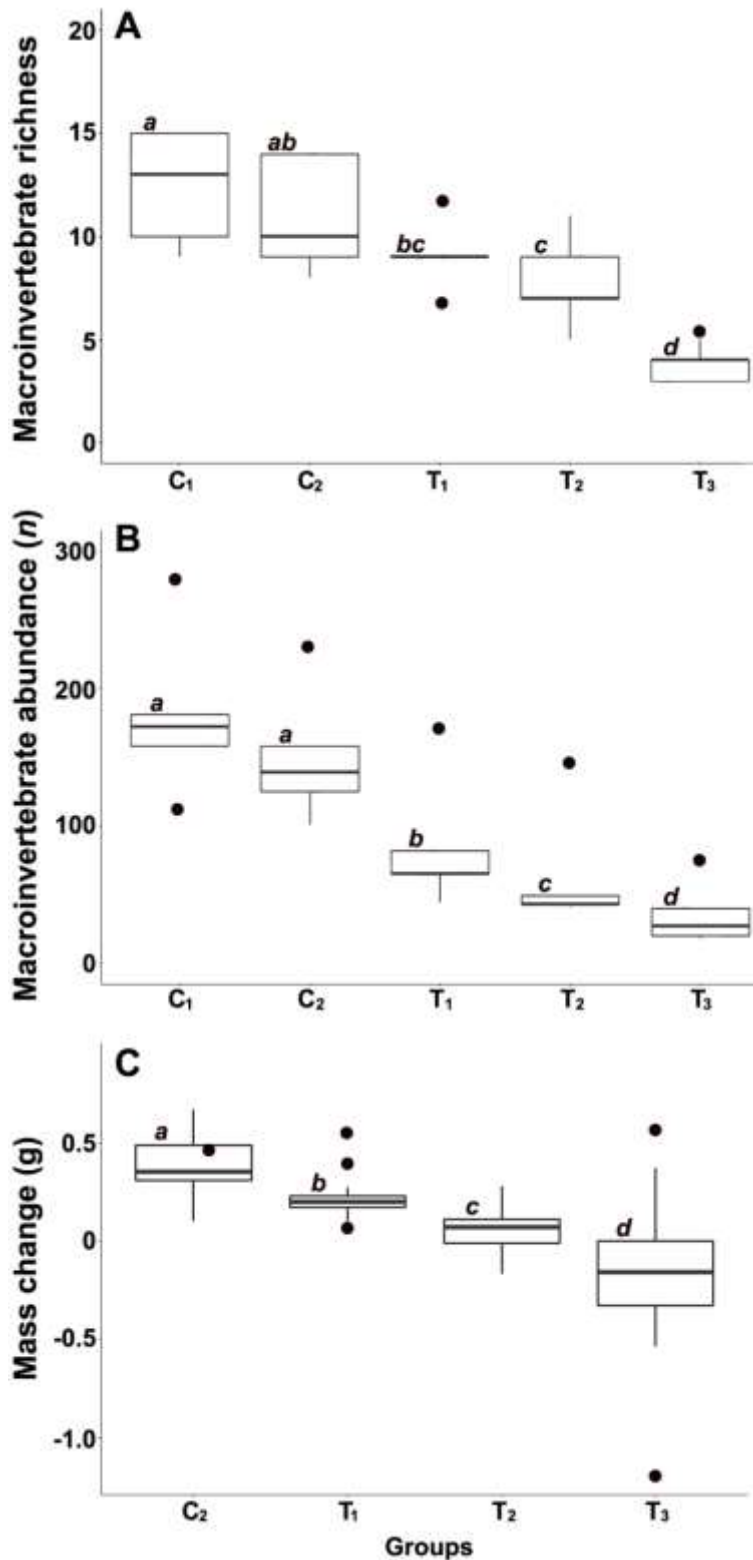
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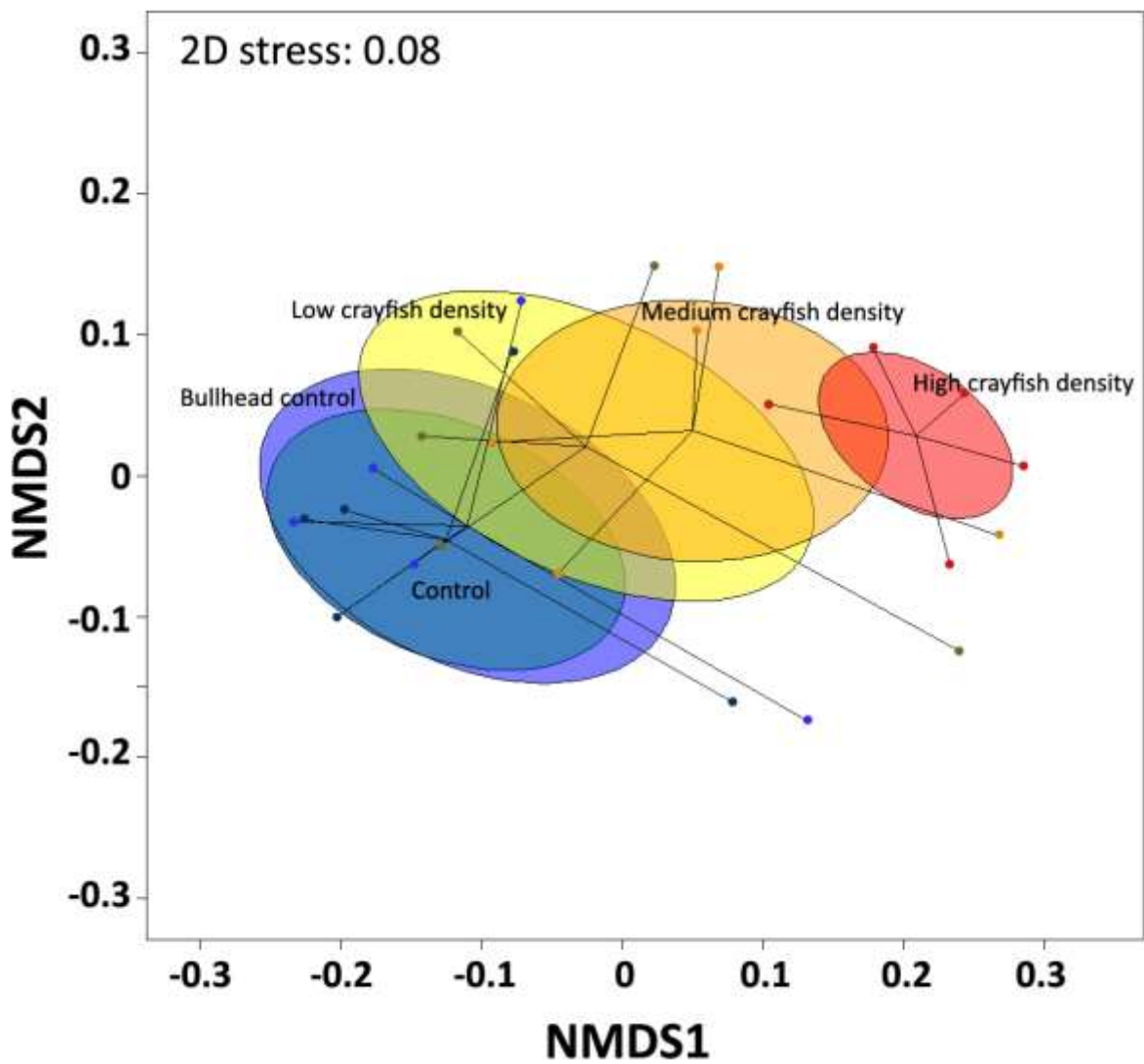
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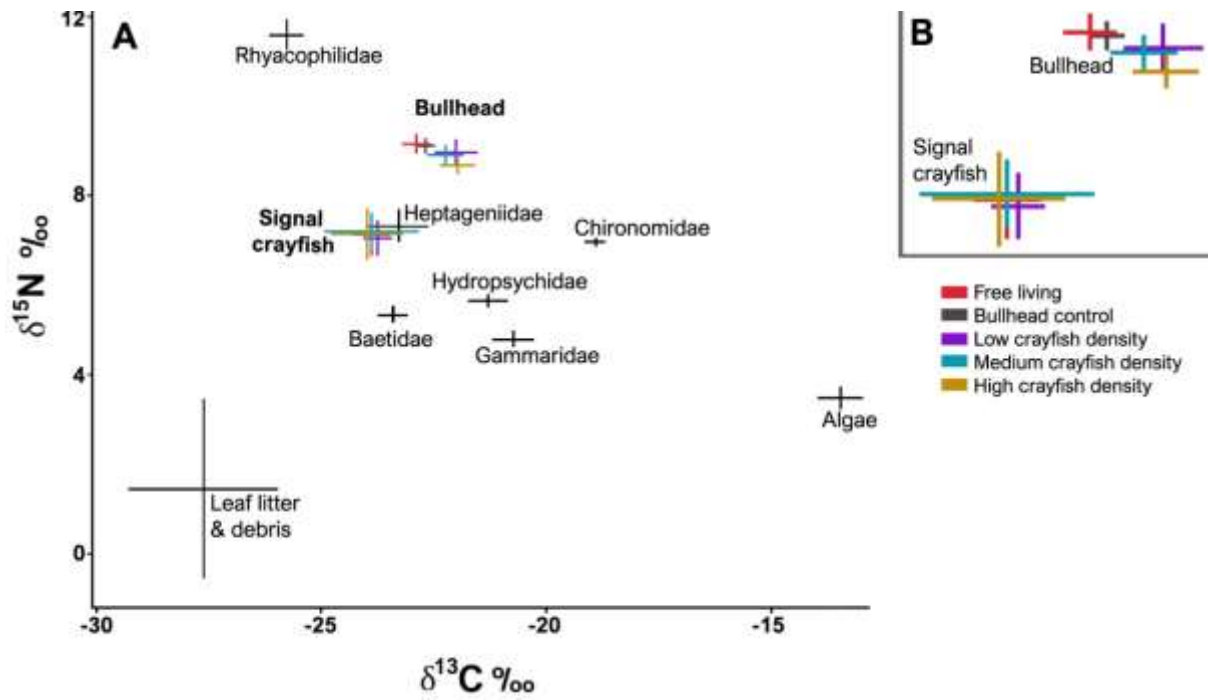
890
 891 **Figure 1:** Macroinvertebrate taxonomic richness (A) and abundance per 0.3 m² Surber (B) at
 892 the end of the experiment, and body mass gain of bullhead (C) belonging to different groups
 893 (C₁, control; C₂, bullhead control; T₁, T₂ and T₃ are low, medium and high crayfish density
 894 treatments respectively). Points are individual data outliers. Different letters on the top of the
 895 boxes are significantly different. *n* = 5 per treatment.



