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3 **Consistent patterns of trophic niche specialisation in host populations infected with a**  
4 **non-native copepod parasite**

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15 Running title: Niche specialisation in parasitized fish

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23 **Summary**

24

25 Populations of generalist species often comprise of smaller sub-sets of relatively specialised  
26 individuals whose niches comprise small sub-sets of the overall population niche. Here, the  
27 role of parasite infections in trophic niche specialisation was tested using five wild fish  
28 populations infected with the non-native parasite *Ergasilus briani*, a copepod parasite with a  
29 direct lifecycle that infects the gill tissues of fish hosts. Infected and uninfected fishes were  
30 sampled from the same habitats during sampling events. Prevalence in the host populations  
31 ranged between 16 and 67 %, with parasite abundances of up to 66 parasites per fish.  
32 Although pathological impacts included hyperplasia and localised haemorrhaging of gill  
33 tissues, there were no significant differences in the length, weight and condition of infected  
34 and uninfected fishes. Stable isotope analyses ( $\delta^{13}\text{C}$ ,  $\delta^{15}\text{N}$ ) revealed that the trophic niche of  
35 infected fishes, measured as standard ellipse area (i.e. the isotopic niche), was consistently  
36 and significantly smaller compared to uninfected conspecifics. These niches of infected fishes  
37 always sat within that of uninfected fish, suggesting trophic specialisation in hosts. These  
38 results suggested trophic specialisation is a potentially important non-lethal consequence of  
39 parasite infection that results from impaired functional traits of the host.

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41 Key words: Non-native parasite, stable isotope analysis, *Rutilus rutilus*, *Abramis brama*,  
42 niche constriction, *Ergasilus briani*

43

## 44 **Key Findings**

- 45 • Measured impact of *Ergasilus briani* in cyprinid fishes
- 46 • Across three sites, parasite prevalence to 67 % and abundance 66 parasites
- 47 • No significant differences in length and condition of infected and uninfected fishes
- 48 • Significantly smaller trophic niches in infected versus uninfected fishes
- 49 • Niches of infected fishes sat within those of uninfected, suggesting diet specialisation.

50

## 51 **Introduction**

52

53 Infections by parasites can have considerable consequences for their free-living hosts,  
54 including alterations in habitat utilisation, and foraging and anti-predator behaviours (Barber  
55 *et al.*, 2000; Lefevre *et al.*, 2009). There remains relatively limited knowledge regarding the  
56 mechanistic basis of these alterations (Clerc *et al.*, 2015), with this also reflected in aspects of  
57 their ecological consequences (Lefevre *et al.*, 2009). It is, however, well established that  
58 parasites can have considerable consequences for food web ecology (e.g. Lafferty *et al.*,  
59 2006; Wood *et al.*, 2007), with the trophic consequences of infections resulting from both  
60 manipulative parasites affecting the strength of trophic links involved in transmission, and  
61 from non-manipulative parasites that impair the functional traits of hosts (Hernandez &  
62 Sukhdeo, 2008; Britton & Andreou, 2016). For example, sticklebacks *Gasterosteus aculeatus*  
63 infected with *Schistocephalus solidus* preferentially ingest smaller prey items of lower quality  
64 compared with uninfected sticklebacks (Cunningham *et al.*, 1994; Jakobsen *et al.*, 1988,  
65 Milinski, 1984). Thus, parasite infections can restrict the prey handling and ingestion abilities  
66 of hosts and/ or reduce the ability of hosts to compete for larger prey items with uninfected  
67 individuals due to factors including energetic constraints that result in shifts in competition

68 symmetry between the infected and uninfected individuals (Barber *et al.*, 2000; Britton,  
69 2013).

70

71 Populations of generalist species are increasingly recognised as comprising smaller sub-sets  
72 of relatively specialised individuals whose niches are then small sub-sets of the overall  
73 population niche (Bolnick *et al.*, 2007; Bolnick *et al.*, 2003; Quevedo *et al.*, 2009). Empirical  
74 studies and foraging models suggest intraspecific competition increases individual trophic  
75 specialisation (Huss *et al.*, 2008; Svanback & Persson, 2004). Whilst other drivers of trophic  
76 specialisation include increased interspecific competition, the exploitation of new ecological  
77 opportunities, and the direct and indirect consequences of predation, there has been little  
78 consideration of how natural enemies, such as parasites, affect the magnitude of individual  
79 trophic specialisation (Araujo *et al.*, 2011; Britton & Andreou, 2016). This is despite the  
80 evidence already outlined that infections can alter host foraging behaviours and diet  
81 composition. Correspondingly, should parasite infections increase levels of competition for  
82 infected individuals then the niche variation hypothesis predicts that their sub-set of the  
83 population would become more specialised in their diet (Van Valen, 1965). Conversely,  
84 under increasing resource competition, a shift to a larger trophic niche by these infected  
85 individuals might maintain their energy requirements (Svanback & Bolnick, 2007).

