Accepted in Parasitology, 02/01/2017

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

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Running title: Niche specialisation in parasitized fish

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Summary

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

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

Key Findings

• Measured impact of *Ergasilus briani* in cyprinid fishes

• Across three sites, parasite prevalence to 67 % and abundance 66 parasites

• No significant differences in length and condition of infected and uninfected fishes

• Significantly smaller trophic niches in infected versus uninfected fishes

• Niches of infected fishes sat within those of uninfected, suggesting diet specialisation.

Introduction

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

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

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

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

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

Materials and Methods

Sample collection and initial data collection

Three freshwater study sites were selected in Southern England where *Ergasilus briani* infections were known to be present in the fish community and infecting *R. rutilus* and *A. brama*. The Basingstoke canal (Site 1; 51.276414N, 0.820642W) was historically supplemented with cyprinid fish through stocking but now has a self-sustaining fish community; it is of 6 to 10 m in width and maximum depth 2.5 m. Henleaze Lake (Site 2; 51.49763N, 2.603867W) is a narrow lake in former quarry of 450 m in length, up to8 m in width and with depths to 6 m. It had been previously stocked with carp *Cyprinus carpio*, *A. brama* and *R. rutilus*, with the latter two species now self-sustaining. Darwell reservoir (Site 3; 50.963617N, 0.440719E) is a water supply reservoir of approximately 63 hectares where the fish community is dominated by *R. rutilus*, perch *Perca fluviatilis* and pike *Esox lucius*. It was the stocking activities on each site in the 1980s and 1990s that resulted in *E. briani* introduction.

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

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

The dried samples were then analysed at the Cornell Isotope Laboratory, New York, USA. They were ground to powder and weighed precisely to ≈1000 μg in tin capsules and analysed on a Thermo Delta V isotope ratio mass spectrometer (Thermo Scientific, USA) interfaced to a NC2500 elemental analyser (CE Elantach Inc., USA). Verification for accuracy was against internationally known reference materials, whose values are determined by the International Association of Atomic Energy (IAEA; Vienna, Austria), and calibrated against the primary reference scales for $\delta^{13}C$ and $\delta^{15}N$ (Cornell University Stable Isotope Laboratory, 2015). The accuracy and precision of the sample runs was tested every 10 samples using a standard animal sample (mink). The overall standard deviation was 0.11 ‰ for $\delta^{15}N$ and 0.09 for $\delta^{13}C$. Linearity correction accounted for differences in peak amplitudes between sample and reference gases (N_2 or CO_2). Analytical precision associated with the $\delta^{15}N$ and $\delta^{13}C$ sample runs was estimated at 0.42 and 0.15 ‰, respectively. The initial data outputs were in the format of delta (□) isotope ratios expressed per thousand (‰). There was no lipid correction applied to the data as C:N ratios indicated very low lipid content and thus lipid extraction or normalization would have little effect on $\delta^{13}C$ (Post *et al*., 2007).

Histopathology

Histopathology of gill tissues was completed to assess the pathological changes associated with *E. briani* infection. Sections of gill were fixed in Bouins fixative for 24 hours before transferring to 70% Industrial Methylated Spirit. The tissues were trimmed, dehydrated in alcohol series, cleared and then embedded in paraffin wax. Transverse and longitudinal sections of 3μm were dried at 50°C, stained using Mayer's haematoxylin and eosin, and examined microscopically for pathological changes and described accordingly.

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 ≈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 (%).

Statistical analyses

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

Results

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

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

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

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

Histopathology

Histopathological examinations revealed consistent pathological changes associated with *E. briani* infection. Parasites attached to the ventral surface of the gill filament, between the hemibranchs, tight to the interbranchial septum. Whilst dissection of the gill was needed to confirm the presence of *E. briani*, their egg strings were often visible prior to removal of the gills (Fig. 1a). During attachment, the parasite's antennae (Fig. 1b) were used to engulf the base of the gill filaments, bringing the head of the parasite tight to the gill septum (Fig. 1c,d). This frequently led to displacement and distortion of filaments to accommodate the body of the parasite (Fig. 1c-e). Parasite attachment led to compression of the gill tissue, with 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 $\delta^{13}C$ and $\delta^{15}N$ between the infected and uninfected fish
250 were not significant for any of the species at any site (Mann Whitney U test: $\delta^{13}C$: 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; $\delta^{15}N$: 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 tropic 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. rutilis* 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

Discussion

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

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

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

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

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

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

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

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

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

Acknowledgements

We thank the fishery owners and managers who gave permission for fish to be sampled on their waters; as well as AES Europe Ltd who assisted with sample collection at site 2; and Ian Wellby and John Wall for assistance with sample collection from site 3. We thank Environment Agency employees for their assistance in obtaining consents for sampling and advising on suitable sites. Views expressed in the paper are those of the authors and not their parent organisations. JP was funded by a studentship from the *Fisheries Society of the British Isles* and Bournemouth University.

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

Site	Species	n	Prevalence (%)	Mean abundance of parasites (± SE)	Range of parasite abundance
1	*A. brama*[1]	45	67	5.71 ± 0.89	0 - 21
1	*R. rutilus*[2]	40	63	6.20 ± 2.09	0 - 66
2	*A. brama*	32	19	1.63 ± 0.85	0 - 16
2	*R. rutilus*	44	16	0.89 ± 0.46	0 - 21
3	*R. rutilus*	64	17	0.40 ± 0.13	0 - 6

[1]Sampled October 2012

[2]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 $\delta^{13}C$ (‰)	Mean $\delta^{15}N$ (‰)
1	Uninfected *A. brama*	15	39.6 ± 3.0	-35.25 ± 0.46	16.06 ± 0.93
	Infected *A. brama*	15	39.5 ± 2.4	-35.40 ± 0.67	16.46 ± 0.81
1	Uninfected *R. rutilus*	10	64.4 ± 23.9	-35.73 ± 1.66	14.44 ± 0.82
	Infected *R. rutilus*	6	69.0 ± 24.0	-35.54 ± 0.61	13.92 ± 0.35
2	Uninfected *A. brama*	10	87.3 ± 14.9	-33.36 ± 0.69	15.74 ± 0.53
	Infected *A. brama*	6	102.7 ± 50.2	-33.08 ± .020	16.09 ± 0.17
2	Uninfected *R. rutilus*	10	100.1 ± 22.1	-32.23 ± 1.44	15.37 ± 0 .78
	Infected *R. rutilus*	7	94.3 ± 14.9	- 31.10 ± 1.87	14.64 ± 1.37
3	Uninfected *R. rutilus*	10	123.5 ± 24.2	-22.64 ± 1.32	13.24 ± 0.48
	Infected *R. rutilus*	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	*A. brama*	1.63	1.13 – 1.87	0.67	0.41 – 0.93	94.70
1	*R. rutilus*	4.71	3.87 – 5.21	0.47	0.29 – 0.76	90.88
2	*A. brama*	1.18	0.81 – 1.27	0.12	0.09 – 0.19	99.99
2	*R. rutilus*	4.52	3.96 – 4.98	3.23	2.99 – 3.79	69.31
3	*R. rutilus*	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.