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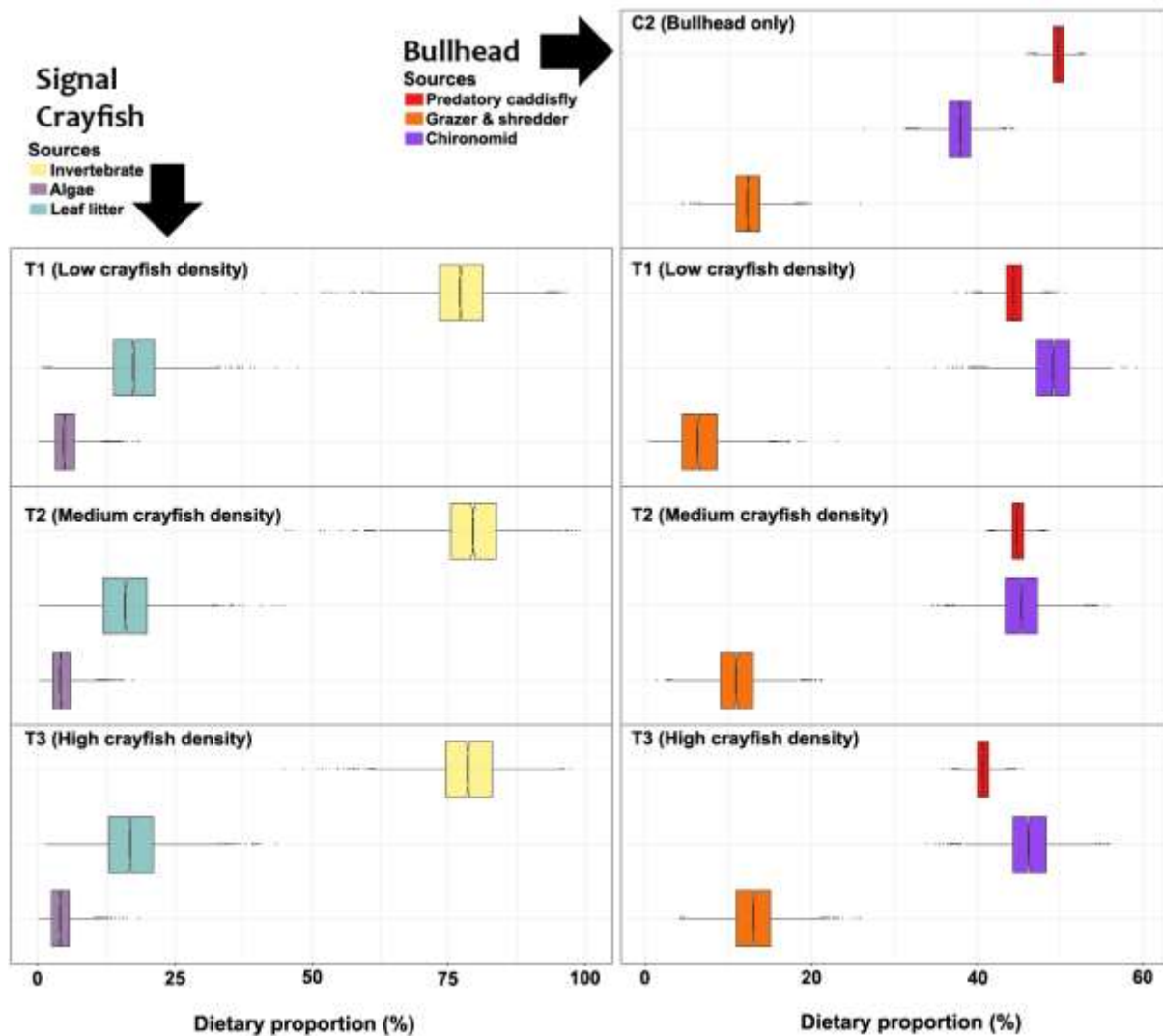
898 **Figure 2:** Non-metric multidimensional scaling (NMDS) ordination plot showing variation of
 899 invertebrate communities in different enclosure groups at the end of the study. Each ellipse
 900 represents 95% confidence interval.