86

87 Consequently, the aim of this study was to identify how the infection of a model parasite  
88 species affects host populations in relation to their trophic niche size and the magnitude of  
89 individual trophic specialisation. The objectives were to: (1) quantify the parasite prevalence,  
90 abundance, histopathology and energetic consequences of the model parasite on two fish  
91 species over five populations; (2) assess the trophic niche size of each fish population, and  
92 those of the two sub-sets of each population: uninfected and infected with the parasite; and

93 (3) assess these outcomes in relation to niche theory and individual trophic specialisation.  
94 The model parasite was *Ergasilus briani*, a non-native copepod gill parasite that originates  
95 from Southeast Asia and was first recorded in Great Britain in 1982 (Alston & Lewis, 1994).  
96 It has a direct lifecycle that involves fish as its only host, with typical hosts in its invasive  
97 range being roach *Rutilus rutilus* and common bream *Abramis brama* of < 100 mm (Alston  
98 and Lewis 1994). The hypothesis tested is that infected individuals will have a reduced  
99 trophic niche size compared with uninfected con-specifics and have impaired growth rates  
100 and energetics.

101

## 102 **Materials and Methods**

103

### 104 *Sample collection and initial data collection*

105 Three freshwater study sites were selected in Southern England where *Ergasilus briani*  
106 infections were known to be present in the fish community and infecting *R. rutilus* and *A.*  
107 *brama*. The Basingstoke canal (Site 1; 51.276414N, 0.820642W) was historically  
108 supplemented with cyprinid fish through stocking but now has a self-sustaining fish  
109 community; it is of 6 to 10 m in width and maximum depth 2.5 m. Henleaze Lake (Site 2;  
110 51.49763N, 2.603867W) is a narrow lake in former quarry of 450 m in length, up to 8 m in  
111 width and with depths to 6 m. It had been previously stocked with carp *Cyprinus carpio*, *A.*  
112 *brama* and *R. rutilus*, with the latter two species now self-sustaining. Darwell reservoir (Site  
113 3; 50.963617N, 0.440719E) is a water supply reservoir of approximately 63 hectares where  
114 the fish community is dominated by *R. rutilus*, perch *Perca fluviatilis* and pike *Esox lucius*. It  
115 was the stocking activities on each site in the 1980s and 1990s that resulted in *E. briani*  
116 introduction.

117

118 The sampling methodology used at each site varied according to the physical habitat. At Site  
119 1, samples of *A. brama* were collected in October 2012 and samples of *R. rutilus* in October  
120 2014 using a combination of use of a 25 x 2.7 m micromesh seine net and electric fishing.  
121 Samples of *R. Rutilus* and *A. brama* were collected from Site 2 in October 2013 using the  
122 micromesh seine net. At Site 3, samples of *R. rutilus* were available from a fishery sampling  
123 programme that captured these fish using a gill net of 30 x 2.5 m and mesh size 33 mm (knot  
124 to knot). Following their capture at all sites, all fish were initially retained in water-filled  
125 containers and for *R. rutilus* and *A. brama*, a random sub-sample of a minimum of 30  
126 individuals per species was taken and transported to the laboratory for processing.

