Context-dependent parasite infection affects trophic niche in populations of sympatric stickleback species

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Abstract

How parasites alter host feeding ecology remains elusive in natural populations. A powerful approach to investigate the link between infection and feeding ecology is quantifying unique and shared responses to parasite infection in related host species within a common environment. Here, 9 pairs of sympatric populations of the three-spined and nine-spined stickleback fishes were sampled across a range of freshwater and brackish habitats to investigate how parasites alter host feeding ecology: (i) biotic and abiotic determinants of parasite community composition, and (ii) to what extent parasite infection correlates with trophic niche specialization of the 2 species, using stable isotope analyses (δ15N and δ13C). It was determined that parasite community composition and host parasite load varied among sites and species and were correlated with dissolved oxygen. It was also observed that the digenean Cyathocotyle sp.’s abundance, a common directly infecting parasite, had a cycle life, correlated with host δ13C in a fish species-specific manner. In 6 sites, correlations were found between parasite abundance and their hosts’ feeding ecology. These effects were location-specific and occasionally host species or host size-specific. Overall, the results suggest a relationship between parasite infection and host trophic niche which may be an important and largely overlooked ecological factor. The population specificity and variation in parasite communities also suggest this effect is multifarious and context-dependent.

Introduction

Parasites are strong selective agents on their hosts, reducing their fitness as well as altering their behaviour, life-history traits and habitat use (Milinski, 1984; Miura et al., 2006; Pagán et al., 2008; Lefèvre et al., 2009; Barber and Huntingford, 1995). Parasites are involved in approximately 75% of food web links (Lafferty et al., 2006), and often exceed total biomass of top predators (Kuris et al., 2008). Despite growing evidence that parasites have a strong impact on food web ecology (Lafferty et al., 2006; Dobson et al., 2009; Anaya-Rojas et al., 2019), their role in altering individual hosts’ trophic niche specialization remains largely unknown (Araújo et al., 2011; Pegg et al., 2015a, 2017; Britton and Androue, 2016; Lockley et al., 2020). Such parasite-mediated effects on trophic niche specialization are unresolved because (i) the effects of parasites on altering host trophic niche may be system-specific, or (ii) parasites with different life-history strategies may alter trophic interactions in different ways. For example, trophically transmitted parasites often have complex life cycles and can manipulate host behaviour, therein modifying trophic interactions (Lafferty et al., 2008; Hammerschmidt et al., 2009). Specifically, through infection and regulation in all trophic levels, including top predators, parasites can induce or inhibit trophic cascades affecting overall ecosystem functioning (Brunner et al., 2017; Anaya-Rojas et al., 2019). It should be noted that hosts are not defenceless, but mounting an immunological response can be energetically costly, and therefore an appropriate feeding strategy is necessary to compensate the costs of infection (Lee et al., 2006; Brunner et al., 2014, 2017).

The theoretical effects of parasite infection on trophic niche specialization have been summarized into 2 main effects: constriction and divergence (Britton and Androue, 2016). Trophic niche constriction occurs when a population subgroup specializes on a restricted breadth of food items that are also consumed by conspecific generalists. Indeed, parasite infection can result in the sub-population of infected hosts being nested within the overall host’s population niche (Britton and Androue, 2016; Pegg et al., 2017; Villalobos et al., 2019). For example, when comparing the trophic niche of the generalist common roach (Rutilus rutilus) and common bream (Abramis brama) infected by Ergasilus briani, infected individuals showed a smaller niche width that is nested within that of uninfected conspecifics (Pegg et al., 2017). Conversely, the overall population niche might expand due to a sub-population of infected individuals shifting diet to food items not previously exploited by conspecifics, resulting in

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trophic niche divergence (Britton and Andreou, 2016). Competitive exclusion of infected individuals would manifest as an overall population niche expansion. For instance, common carp (Cyprinus carpio) infected with the non-native Asian tapeworm (Bothriocephalus acheilognathi) can become competitively disadvantaged upon infection, and alter their diet compared to uninfected conspecifics (Britton et al., 2011; Pegg et al., 2015a).