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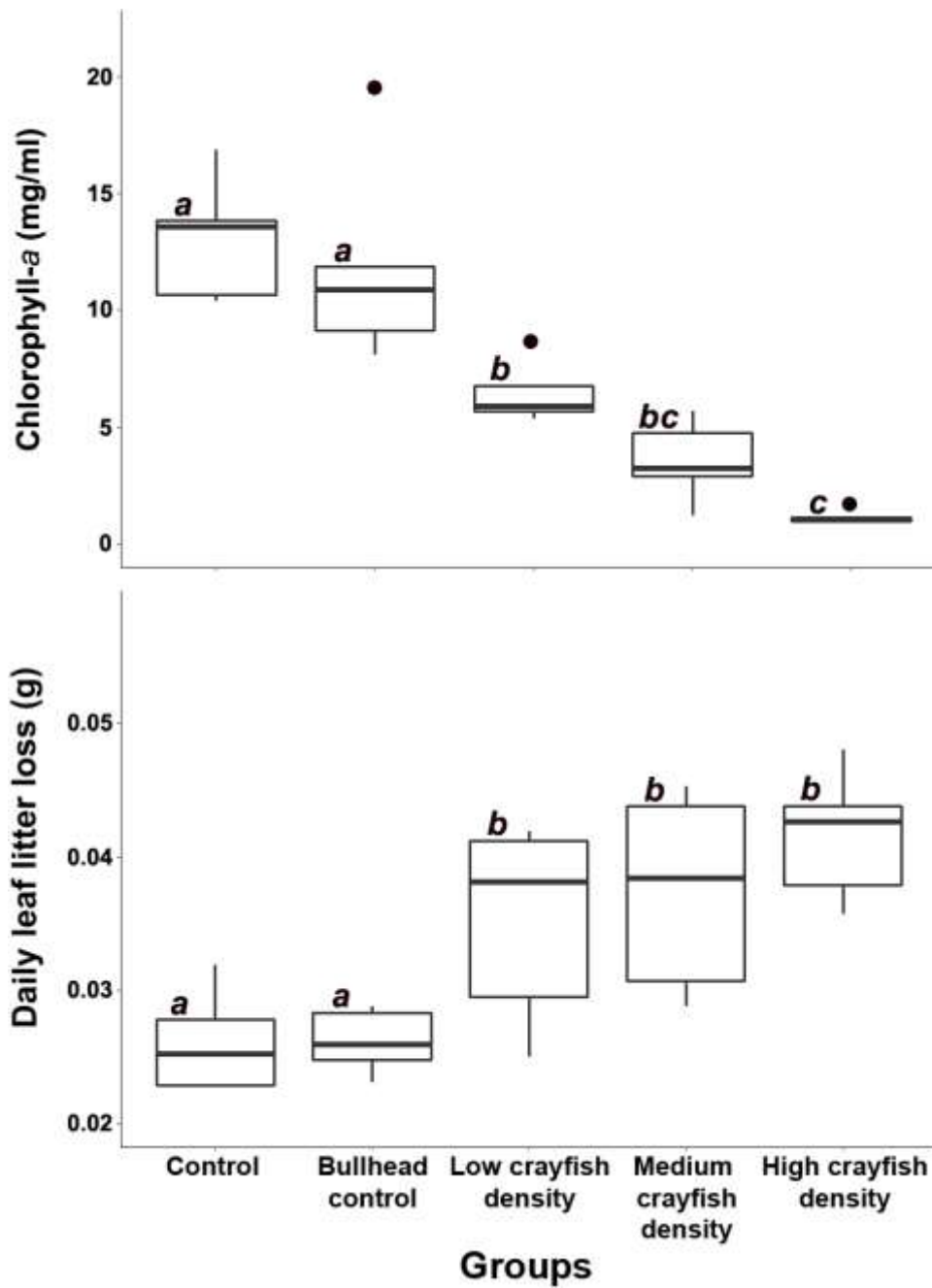
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903 **Figure 3:** Isospace plot for signal crayfish, bullhead and their potential dietary items (A). Data
 904 are represented as mean and standard deviation. B, 'Zoom in' on isospace plot for bullhead
 905 and signal crayfish treatments.



906

907 **Figure 4:** Boxplots showing comparisons of signal crayfish and bullhead dietary proportions
 908 for different prey sources in different treatment groups. Midline within the box is the median;
 909 upper and lower limits of the box represent the third and first quartile (75th and 25th
 910 percentile) respectively. Points are individual enclosure data.



911

912 **Figure 5:** Chlorophyll- α concentration as a marker of algal biomass at the end of the
 913 mesocosm study duration (above) and daily leaf litter loss (below) in different experimental
 914 groups. Points are individual data outliers. Different letters on the top of the boxes are
 915 significantly different. $n = 5$ per treatment.

916

917 **Table 1:** Macroinvertebrate richness, density and community in different control and treatment
 918 groups over time.