127

128 In the laboratory, all fish were euthanized (anaesthetic overdose; MS-222), with weight (W;  
129 to 0.01 g), and fork length (L; nearest mm) recorded. A detailed post-mortem was then  
130 conducted on each individual *R. rutilus* and *A. brama* for detecting the presence of infections  
131 of native and non-native parasites using a standard protocol adapted from Hoole et al. (2001).  
132 Skin scrapes and internal organs were examined with aid of low and high power microscopy  
133 to enable parasite identification. Gill arches from both gill cavities were removed and  
134 examined under low power for parasites, including *E. brianii*. Following detection of a  
135 parasite, identification was to species level where possible. When *E. brianii* was detected as  
136 present in a fish then their intensity of infection was recorded (i.e. number of parasites  
137 present). Hereafter, where an individual *R. rutilus* or *A. brama* is referred to as either infected  
138 or non-infected, it refers to the presence/ absence of *E. brianii* in that individual during this  
139 process. Gill tissue from infected and uninfected individuals was retained and prepared for  
140 histopathology. On completion of the post-mortem, a sample of dorsal muscle was taken  
141 from a proportion of the fish samples (sample sizes 6 to 15 per sub-set of fish per population).  
142 These samples were then dried at 60°C to constant weight.

143 The dried samples were then analysed at the Cornell Isotope Laboratory, New York, USA.  
144 They were ground to powder and weighed precisely to  $\approx 1000 \mu\text{g}$  in tin capsules and analysed  
145 on a Thermo Delta V isotope ratio mass spectrometer (Thermo Scientific, USA) interfaced to  
146 a NC2500 elemental analyser (CE Elantach Inc., USA). Verification for accuracy was against  
147 internationally known reference materials, whose values are determined by the International  
148 Association of Atomic Energy (IAEA; Vienna, Austria), and calibrated against the primary  
149 reference scales for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  (Cornell University Stable Isotope Laboratory, 2015). The  
150 accuracy and precision of the sample runs was tested every 10 samples using a standard  
151 animal sample (mink). The overall standard deviation was 0.11 ‰ for  $\delta^{15}\text{N}$  and 0.09 for  $\delta^{13}\text{C}$ .  
152 Linearity correction accounted for differences in peak amplitudes between sample and  
153 reference gases ( $\text{N}_2$  or  $\text{CO}_2$ ). Analytical precision associated with the  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  sample  
154 runs was estimated at 0.42 and 0.15 ‰, respectively. The initial data outputs were in the  
155 format of delta ( $\square$ ) isotope ratios expressed per thousand (‰). There was no lipid correction  
156 applied to the data as C:N ratios indicated very low lipid content and thus lipid extraction or  
157 normalization would have little effect on  $\delta^{13}\text{C}$  (Post *et al.*, 2007).

158

### 159 *Histopathology*

160 Histopathology of gill tissues was completed to assess the pathological changes associated  
161 with *E. briani* infection. Sections of gill were fixed in Bouins fixative for 24 hours before  
162 transferring to 70% Industrial Methylated Spirit. The tissues were trimmed, dehydrated in  
163 alcohol series, cleared and then embedded in paraffin wax. Transverse and longitudinal  
164 sections of  $3\mu\text{m}$  were dried at  $50^\circ\text{C}$ , stained using Mayer's haematoxylin and eosin, and  
165 examined microscopically for pathological changes and described accordingly.

166

167

168 *Data analyses*

169 Infection levels of *E. briani* in *R. rutilus* and *A. brama* were described as their prevalence  
170 (number of infected individuals/total number of individuals x 100) and abundance (number of  
171 *E. briani* per host).

172

173 The stable isotope data of *R. rutilus* and *A. brama* were used to assess their trophic niche size.  
174 Trophic niche size was calculated using the metric standard ellipse area (SEA) and thus  
175 represented the isotopic niche. Whilst the isotopic niche is closely related to the trophic niche,  
176 it is also influenced by factors including growth rate and metabolism, and thus is used only as  
177 a proxy of the trophic niche (Jackson *et al.*, 2011). Standard ellipse areas were calculated in  
178 the SIAR and SIBER packages (Jackson *et al.*, 2011) in the R computing program (R Core  
179 Team 2013). Standard ellipse areas are bivariate measures of the distribution of individuals in  
180 trophic space. As each ellipse encloses  $\approx 40\%$  of the data, they represent the core dietary  
181 breadth and thus reveal the typical resource use within a species or population (Jackson *et al.*,  
182 2011, 2012). Due to small sample sizes, then a Bayesian estimate of SEA ( $SEA_B$ ) was used  
183 for testing differences in niche size between analysed groups, with this calculated using a  
184 Markov chain Monte Carlo simulation with  $10^4$  iterations for each group (Jackson *et al.*,  
185 2011; R Core Team 2014; Tran *et al.*, 2015). This generated 95 % confidence intervals  
186 around the  $SEA_B$  estimates and thus where these confidence intervals did not overlap  
187 between comparator groups, the niche sizes were interpreted as significantly different. For  
188 each population of *R. rutilus* and *A. brama* in each site,  $SEA_B$  was calculated for two sub-sets  
189 of individuals: those infected with *E. briani* and those uninfected. In addition, to enable  
190 calculation of the extent of the overlap of niches within each species,  $SEAc$  had to be  
191 calculated. The overlap in niche size was calculated as the extent to which the respected  
192 niches shared isotopic space (%).