Identifying whether parasite-induced niche constriction or divergence are a function of environmental conditions represents a logical next step in uncovering the causal mechanisms that underlie the relationship between parasitism and host trophic ecology. Indeed, abiotic environmental conditions such as variation in physico-chemistry affect important aspects of the ecology of host–parasite interactions, including host community structure, and parasite abundance and virulence (Bianar et al., 2009; Karvonen et al., 2013; Mahmud et al., 2017; Deflem et al., 2021). For example, reductions in dissolved oxygen (DO) can induce hypoxic stress in hosts which can result in ineffective immune responses, and is associated with increased parasite diversity and infection load in fish (Lacerda et al., 2018; Ojwala et al., 2018; Abdel-Tawwab et al., 2019). Conversely, acidification of aquatic environments can deplete intermediate host populations as in the case in many trematode parasites and their snail hosts, and is associated with reductions in parasite richness and virulence (Lafferty and Kuris, 1999; Young and MacColl, 2017).

To disentangle the relative contributions of host-related and environmental drivers of parasite-mediated shifts in host trophic niche, studies can focus on natural systems with environmental gradients and multiple related species persisting in a common landscape. In general, if genetically and ecologically similar sympatric species respond in a similar manner, the response is likely constrained by niche availability in the environment. However, if species living in sympathy respond differently to a shared environment, the responses are likely linked to mechanisms unique to a species (Raeymaekers et al., 2017; Bal et al., 2021).

The three-spined (Gasterosteus aculeatus Linnaeus, 1758; Gasterosteidae) and nine-spined (Pungitius pungitius Linnaeus, 1758; Gasterosteidae) sticklebacks are 2 small teleost fishes, which diverged around 26 million years ago (Varadharan et al., 2019). Often found living in sympathy, both species demonstrate examples of overlapping dietary preference and habitat use, similar population genetic structure and parallel phenotypic divergence (Copp and Kovač, 2003; Raeymaekers et al., 2017; Bal et al., 2021). Their macroparasites are well documented, with several parasites able to infect both species, and some that are host-specific (Kalbe et al., 2002; Zander, 2007; Henrich et al., 2013). Responses to infection in both species use a similar set of immune genes (Lenz et al., 2013). The shared ecological context among the species provides a good opportunity to investigate the relative roles of host species, their environment and parasite infection on trophic niche ecology.

Using natural coexisting populations of G. aculeatus and P. pungitius across a range of habitats in Belgium and the Netherlands, we investigated the role of parasites in altering the trophic niche of host species, including (i) quantifying parasite diversity and abundance among host populations and their relationship with abiotic environmental variables, (ii) using stable isotope analysis (SIA; δ15N and δ13C) to assess the size of host trophic niche and investigate its relationship with parasite infection and (iii) determine whether these effects are consistent among species and sampling site, or more context-dependent. These aims test our hypotheses that infected individuals will have constricted or divergent trophic niches compared with uninfected conspecifics, and whether constriction or divergence will be linked to additional environmental variables.

Materials and methods

Sample and data collection

The coastal lowlands of the Netherlands and Belgium contain both freshwater and brackish habitats. These habitats are often dominated by 2 coexisting and phylogenetically related fish species: the 3-spined (G. aculeatus) and the 9-spined (P. pungitius) sticklebacks. Five freshwater and 4 brackish sites were sampled in March–April 2018 using dipnets (Fig. 1). Sites were selected to represent a range of salinity and were only sampled if the target species were identified living in sympathy. Sampling started with recording conductivity (μS cm−1), pH, temperature (°C), DO (mg L−1), turbidity and average water depth of the site. Fish densities were then recorded across a 100 m transect using a standardized approach of dipnetting once per meter. A total of 30 individuals per species were taken from the Belgian sites, and 25 individuals per species from the Dutch sites. In the laboratory, fish from each site and species were kept separately for up to 48 h in aerated aquaria. Lighting was controlled under a 12:12 day:night regime. The fish were euthanized with a lethal dose of tricaine methanesulphonate (MS-222) and weight (g ± 0.001), length (cm ± 0.1 cm) and sex were recorded. A detailed evaluation of parasite infection was conducted for each individual, detecting the presence of parasites using a protocol developed for sticklebacks (Kalbe et al., 2002). First, ectoparasites were identified scanning the entire body using a stereomicroscope. Then, endoparasites were identified using a microscope and high-pressure compressorium containing the liver, gut, intestines, swim bladder, body and head, kidneys, gill arches and right eye. Finally, a muscle sample without skin was removed from the right flank of each individual, and frozen for SIA. The muscle was chosen due to the lower stable isotope turnover rate when compared with other tissues such as the liver (Guelinckx et al., 2008).