Categories	Comparisons	Mean square	df	F-value	P-value
Richness	Groups	0.090	4, 36	31.4	<0.001
	Time	0.326	1, 36	113.5	<0.001
	Interaction	0.084	4, 36	29.3	<0.001
	Time: Before	0.001	4, 16	0.7	0.690
	Time: After	0.174	4, 16	38.3	<0.001
Density	Groups	0.614	4, 36	58.1	<0.001
	Time	4.364	1, 36	413.1	<0.001
	Interaction	0.724	4, 36	68.5	<0.001
	Time: Before	0.004	4, 16	2.1	0.130
	Time: After	1.333	4, 16	85.7	<0.001
Community	Groups	0.173	4, 40	1.8	0.005
	Time	1.005	1, 40	10.6	0.001
	Interaction	0.178	4, 40	1.9	0.003
	Time: Before	0.005	4, 20	0.06	0.099
	Time: After	0.345	4, 20	3.04	0.001

919 Richness and abundance data were subjected to Linear Mixed-Effects Modelling (LMM) and
 920 community data were analysed by Permutational Multivariate Analysis of Variance. Pairwise
 921 comparisons were made by LMM. Statistically significant values are shown in bold.

922

923 **Table 2:** Results of SIMPER analysis indicating the primary benthic invertebrate taxa (the
 924 top five ranked by contribution to community dissimilarity) in different experimental groups.

Group	Taxon	Mean (\pm SD) abundance		Contribution to dissimilarity (%)	Cumulative contribution (%)
		Before	After		
Control	Heptageniidae	88.8 \pm 49.3	91.6 \pm 57.2	10.57	10.57
	Chironomidae	9.2 \pm 8.4	12.8 \pm 12.5	10.55	21.12
	Simulidae	7.2 \pm 8.7	8.0 \pm 7.3	8.80	29.92
	Baetidae	11.4 \pm 8.5	11.4 \pm 9.4	8.11	38.03
	Dixidae	5.0 \pm 6.8	5.0 \pm 6.9	7.80	45.83
Fish	Chironomidae	11.8 \pm 11.1	14.4 \pm 13.6	11.56	11.56
control	Heptageniidae	92.0 \pm 49.5	80.2 \pm 43.5	10.61	22.17
	Simulidae	8.0 \pm 7.6	8.2 \pm 8.6	8.55	30.72
	Baetidae	11.0 \pm 8.6	11.4 \pm 7.6	8.17	38.89
	Nemouridae	4.8 \pm 5.1	3.4 \pm 4.2	7.12	46.01
Low- density treatment	Heptageniidae	89.8 \pm 52.4	45.8 \pm 43.5	15.27	15.27
	Chironomidae	12.4 \pm 11.4	12.0 \pm 10.9	8.95	24.22
	Simulidae	7.4 \pm 8.2	3.2 \pm 4.1	7.29	31.51
	Gammaridae*	12.4 \pm 3.6	3.0 \pm 1.6	7.19	38.7
	Baetidae	10.4 \pm 8.7	5.0 \pm 4.0	6.97	45.67
Medium- density treatment	Heptageniidae	97.8 \pm 52.7	38.6 \pm 36.1	15.84	15.84
	Gammaridae*	11.8 \pm 2.9	1.2 \pm 0.8	8.88	24.72
	Baetidae	11.4 \pm 8.1	3.8 \pm 3.7	7.29	32.01
	Chironomidae	14.0 \pm 10.6	11.2 \pm 10.0	7.18	39.19
	Simulidae	8.4 \pm 9.8	1.6 \pm 2.1	6.94	46.13
High- density treatment	Heptageniidae	91.6 \pm 52.0	23.2 \pm 17.5	15.49	15.49
	Gammaridae*	12.4 \pm 2.1	0.2 \pm 0.4	10.66	26.15
	Hydropsychidae*	9.0 \pm 3.5	0 \pm 0	9.42	35.57
	Baetidae	11.6 \pm 9.1	2.4 \pm 3.4	7.39	42.96
	Chironomidae	10.8 \pm 9.9	6.6 \pm 7.8	7.06	50.02

925 *, taxon differed significantly between before and after conditions. Abundance is based on
 926 0.3 m² Surber samplings.

927

928

929 **Table 3:** Quantification of signal crayfish and bullhead diets, based on Stable Isotope Mixing
 930 Models.

Groups	Diets (% , mean \pm SD; 95% CI)					
	Crayfish			Bullhead		
	Leaf litter & debris	Algae	Macro-invertebrate	Chironomids	Grazers & shredders	Predatory caddis
C ₂	–	–	–	37.9 \pm 0.02 ^a (34.1–41.6)	12.3 \pm 0.02 ^a (8.3–16.7)	49.8 \pm 0.01 ^a (47.8–51.7)
T ₁	18.9 \pm 0.05 ^a (7.9–29.5)	5.2 \pm 0.03 ^a (1.2–11)	75.9 \pm 0.06 ^a (64–88)	49.0 \pm 0.03 ^b (42.6–54.1)	6.6 \pm 0.03 (1.7–13.0) ^b	44.4 \pm 0.01 ^b (41.4–47.1)
T ₂	17.4 \pm 0.06 ^a (6.5–28.1)	4.5 \pm 0.03 ^a (1–10.2)	78.1 \pm 0.06 ^a (66.2–90.1)	44.9 \pm 0.03 ^b (38.1–49.6)	10.9 \pm 0.03 ^a (5.2–16.7)	44.2 \pm 0.01 ^b (43.1–47.2)
T ₃	18.7 \pm 0.06 ^a (7.2–29.3)	4.4 \pm 0.02 ^a (0.9–10.1)	76.9 \pm 0.06 ^a (65.2–89.2)	46.3 \pm 0.03 (40.4–52.3) ^b	13.0 \pm 0.03 ^a (7.2–18.9)	40.7 \pm 0.01 ^c (38.5–42.8)

931 Different superscript letters within each column indicates significant differences.

932

933

934 **Supplementary information**

935

936 **METHOD S1: Enclosures set up**

937 The enclosures were manually dug into the streambed, to a depth of about 30 cm, and 1.5-m
938 holding stakes were driven in place at each corner. Enclosures were then refilled, covering
939 the bottom mesh with substrate (see below) to a depth that matched the level outside.
940 Enclosures were allowed to condition for about four weeks to facilitate natural algal growth
941 and macroinvertebrate colonisation prior to the introduction of fish or crayfish to the
942 enclosures.

943 Prior to setting up enclosures, substrate characteristics at the site were recorded by counting
944 and measuring boulders (>256 mm), cobbles (64 – 256 mm) and pebbles (16 – 64 mm)
945 (following a simplified version of the Wentworth Scale; Wentworth, 1922) using a 1 m × 1 m
946 quadrat ($N = 20$). At this site, finer sediments (< 16 mm, gravel, sand, silt) were incidental and
947 mainly occurred in pockets within the larger sediment interstices. The mean number of larger
948 substrate particles, i.e. boulders and cobbles, and their size (area) were calculated per m²
949 quadrat. Based upon this, equal numbers of larger particles of similar sizes (boulders, $n = 4$,
950 mean area 559 cm²; cobbles, $n = 78$, mean area 124 cm²; pebbles, $n = 50$, mean area 16 cm²)
951 were used to refill every enclosure. Approximately equal amounts of smaller substrates (i.e.
952 gravel and smaller substrates, total ~5000 ml) were also added. This ensured similar shelter
953 opportunities within the enclosures for study animals to those of outside habitat per unit area.
954 Substrate particle volume and composition may have differed to a small extent across
955 enclosures but careful attempts were made to minimise variations.