193 *Statistical analyses*

194 For each fish species and population infected with *E. brianii*, differences between the infected  
195 and uninfected hosts were tested for length using ANOVA, and their stable isotopes of  $\delta^{13}\text{C}$   
196 and  $\delta^{15}\text{N}$  using Mann Whitney U tests. Condition was calculated as Fulton's Condition Factor  
197 K, where  $K = 100 \times W/L^3$ , where L was measured in cm, with differences between infected  
198 and uninfected fishes also tested using Mann Whitney U tests. Differences in weight between  
199 the infected and uninfected fish per population and species were then tested in a generalized  
200 linear model (GLM) where the effect of length on weight was controlled as a co-variate;  
201 outputs included estimated marginal means of weight controlled for length for each sub-set of  
202 fish and the significance of their differences through pairwise comparisons with Bonferroni  
203 correction for multiple comparisons. Other than the stable isotope mixing models, all  
204 analyses were completed in SPSS v. 22.0. In all analyses, where parametric tests were used,  
205 the assumptions of normality of residuals and homoscedasticity were checked, and response  
206 variables were log-transformed to meet the assumption if necessary.

207

208 **Results**

209

210 *Parasite prevalence and abundance, and effect on fish length and weight*

211 Prevalence and mean parasite abundance was highest at Site 1 for both fishes, with the  
212 maximum abundance recorded being 66 *E. brianii* in an individual *R. rutilus* (Table 1). The  
213 native parasites recorded were 13 species that would be considered as the expected parasite  
214 fauna of these fishes in a British community. These parasites were recorded at levels that  
215 were considered as not high enough to cause clinical pathology (Hoole et al., 2001) and  
216 included species of Diplozoa, Piscicola, Dactylogyrus and Myxosporida. Across the dataset,  
217 there was no relationship between *E. brianii* abundance and their number of native parasites

218 per host (*R. rutilus*:  $R^2 = 0.02$ ,  $F_{1,94} = 0.49$ ,  $P > 0.05$ ; *A. brama*:  $R^2 = 0.03$ ,  $F_{1,94} = 0.52$ ,  $P >$   
219  $0.05$ ). At Site 1, the non-native parasite *Ergasilus sieboldi* was also detected in the gills of  
220 two *A. brama*. Due to the potential for *E. sieboldi* to confound subsequent analyses, these fish  
221 were omitted from the dataset.

222

223 Differences in fish lengths between the infected and uninfected fish were not significant at  
224 any site (ANOVA: Site 1: *R. rutilus*  $F_{1,19} = 0.11$ ,  $P > 0.05$ ; *A. brama*  $F_{1,29} = 0.01$ ,  $P > 0.05$ , Site  
225 2: *R. rutilus*  $F_{1,14} = 0.84$ ,  $P > 0.05$ ; *A. brama*  $F_{1,15} = 0.42$ ,  $P > 0.05$ , Site 3: *R. rutilus*  $F_{1,19} =$   
226  $0.01$ ,  $P > 0.05$ ; Table 2). Similarly, there were no significant differences between the body  
227 weight of infected and uninfected fish at any site when the effect of total length was  
228 controlled (GLM: Site 1: *A. brama*: Wald  $\chi^2 = 1.27$ ,  $P > 0.05$ ; *R. rutilus* Wald  $\chi^2 = 0.91$ ,  $P >$   
229  $0.05$ ; Site 2: *A. brama*: Wald  $\chi^2 = 0.001$ ,  $P > 0.05$ ; *R. rutilus*: Wald  $\chi^2 = 0.67$ ,  $P > 0.05$ ), or in  
230 Fulton's condition factor, K (Mann Whitney U tests: Site 1: *A. brama*:  $Z = 1.16$ ,  $P > 0.05$ ; *R.*  
231 *rutilus*  $Z = 0.83$ ,  $P > 0.05$ ; Site 2: *A. brama*:  $Z = 0.82$ ,  $P > 0.05$ ; *R. rutilus*:  $Z = 0.48$ ,  $P > 0.05$ ).