956 Mesh lids, shut tightly with cable ties, were employed on the top of each enclosure to make
957 sure that crayfish or fish could not escape or enter through the top. The lid was 0.1 – 0.2 m
958 above the normal water surface, depending on the natural gradient of the river, to minimise
959 the chance of fish escape during checking of the enclosure. The heavy duty plastic mesh used
960 was aimed at minimising the probability of enclosure damage due to abrasion by substrate,
961 and resultant escape of study animals. During the experiment, the sides and tops of the
962 enclosures were brushed biweekly to prevent debris build up and maintain flow through the
963 enclosure.

964

965 **METHOD S2: Collection of signal crayfish and bullhead and individual marking**

966 Signal crayfish and bullhead used in the experimental enclosures were collected from the
967 River Lune in and around (within 200 m) of the study site where they exist in sympatry. Some
968 of the planned components of this study (e.g. stable isotope analyses) may be affected if study
969 animals are collected from outside of the immediate study locality. This is due to potential slow
970 turnover rate of crayfish or bullhead tissues as the influence of previous diet on stable isotope
971 ratios can be long lasting (McCutchan *et al.*, 2003).

972 Bullhead were collected by electrofishing (using a land-based generator, Honda EU inverter
973 10i; and an electrofishing control unit, model Electracatch WFC4, Electracatch International,
974 Wolverhampton, England). Captured bullhead individuals were kept at a very low density in
975 semi-transparent plastic tanks (at two individuals per tub with shelters; tank size: 35 cm long
976 × 21 cm wide × 21 cm high) in shade, filled with river water, until further processing on the
977 same day. After measurements, bullhead were sedated in buffered tricaine methansulphonate
978 (0.1 g L⁻¹, using river water) and individually marked to determine changes in individual length
979 and weight at the end of the experiment by using Visible Implant Elastomer (VIE; Northwest
980 Marine Technology, Inc., Shaw Island, WA, USA) tags, coded by mark location on the ventral
981 side, and kept in the plastic tubs again for further observations. After about one hour, they
982 were checked again (all behaving normally) and introduced to the enclosures.

983 Signal crayfish were caught by hand-net searching from the river and kept in plastic tanks at
984 a low density (three crayfish per tank with shelters) until further processing, outlined above.
985 Carapace length of crayfish (CL, length from rostral apex to the posterior median edge of the
986 carapace; Brewis and Bowler, 1982) and weight were recorded using the same instruments
987 described above. Sex and any obvious marks on the crayfish's body (e.g. leg loss or other
988 body marks) were also noted. Following physical examinations, crayfish were marked
989 individually by VIE, coded by mark location on abdominal somites. VIEs are an effective
990 tagging technique for both adult and juvenile crayfish that perform well without affecting
991 crayfish biology, and are retained after moulting (Clark & Kershner, 2006).

992

993 **METHOD S3: Determination of algal growth**

994 Each biofilm sample was thawed and filtered on a glass fibre filter paper (GF/C Whatman) and
995 added to a 10 ml solution of 90% acetone. These were placed at 5°C for 24 h in a lightless
996 refrigerator for chlorophyll- α extraction to occur, centrifuged at 2530 rpm for 5 minutes. The
997 absorbance of subsamples in 5 ml cuvettes was measured at 630, 647 and 664 nm in a

998 spectrophotometer (GENESYS™ 10S UV-Vis, Thermo Scientific, USA), calibrated with a 90%
999 acetone solution.

1000

1001 **METHOD S4: Stable isotope mixing models**

1002 For crayfish, DTDF values of +2.0‰ and +2.3‰ were used for carbon and nitrogen
1003 respectively (Rudnick and Resh, 2005; Wood *et al.*, 2017). These values were added to
1004 different food sources including leaf litter, organic debris, algae and various families of
1005 macroinvertebrates. Although cannibalism in signal crayfish is common (Houghton, Wood &
1006 Lambin, 2017) crayfish was not included in the model as a potential food source as no signal
1007 crayfish including young of the year (YoY) was recorded during invertebrate sampling and
1008 there were no missing crayfish in any of the enclosures. For bullhead, a DTDF value of +2.1‰
1009 was used for carbon (McCutchan *et al.*, 2003). A DTDF value of +2.3‰ for the nitrogen isotope
1010 ($\delta^{15}\text{N}$) was obtained through the calculation of the mean DTDF value from those reported in
1011 fishes feeding on prey items similar to those of bullhead (i.e. primarily macroinvertebrates).
1012 These fishes were *Coregonus nasus* (+2.0‰; Hesslein *et al.*, 1993), *Oncorhynchus mykiss*
1013 (+1.3‰ and +1.9‰; Rounick and Hicks, 1985; McCutchan *et al.*, 2003) and *Salvelinus*
1014 *fontinalis* (+3.3‰; McCutchan *et al.*, 2003).

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1020 **Table S1:** Recorded bullhead densities in UK rivers and crayfish density in Westholme Beck.

River	Density (m ⁻²)	Reference
Bullhead		
Devil's Brook, Dorset	5.3	Mills and Mann (1983)
River Tarrant, Dorset	75	Mann (1971); Mills and Mann (1983)
Great Ouse	1.3–14.7	Guan and Wiles (1997)
Mill Stream	0.8	Prenda <i>et al.</i> (1997)
Bere Stream	2.2	Prenda <i>et al.</i> (1997)
All UK rivers	0.00002–11.1	Environment Agency (2016)
Signal crayfish		
River Great Ouse	up to 21	Guan and Wiles (1997)
River Wharfe	9–24	Bubb <i>et al.</i> (2009)
Bookill Gill Beck, Yorkshire Dales,	20.5–110.4	Chadwick <i>et al.</i> (2021)
Westholme Beck (a tributary of the River Tees, similar to the River Lune)	4–24 (12.2±5.3)	Based on Surber (0.5 m ⁻²) samplings (<i>n</i> = 26) carried out by the authors in 2017.

1021

1022 **Table S2:** Various water quality parameters across control and treatment groups over the
 1023 duration of study, measured weekly (10:00–12:00) in enclosures during site visits.