232

### 233 *Histopathology*

234 Histopathological examinations revealed consistent pathological changes associated with *E.*  
235 *briani* infection. Parasites attached to the ventral surface of the gill filament, between the  
236 hemibranchs, tight to the interbranchial septum. Whilst dissection of the gill was needed to  
237 confirm the presence of *E. briani*, their egg strings were often visible prior to removal of the  
238 gills (Fig. 1a). During attachment, the parasite's antennae (Fig. 1b) were used to engulf the  
239 base of the gill filaments, bringing the head of the parasite tight to the gill septum (Fig. 1c,d).  
240 This frequently led to displacement and distortion of filaments to accommodate the body of  
241 the parasite (Fig. 1c-e). Parasite attachment led to compression of the gill tissue, with  
242 flattening of the epithelium (Fig 1d,e). This was often accompanied by hyperplasia, localised

243 haemorrhaging, epithelial erosion and compression of blood vessels underlying the body of  
244 the parasite (Fig 1e). Although no direct evidence for parasite feeding was observed,  
245 localised loss and compression of gill epithelium was often apparent adjacent to the mouth  
246 (Fig. 1f).

247

#### 248 *Stable isotope metrics*

249 The differences in the mean values of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  between the infected and uninfected fish  
250 were not significant for any of the species at any site (Mann Whitney U test:  $\delta^{13}\text{C}$ : Site 1: *A.*  
251 *brama*  $Z = 0.57$ ,  $P > 0.05$ ; *R. rutilus*  $Z = 0.23$ ,  $P > 0.05$  Site 2: *A. brama*  $Z = 1.19$ ,  $P > 0.05$ ;  
252 *R. rutilus*  $Z = 1.80$ ,  $P > 0.05$ ; Site 3: *R. rutilus*  $Z = 0.01$ ,  $P > 0.05$ ;  $\delta^{15}\text{N}$ : Site 1: *A. brama*  $Z =$   
253  $0.57$ ,  $P > 0.05$ ; *R. rutilus*  $Z = 0.16$ ,  $P > 0.05$ ; Site 2: *A. brama*  $Z = 1.30$ ,  $P > 0.05$ ; *R. rutilus*  $Z$   
254  $= 1.03$ ,  $P > 0.05$ ; Site 3: *R. rutilus*  $Z = 1.48$ ,  $P > 0.05$ ) (Table 2). There was, however, a  
255 consistent pattern of trophic (isotopic) niche size being considerably higher in the uninfected  
256 sub-set of fish when compared to their infected conspecifics (Table 3; Fig. 2). The extent of  
257 the overlap between the trophic niches of each sub-set of the populations was high, with  
258 infected *A. brama* sharing 95 and 100 % of trophic space with uninfected *A. brama* in Sites 1  
259 and 2 respectively, and infected *R. rutilus* shared 91, 69 and 73 % of trophic niche space with  
260 uninfected *R. rutilus* in Sites 1, 2 and 3 respectively (Fig. 2-4). Where *R. rutilus* and *A. brama*  
261 were present in sympatry at Site 2, there was minimal overlap in the trophic niches of their  
262 uninfected individuals (16.7 %), but this increased between their infected sub-sets of  
263 individuals (89.2 %) (Table 3; Fig. 3).

264

265

266 **Discussion**

267

268 Across the *R. rutilus* and *A. brama* infected by *E. briani*, infections by native parasites were  
269 relatively minor, were not associated with major pathological changes and were no related to  
270 *E. briani* infection. In contrast, infections in the gills of both fishes by *E. briani* resulted in  
271 gross pathological changes characterised by displacement gill filaments, loss and  
272 compression of epithelium, hyperplasia and localised haemorrhaging within the filaments as a  
273 consequence of parasite attachment. This is consistent with pathological changes associated  
274 with other ergasilid parasites (Alston & Lewis, 1994; Dezfuli *et al.*, 2003). When the trophic  
275 niche widths of infected and uninfected fishes were compared, these revealed a general and  
276 consistent pattern of trophic niche constriction in the infected fishes, as per the hypothesis,  
277 suggesting that the infected fishes were consuming specific food items that were also within  
278 the dietary range of uninfected individuals. Despite this diet specialisation resulting in the  
279 trophic niche of infected individuals overlapping with the niche width of the subset of the  
280 infected individuals of the other species, this dietary specialisation appeared sufficient to  
281 maintain their energetic requirements, given that infection did not adversely affect their  
282 individual condition, contrary to the hypothesis. Whilst it would have been advantageous to  
283 then investigate the diet of each sub-set of fish using, for example, stable isotope mixing  
284 models (Phillips *et al.* 2005), the putative food resource data collected at the sites did not  
285 enable adequate separation of dietary resources when applied to mixing models in SIAR  
286 (Jackson *et al.* 2011, 2012).

287

288 Optimum foraging theory models typically assume that individuals rank alternative resources  
289 according to their energetic value per unit handling time, with this dependent on the resource  
290 traits and phenotypic capacity of individuals to capture, handle and to digest those resources

291 (Araujo *et al.*, 2011). This suggests individuals will feed on the most valuable resources,  
292 ignoring lower-value resources when search and handling time could be better spent  
293 searching for more valuable ones (Bolnick *et al.*, 2003). Thus, niche variation between  
294 individuals is largely dependent on the diversity and abundance of available resources versus  
295 the phenotypic traits of the individual (Crowden & Broom, 1980; Stephens & Krebs, 1986).  
296 Our outputs, revealing that infected fishes had increased specialisation in their trophic niche,  
297 it can be argued that this was associated with the phenotypic changes resulting from the  
298 infection pathology. However, given that the study was based on field studies rather than  
299 manipulative experiments and thus identified correlative relationships rather than definitive  
300 causal mechanisms, then an alternative explanation for the patterns detected in the data was  
301 that dietary specialisations was a driver of infection (Pegg *et al.*, 2015; Britton & Andreou,  
302 2016). Indeed, contrasting parasite infections can develop between individuals in a  
303 population as a consequence of existing trophic niche specialisations (Britton & Andreou,  
304 2016). This is because dietary specialisations that result from variability in, for example, fish  
305 body and/ or gape size, or their habitat utilisation (e.g. littoral versus open-water), can elevate  
306 the exposure of individuals to some parasites (Bolnick *et al.* 2003; Britton & Andreou, 2016).  
307 Although more often associated with parasites with complex lifecycles, where dietary  
308 specialisations increase the exposure to intermediate hosts (Pegg *et al.*, 2015), differences in  
309 the behavioural traits of fish can also result in the acquisition of higher numbers of copepod  
310 parasites (Poulin *et al.*, 1991). Consequently, whilst we suggest that infection was the driver  
311 of the dietary specialisation due to the histopathological impacts of infection detected, that  
312 specialisation in habitat use, diet or behaviour was a driver of *E. brianii* infection cannot be  
313 ruled out. It is recommended that experimental studies completed in more controlled  
314 conditions are used to test this in future.

315

316 That *E. briani* infection was the driver of trophic niche specialisation, rather than a  
317 consequence, is supported by a number of studies that have revealed parasites impact host  
318 foraging efficiency through a variety of physiological, pathological and behavioural  
319 mechanisms (Britton & Andreou, 2016). These infection consequences resulting in, for  
320 example, altered time budgets through increased time spent foraging (Giles 1983; Barber *et*  
321 *al.*, 1995; Britton & Andreou, 2016), and alterations in diet composition compared with non-  
322 infected individuals (Milinski, 1984). Moreover, in other animals infected with gill parasites,  
323 shifts in heart rate and oxygen consumption have been recorded (Schuwerack *et al.*, 2001),  
324 along with reduced haemoglobin levels (Montero *et al.*, 2004), which impact swimming  
325 efficacy (Duthie & Hughes, 1987) and the ability to maintain normal intestinal function while  
326 swimming (Thorarensen *et al.*, 1993). In other Ergasilid parasites, gill damage also results in  
327 respiratory dysfunction, osmoregulatory failure, and haematological disruption (e.g.  
328 Abdelhalim *et al.*, 1991, Alston & Lewis, 1994, Dezfuli *et al.*, 2003). As a consequence of  
329 these studies, we thus speculate that the infected fishes in our study increased their predation  
330 of prey that were highly abundant and/ or relatively slow moving, and thus required relatively  
331 low energy expenditure to capture and handle during foraging, as a consequence of some  
332 energetic costs associated with infection. However, this was not quantified experimentally in  
333 our study and thus is another recommendation for further work.