Parameters	Mean (± SD)	LMM results	
		F-values	p-values
Water depth (cm)	23.0 ± 6.4	0.56	0.697
Water temperature (°C)	14.8 ± 0.9	1.15	0.369
Dissolved oxygen (mg L ⁻¹)	8.6 ± 0.7	1.24	0.342
pH	8.2 ± 0.2	1.74	0.190
Flow velocity (m s ⁻¹)	0.2 ± 0.1	1.65	0.217

1024

1025 **Table S3:** Abundance and taxonomic richness (mean ± SD) in different experimental groups
 1026 (C₁, without crayfish or bullhead; C₂, bullhead only; T₁, T₂, and T₃ are low, medium and high
 1027 crayfish density treatments respectively). Abundance is based on combined 3 × 0.1 m²
 1028 Surbers per enclosure.

Groups	Taxonomic richness		Abundance	
	Before	After	Before	After
C ₁ (Ctrl)	12.1 ± 2.8	12.4 ± 2.8	179.5 ± 50.8	180.4 ± 61.2
C ₂ (Bull ctrl)	12.0 ± 2.5	11.0 ± 2.8	176.6 ± 54.9	150.6 ± 49.0
T ₁ (Low)	12.2 ± 3.3	9.2 ± 1.8	180.6 ± 60.3	85.4 ± 49.7
T ₂ (Medium)	12.4 ± 3.3	7.8 ± 2.3	187.4 ± 54.1	64.2 ± 45.3
T ₃ (High)	11.8 ± 2.9	3.8 ± 0.8	180.6 ± 55.1	35.8 ± 23.0

1029

1030

1031 **Table S4:** Pairwise comparisons of macroinvertebrate richness and abundance within and
 1032 between control and treatment groups (C₁, control without fish or crayfish; C₂, bullhead
 1033 control; T₁, T₂ and T₃ are low, medium and high crayfish density treatments respectively) at
 1034 the end of the study. Bold values indicate significant differences.

Comparisons	Richness		Abundance	
	z-value	p-value	z-value	p-value
C ₂ -C ₁	-1.3	0.670	-2.4	0.112
T ₁ -C ₁	-3.1	0.016	-10.1	<0.001
T ₂ -C ₁	-4.9	<0.001	-13.5	<0.001
T ₃ -C ₁	-11.2	<0.001	-19.2	<0.001
T ₁ -C ₂	-1.8	0.393	-7.7	<0.001
T ₂ -C ₂	-3.6	0.003	-11.1	<0.001
T ₃ -C ₂	-9.9	<0.001	-16.8	<0.001
T ₂ -T ₁	-1.8	0.378	-3.4	0.006
T ₃ -T ₁	-8.1	<0.001	-9.1	<0.001
T ₃ -T ₂	-6.3	<0.001	-5.7	<0.001

1035

1036 **Table S5:** Similarity Percentage Analysis (SIMPER), based on Bray-Curtis dissimilarity index
 1037 on abundance data of macroinvertebrate families between two control groups C₁ (without
 1038 benthic fish or crayfish) and C₂ (benthic fish only) at the end of the study.

Families	Average	Ratio	Average abundance		p-value	Cumulative contribution
			C ₁	C ₂		
Chironomidae	0.035	1.11	2.75	2.93	0.976	11.51
Heptageniidae	0.032	1.02	9.27	8.73	0.966	21.91
Simuliidae	0.025	1.21	2.43	2.38	0.868	30.17
Baetidae	0.024	0.96	2.97	2.99	0.804	38.05
Dixidae	0.022	1.13	1.41	0.69	0.485	45.12
Ephemerellidae	0.020	1.49	2.20	1.41	0.422	51.66
Nemouridae	0.019	1.25	1.40	1.36	0.996	57.89
Caenidae	0.016	1.19	1.29	1.00	0.613	62.93
Hydropsychidae	0.014	1.14	2.27	1.43	0.133	67.42
Leptophebiidae	0.013	0.69	0.57	0.40	0.490	71.57
Rhyacophilidae	0.012	1.17	0.91	0.00	0.077	75.58
Leuctridae	0.012	1.32	2.86	2.74	0.940	79.45
Elmidae	0.011	1.13	1.68	1.09	0.478	83.12
Perlodidae	0.011	1.28	1.26	1.08	0.811	86.72
Gammaridae	0.010	1.59	3.65	3.28	0.471	89.87
Culicidae	0.009	1.07	0.57	0.40	0.524	92.74
Tipulidae	0.008	0.67	0.40	0.40	0.945	95.44
Hydrophilidae	0.006	0.68	0.35	0.20	0.521	97.54
Perlidae	0.005	0.49	0.35	0.00	0.166	99.1
Polycentropodidae	0.003	0.49	0.20	0.00	0.153	100

1039 Average, Average dissimilarity; Ratio, Average dissimilarity / SD; Cumulative contribution,
 1040 contribution to overall dissimilarity between communities of two groups

1041

1042 **Table S6:** Similarity Percentage Analysis (SIMPER), based on Bray-Curtis dissimilarity index
 1043 on abundance data of macroinvertebrate families between C₁ (without benthic fish or
 1044 crayfish) and T₁ (low density treatment, benthic fishes and four crayfish) at the end of the
 1045 study. Bold values indicate significant differences.

Families	Average	Ratio	Average abundance		p-value	Cumulative contribution
			C ₁	T ₁		
Heptageniidae	0.065	1.56	9.27	6.27	0.124	15.64
Chironomidae	0.040	1.09	2.75	2.68	0.972	25.15
Gammaridae	0.032	3.72	3.65	1.68	0.014	32.91
Baetidae	0.031	1.32	2.97	1.95	0.485	40.25
Simuliidae	0.030	1.35	2.43	1.29	0.553	47.4
Ephemerellidae	0.027	1.57	2.20	0.97	0.210	53.98
Dixidae	0.023	0.79	1.41	0.00	0.023	59.43
Hydropsychidae	0.020	1.46	2.27	1.40	0.472	64.3
Nemouridae	0.020	1.46	1.40	1.28	0.745	69.03
Caenidae	0.019	1.20	1.29	0.69	0.410	73.52
Perlodidae	0.018	1.40	1.26	0.28	0.102	77.96
Elmidae	0.017	1.42	1.68	0.60	0.022	82.14
Leptophebiidae	0.015	0.68	0.57	0.35	0.503	85.66
Rhyacophilidae	0.014	1.24	0.91	0.20	0.071	88.98
Leuctridae	0.012	1.28	2.86	2.40	0.198	91.97
Culicidae	0.009	0.80	0.57	0.00	0.023	94.05
Tipulidae	0.009	0.69	0.40	0.28	0.991	96.12
Perlidae	0.007	0.67	0.35	0.20	0.441	97.89
Hydrophilidae	0.006	0.49	0.35	0.00	0.023	99.22
Polycentropodidae	0.003	0.49	0.20	0.00	0.023	100

1046 Average, Average dissimilarity; Ratio, Average dissimilarity / SD; Cumulative contribution,
 1047 contribution to overall dissimilarity between communities of two groups

1048

1049 **Table S7:** Similarity Percentage Analysis (SIMPER), based on Bray-Curtis dissimilarity index
 1050 on abundance data of macroinvertebrate families between C₁ (without benthic fish or
 1051 crayfish) and T₂ (medium crayfish density treatment, benthic fishes and eight crayfish) at the
 1052 end of the study. Bold values indicate significant differences.