334

335 For predator populations containing infected individuals, whilst specialisation may be  
336 beneficial at the population level as it appears to facilitate the survival of infected individuals  
337 despite the pathological impacts incurred (Lomnicki, 1988), the sub-set of specialised  
338 individuals might be at greater risk from external pressures (Durell, 2000). For example, the  
339 increased time spent foraging and/ or the utilisation of different habitats to preferentially  
340 forage on specific prey items, allied with the potential for their anti-predator behaviours being

341 modified, might result in increased predation risk (Barber *et al.*, 2000; Lafferty, 1999; Ward  
342 *et al.*, 2002). Indeed, when infected with *S. solidus*, three-spined stickleback *G. aculeatus*  
343 spend more time foraging as a compensatory mechanism (Giles, 1987), resulting in a trade-  
344 off with anti-predator behaviours (Giles, 1983), and thus incurring a greater likelihood of  
345 being predated by a piscivorous bird (Milinski, 1985). Similarly, infected banded killifish  
346 *Fundulus diaphanous* are more likely to occupy the front of shoals, a position that optimises  
347 feeding opportunities but also carries the greatest risk of predation (Ward *et al.*, 2002).

348

349 The focal parasite of this study, *E. brianii*, is an introduced parasite to the UK, arriving as a  
350 consequence of fish being moved within aquaculture and fisheries (Fryer & Andrews, 1983).  
351 It thus represents a parasite that was successfully introduced into the UK, despite such  
352 movements often resulting in non-native parasites failing to establish through, for example,  
353 enemy release (Sheath *et al.*, 2015). The consequences of introduced parasites within native  
354 communities can be varied, but can result in disease outbreaks resulting in high fish losses.  
355 For example, the rosette agent *Sphareothecum destruens*, spread via the invasive topmouth  
356 gudgeon *Pseudorasbora parva*, can cause high mortality rates in naïve fishes (Andreou *et al.*,  
357 2012) and the impact of the introduced *Gyrodactylus salaris* in Norway was the collapse of  
358 wild salmon populations in 45 Norwegian rivers (Peeler & Thrush, 2004) with an economic  
359 cost the in excess of US \$500,000,000 (Hansen *et al.*, 2003). Whilst the impact of *E. brianii*  
360 here was much less dramatic, our outputs suggested that ecological alterations did occur as a  
361 potential cost of infection, with modification of host diet composition that constricted the  
362 trophic niche of the host component of the population.

363

364 Studies on trophic niche specialisation have identified a range of causal factors, particularly  
365 inter- and intra-specific competitive processes, predation pressure and impact and the

366 exploitation of new ecological opportunities (Araujo *et al.*, 2011). The role of parasitism in  
367 trophic niche specialisation has, conversely, received very little attention (Britton & Andreou,  
368 2016). Consequently, our findings that the trophic niches of individuals infected with *E.*  
369 *briani* were consistently constricted and specialised across five fish populations are  
370 important. They suggest that the host consequences of infection, including pathological  
371 impacts, could also be an important driver of niche constriction that has been largely  
372 overlooked and thus should be incorporated into future studies on the ecological drivers of  
373 trophic niche specialisation.

374

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376

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384

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540 Table 1. Prevalence and abundance of *Ergasilus briani* per site and species

Site	Species	n	Prevalence (%)	Mean abundance of parasites ( $\pm$ SE)	Range of parasite abundance
1	<i>A. brama</i> <sup>1</sup>	45	67	5.71 $\pm$ 0.89	0 - 21
1	<i>R. rutilus</i> <sup>2</sup>	40	63	6.20 $\pm$ 2.09	0 - 66
2	<i>A. brama</i>	32	19	1.63 $\pm$ 0.85	0 - 16
2	<i>R. rutilus</i>	44	16	0.89 $\pm$ 0.46	0 - 21
3	<i>R. rutilus</i>	64	17	0.40 $\pm$ 0.13	0 - 6

541 <sup>1</sup>Sampled October 2012

542 <sup>2</sup>Sampled October 2014



Table 2. Sample sizes, mean lengths of subsampled fish and their mean stable isotope data at each study site.