Families	Average	Ratio	Average abundance		p-value	Cumulative contribution
			C ₁	T ₂		
Heptageniidae	0.074	1.66	9.27	5.79	0.067	14.73
Gammaridae	0.049	3.46	3.65	0.97	0.006	24.4
Chironomidae	0.042	1.21	2.75	2.76	0.989	32.76
Baetidae	0.036	1.56	2.97	1.66	0.402	39.92
Ephemerellidae	0.034	1.59	2.20	0.55	0.058	46.68
Simuliidae	0.033	1.47	2.43	0.93	0.254	53.2
Leuctridae	0.032	1.65	2.86	1.21	0.057	59.49
Elmidae	0.027	2.05	1.68	0.20	0.018	64.77
Hydropsychidae	0.025	1.58	2.27	0.97	0.283	69.82
Dixidae	0.025	0.79	1.41	0.00	0.006	74.73
Nemouridae	0.021	1.76	1.40	1.05	0.266	78.97
Caenidae	0.021	1.25	1.29	0.28	0.057	83.2
Perlodidae	0.020	1.49	1.26	0.20	0.041	87.25
Rhyacophilidae	0.016	1.17	0.91	0.00	0.006	90.32
Leptophebiidae	0.015	0.66	0.57	0.28	0.491	93.4
Culicidae	0.011	1.09	0.57	0.40	0.952	95.64
Tipulidae	0.006	0.49	0.40	0.00	0.006	96.9
Perlidae	0.006	0.49	0.35	0.00	0.006	98.1
Hydrophilidae	0.006	0.49	0.35	0.00	0.006	99.3
Polycentropodidae	0.004	0.49	0.20	0.00	0.006	100

1053 Average, Average dissimilarity; Ratio, Average dissimilarity / SD; Cumulative contribution,
 1054 contribution to overall dissimilarity between communities of two groups

1055

1056 **Table S8:** Similarity Percentage Analysis (SIMPER), based on Bray-Curtis dissimilarity index
 1057 on abundance data of macroinvertebrate families between C₁ (without benthic fish or
 1058 crayfish) and T₃ (high density treatment, benthic fishes and 12 crayfish) at the end of the
 1059 study.

Families	Average	Ratio	Average abundance		p-value	Cumulative contribution
			C ₁	T ₃		
Heptageniidae	0.098	1.63	9.27	4.56	0.092	15.33
Gammaridae	0.072	5.41	3.65	0.20	0.012	26.56
Chironomidae	0.051	1.23	2.75	1.89	1	34.5
Baetidae	0.047	1.63	2.97	1.11	0.472	41.86
Hydropsychidae	0.046	6.80	2.27	0.00	0.012	49.08
Simuliidae	0.045	1.59	2.43	0.28	0.036	56.04
Ephemerellidae	0.043	1.67	2.20	0.28	0.046	62.69
Leuctridae	0.033	1.47	2.86	1.32	0.799	67.87
Elmidae	0.031	2.06	1.68	0.20	0.053	72.67
Dixidae	0.028	0.80	1.41	0.00	0.012	77.1
Nemouridae	0.027	1.16	1.40	0.00	0.012	81.3
Perlodidae	0.026	1.65	1.26	0.00	0.012	85.36
Caenidae	0.025	1.19	1.29	0.00	0.012	89.28
Rhyacophilidae	0.018	1.17	0.91	0.00	0.012	92.04
Leptophebiidae	0.015	0.49	0.57	0.00	0.012	94.41
Culicidae	0.011	0.80	0.57	0.00	0.012	96.08
Tipulidae	0.007	0.49	0.40	0.00	0.012	97.20
Perlidae	0.007	0.49	0.35	0.00	0.012	98.29
Hydrophilidae	0.007	0.49	0.35	0.00	0.012	99.37
Polycentropodidae	0.004	0.49	0.20	0.00	0.012	100

1060 Average, Average dissimilarity; Ratio, Average dissimilarity / SD; Cumulative contribution,
 1061 contribution to overall dissimilarity between communities of two groups

1062

1063 **Table S9:** Similarity Percentage Analysis (SIMPER), based on Bray-Curtis dissimilarity index
 1064 on abundance data of macroinvertebrate families between C₂ (benthic fish only) and T₁ (low
 1065 density treatment, benthic fishes and four crayfish) at the end of the study. Bold values indicate
 1066 significant differences.

Families	Average	Ratio	Average abundance		p-value	Cumulative contribution
			C ₂	T ₁		
Heptageniidae	0.065	1.60	8.73	6.27	0.126	16.97
Chironomidae	0.044	1.03	2.93	2.68	0.928	28.45
Simuliidae	0.035	1.32	2.38	1.29	0.465	37.58
Baetidae	0.035	1.41	2.99	1.95	0.428	46.59
Gammaridae	0.030	1.97	3.28	1.68	0.010	54.31
Nemouridae	0.022	1.42	1.36	1.28	0.758	60.01
Ephemerellidae	0.020	1.34	1.41	0.97	0.963	65.28
Hydropsychidae	0.020	1.29	1.43	1.40	0.913	70.49
Perlodidae	0.018	1.49	1.08	0.28	0.116	75.21
Caenidae	0.018	1.19	1.00	0.69	0.757	79.83
Elmidae	0.014	1.20	1.09	0.60	0.367	83.36
Dixidae	0.013	0.79	0.69	0.00	0.026	86.77
Leptophebiidae	0.012	0.69	0.40	0.35	0.992	90.01
Leuctridae	0.011	1.22	2.74	2.40	0.367	93.01
Tipulidae	0.009	0.69	0.40	0.28	0.986	95.46
Culicidae	0.007	0.79	0.40	0.00	0.042	97.22
Rhyacophilidae	0.004	0.49	0.00	0.20	0.968	98.21
Hydrophilidae	0.004	0.49	0.20	0.00	0.042	99.14
Perlidae	0.003	0.49	0.00	0.20	0.990	100

1067 Average, Average dissimilarity; Ratio, Average dissimilarity / SD; Cumulative contribution,
 1068 contribution to overall dissimilarity between communities over time

1069

1070 **Table S10:** Similarity Percentage Analysis (SIMPER), based on Bray-Curtis dissimilarity index
 1071 on abundance data of macroinvertebrate families between C₂ (benthic fish only) and T₂
 1072 (medium density treatment, benthic fishes and eight crayfish) at the end of the study. Bold
 1073 values indicate significant differences.