Site	Species	n	Mean length (mm)	Mean $\delta^{13}\text{C}$ (‰)	Mean $\delta^{15}\text{N}$ (‰)
1	Uninfected <i>A. brama</i>	15	39.6 ± 3.0	-35.25 ± 0.46	16.06 ± 0.93
	Infected <i>A. brama</i>	15	39.5 ± 2.4	-35.40 ± 0.67	16.46 ± 0.81
1	Uninfected <i>R. rutilus</i>	10	64.4 ± 23.9	-35.73 ± 1.66	14.44 ± 0.82
	Infected <i>R. rutilus</i>	6	69.0 ± 24.0	-35.54 ± 0.61	13.92 ± 0.35
2	Uninfected <i>A. brama</i>	10	87.3 ± 14.9	-33.36 ± 0.69	15.74 ± 0.53
	Infected <i>A. brama</i>	6	102.7 ± 50.2	-33.08 ± .020	16.09 ± 0.17
2	Uninfected <i>R. rutilus</i>	10	100.1 ± 22.1	-32.23 ± 1.44	15.37 ± 0.78
	Infected <i>R. rutilus</i>	7	94.3 ± 14.9	- 31.10 ± 1.87	14.64 ± 1.37
3	Uninfected <i>R. rutilus</i>	10	123.5 ± 24.2	-22.64 ± 1.32	13.24 ± 0.48
	Infected <i>R. rutilus</i>	10	122.7 ± 23.4	-22.43 ± 1.08	12.94 ± 0.34

Table 3. Trophic niche width (standard ellipse areas, as  $SEA_B$  (95 % confidence intervals) and  $SEAc$  of the uninfected and infected sub-sets of fish per site, and their relative size and extent of isotopic niche overlap between the infected and uninfected sub-sets of fish ( $SEAc$ ).

Site	Species	$SEAc$ uninfected ( $\%{}^2$ )	$SEA_B$ uninfected ( $\%{}^2$ )	$SEAc$ infected ( $\%{}^2$ )	$SEA_B$ infected ( $\%{}^2$ )	Niche overlap (%)
1	<i>A. brama</i>	1.63	1.13 – 1.87	0.67	0.41 – 0.93	94.70
1	<i>R. rutilus</i>	4.71	3.87 – 5.21	0.47	0.29 – 0.76	90.88
2	<i>A. brama</i>	1.18	0.81 – 1.27	0.12	0.09 – 0.19	99.99
2	<i>R. rutilus</i>	4.52	3.96 – 4.98	3.23	2.99 – 3.79	69.31
3	<i>R. rutilus</i>	1.99	1.54 – 2.43	1.26	0.99 – 1.45	73.25

## Figure captions

Figure 1. Pathology of *Rutilus rutilus* infected with *Ergasilus briani*. a) Presence of two *E. briani* (arrows) attached between the gill filaments following removal of the operculum. b) Whole *E. briani* following dissection of the gill tissue, showing antennae used for attachment (arrows). c) Histopathology of *R. rutilus* gill, with attachment of two *E. briani* (\*) tight to interbranchial septum with displacement of filaments. The antennae can be seen engulfing multiple filaments (arrow). d) Compression and distortion of gill tissue (arrow) adjacent to *E. briani*, indicative of forceful attachment to the base of the gill filaments. e) Transverse section through infected gill arch, with multiple *E. briani* (\*) attached between the hemibranchs, with compression and erosion of epithelium, localised haemorrhage (arrow) and displacement of filaments. f) Gill tissue adjacent to *E. briani*, showing epithelial loss and compression, with constriction of blood vessel underlying the parasite (arrow). Normal vessel shown away from the immediate site of parasite attachment (\*).

Figure 2. Trophic niche width (as standard ellipse area, SEAc) of infected and uninfected *Abramis brama* and *Rutilus rutilus* from Site 1. a) *A. brama* sampled May 2012, b) *R. rutilus* sampled October 2014. The black line represents the infected individuals and the grey line represents uninfected individuals.

Figure 3. Trophic niche width (as standard ellipse area, SEAc) of infected and uninfected *Abramis brama* and *Rutilus rutilus* from Site 2. The black line represents the infected individuals and the grey line represents uninfected individuals.

Figure 4. Trophic niche width (as standard ellipse area, SEAc) of infected and uninfected *Abramis brama* and *Rutilus rutilus* from Site 3. The black line represents the infected individuals and the grey line represents uninfected individuals.