Families	Average	Ratio	Average abundance		p-value	Cumulative contribution
			C ₂	T ₂		
Heptageniidae	0.075	1.78	8.73	5.79	0.070	16.39
Chironomidae	0.048	1.19	2.93	2.76	1	26.93
Gammaridae	0.048	2.31	3.28	0.97	0.007	37.37
Baetidae	0.041	1.65	2.99	1.66	0.221	46.3
Simuliidae	0.038	1.31	2.38	0.93	0.223	54.62
Leuctridae	0.033	1.69	2.74	1.21	0.040	61.81
Ephemerellidae	0.024	1.32	1.41	0.55	0.266	67.08
Nemouridae	0.023	1.51	1.36	1.05	0.334	72.09
Hydropsychidae	0.022	1.38	1.43	0.97	0.953	76.84
Elmidae	0.020	1.39	1.09	0.20	0.064	81.16
Perlodidae	0.020	1.46	1.08	0.20	0.055	85.43
Caenidae	0.019	1.18	1.00	0.28	0.221	89.57
Rhyacophilidae	0.014	0.79	0.69	0.00	0.007	92.73
Leptophebiidae	0.013	0.69	0.40	0.28	1	95.55
Culicidae	0.010	0.93	0.40	0.40	1	97.65
Tipulidae	0.007	0.49	0.40	0.00	0.007	99.14
Hydrophilidae	0.004	0.49	0.20	0.00	0.007	100

1074 Average, Average dissimilarity; Ratio, Average dissimilarity / SD; Cumulative contribution,
 1075 contribution to overall dissimilarity between communities over time

1076

1077 **Table S11:** Similarity Percentage Analysis (SIMPER), based on Bray-Curtis dissimilarity index
 1078 on abundance data of macroinvertebrate families between C₂ (benthic fish only) and T₃ (high
 1079 density treatment, benthic fishes and 12 crayfish) at the end of the study. Bold values indicate
 1080 significant differences.

Families	Average	Ratio	Average abundance		p-value	Cumulative contribution
			C ₂	T ₃		
Heptageniidae	0.101	1.65	8.73	4.56	0.085	17.14
Gammaridae	0.074	3.35	3.28	0.20	0.006	29.7
Chironomidae	0.061	1.34	2.93	1.89	1	39.96
Baetidae	0.055	1.70	2.99	1.11	0.346	49.21
Simuliidae	0.052	1.31	2.38	0.28	0.037	57.98
Leuctridae	0.035	1.46	2.74	1.32	0.740	63.82
Hydropsychidae	0.033	1.80	1.43	0.00	0.006	69.35
Ephemerellidae	0.031	1.39	1.41	0.28	0.172	74.61
Nemouridae	0.029	1.11	1.36	0.00	0.006	79.51
Perlodidae	0.026	1.71	1.08	0.00	0.006	83.89
Elmidae	0.023	1.37	1.09	0.20	0.120	87.83
Caenidae	0.022	1.13	1.00	0.00	0.006	91.60
Rhyacophilidae	0.017	0.79	0.69	0.00	0.006	94.49
Leptophebiidae	0.012	0.49	0.40	0.00	0.006	96.46
Culicidae	0.009	0.79	0.40	0.00	0.006	97.9
Tipulidae	0.008	0.49	0.40	0.00	0.006	99.22
Hydrophilidae	0.005	0.49	0.20	0.00	0.006	100

1081 Average, Average dissimilarity; Ratio, Average dissimilarity / SD; Cumulative contribution,
 1082 contribution to overall dissimilarity between communities of two groups

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1085 **Table S12:** Pairwise post-hoc comparisons of stable isotopes ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) in muscle
 1086 among different groups of bullhead (W, wild; C₂, bullhead control; T₁, T₂ and T₃ are low,
 1087 medium and high crayfish density treatments respectively). Bold values indicate significant
 1088 differences.

Groups	$\delta^{15}\text{N}$		$\delta^{13}\text{C}$	
	z	p	z	p
W vs. C ₂	0.06	1.000	-0.61	0.972
T ₁ vs. C ₂	-1.82	0.347	4.96	<0.001
T ₂ vs. C ₂	-2.79	0.039	3.53	0.003
T ₃ vs. C ₂	-5.38	<0.001	4.97	<0.001
T ₂ vs. T ₁	-0.71	0.952	-1.42	0.600
T ₁ vs. T ₃	3.54	0.003	-0.02	1.000
T ₂ vs. T ₃	2.83	0.035	-1.44	0.589
W vs. T ₁	1.06	0.818	-3.47	0.005
W vs. T ₂	1.48	0.563	-2.65	0.050
W vs. T ₃	3.12	0.015	-3.48	0.004

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1091 **Table S13:** Chlorophyll- α and leaf litter responses between groups (C₁, control without fish
 1092 and crayfish; C₂, bullhead control; T₁, T₂ and T₃ are low, medium and high crayfish density
 1093 treatments respectively). Bold values indicate significant differences.

Groups	ANOVA post-hoc (<i>p</i> -value)		Effect size ¹ (Hedges' <i>g</i>) [95% CI]	
	Chlorophyll- α	Leaf-litter	Chlorophyll- α	Leaf litter
C ₁ vs. C ₂	0.839	1.00	0.26 (S) [-1.18 to 1.70]	-0.01 (N) [-0.55 to 0.53]
C ₁ vs. T ₁	<0.001	0.048	2.60 (L) [-0.47 to 5.67]	-1.27 (L) [-3.43 to 0.89]
C ₁ vs. T ₂	<0.001	0.008	3.42 (L) [-0.003 to 6.85]	-1.60 (L) [-4.06 to 0.86]
C ₁ vs. T ₃	<0.001	<0.001	2.03 (L) [1.31 to 2.76]	-2.82 (L) [-5.31 to -0.33]
C ₂ vs. T ₁	<0.001	0.050	0.31 (S) [0.12 to 0.50]	-1.44 (L) [-3.73 to 0.86]
C ₂ vs. T ₂	<0.001	0.010	2.04 (L) [-0.31 to 4.40]	-1.80 (L) [-4.34 to 0.75]
C ₂ vs. T ₃	<0.001	<0.001	2.85 (L) [0.05 to 5.65]	-2.84 (L) [-4.58 to -1.11]
T ₁ vs. T ₂	0.078	0.970	1.51 (L) [-0.29 to 3.32]	-0.24 (S) [-0.68 to 0.19]
T ₁ vs. T ₃	<0.001	0.388	4.59 (L) [0.15 to 9.03]	-0.83 (L) [-2.45 to 0.80]
T ₂ vs. T ₃	0.205	0.783	1.65 (L) [-0.34 to 3.64]	-0.53 (M) [-1.80 to 0.73]

1094 ¹Effect size: L, large; M, medium; N, negligible; S, small

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