Stable isotope analysis

The energy flow among trophic links can be estimated through the use of stable isotopes. In particular, nitrogen stable isotopes (δ15N) are typically enriched by 3–4‰ between consumers and their prey (Post, 2002). Carbon stable isotopes (δ13C) on the other hand inform on the initial source of carbon (i.e. littoral vs
pelagic), enabling inferences on the feeding ecology of organisms (Post, 2002). Hence, with SIA it is possible to determine the trophic niche of individuals within and among populations and explore the link between trophic niche and parasite infection. Specifically, the study of stable isotopes and parasite infection in populations of sympatric host species can reveal whether shared parasite infections consistently alter a host’s trophic niche. Here, muscle samples were dried at 60°C for 48 h, ground into a fine powder using a ball mill (Retsch UK Ltd., Hope, UK), and weighed into tin capsules (~0.8 mg; Elemental Microanalysis Ltd., Oakhampton, UK) using an ultra-microbalance (±0.001 mg; Sartorius Lab Instruments, Göttingen, Germany). Isotope ratios were analysed using continuous flow isotope mass spectrometry (Sercon, Crewe, UK). Isotope ratios are expressed in per mil (‰) relative to known reference materials for both carbon (δ13C) and nitrogen (δ15N). The C:N ratio is a proxy of body composition with respect to lipids, and in the data it indicates an overall low lipid content, negating the need to apply lipid correction protocols (Post et al., 2007).

**Parasite infection and the environment**

Environmental conditions affect the distribution of parasites. Hence, the variation in parasite abundance among host populations and the relationship between parasite community composition and abiotic environmental variables was investigated. To conduct the analyses effectively, 2 datasets were created. The first dataset used square-root-transformed aggregated mean parasite site abundance for each site and species (hereafter aggregated dataset), and the second was kept as an individual-based dataset which permitted site-specific inferences. The aggregated dataset was created to avoid pseudo-replication when comparing parasite community data to environmental variables, upon which a principal coordinates analysis (PCoA) was performed based on Bray-Curtis dissimilarities. Then a permutational multivariate analysis of variance (PERMANOVA) on the same Bray-Curtis dissimilarity matrix was performed to assess the effect of site, host species and the interaction between host species and site using the adonis function in the R package vegan v.2.5.5. A post hoc test was run using the pairwise.adonis function (pairwiseAdonis package v.0.4) to identify all significant pairwise combinations (Martinez Arbizu, 2020). Next, a similarity percentages test based on permutations (SIMPER) was used to identify which parasites contributed most to the differences between species and site. Finally, differences were correlated among environmental conditions (i.e. DO, pH, temperature, conductivity and total stickleback density), and the parasite community that used the PCoA axes’ scores as response variables in linear models.

**Linking parasite infection and trophic niche**

An index of parasite load, the Individual Parasite Index (IPI; Kalbe et al., 2002), was calculated for each individual fish comparing intra-individual parasite abundance to abundances. Two datasets were created. First, all samples were pooled and IPI was calculated across all sites (IPIAll), which enables comparison of infection load across fish from all sites. However, to detect patterns of trophic niche specialization across the entire dataset, the impact of parasite infections needs to be consistent and strong. Differences in parasite community, coevolutionary histories and environmental heterogeneity all decrease the likelihood of detecting such patterns in natural systems. Hence, the second dataset was created by calculating IPI within each site separately (IPIsite), permitting the investigation of site-specific patterns of parasite infection that would be obscured using the whole dataset. This is needed as IPI considers variation in parasite count in the total sample.

To link parasite infection and trophic niche, the stable isotopes δ15N and δ13C of both species were used to investigate the hypothesis that parasite infection affects host trophic niche specialization. Firstly, collinearity was removed from IPIsite and fish length by taking the residuals of a linear model between those 2 factors. Models were then created using δ13C as the response variable, an interaction between IPIsite, stickleback species and fish length as the fixed factor, and sampling site as a random factor. To permit comparisons among fish sizes, we grouped fish by species and size evenly into 3 groups based on length for each site. The same model was created but changing the response variable to δ15C. Stepwise model selections were performed based on the AIC criterion using the step function in the lmerTest package v.3.1 (Kuznetsova et al., 2017). In addition, previous work has shown that patterns of parasite-mediated trophic niche specialization can be achieved by a single parasite species (Pegg et al., 2015a, 2017). To investigate whether a single parasite putatively induced trophic niche specialization here, IPIsite was swapped for the square-root-transformed abundance of the 3 most important parasites as indicated by the SIMPER analysis (Cyathocotyle sp., Neochinorhynchus sp. and Cyathocotyle sp.)., and Glochidia in L05 due to its extreme abundance only in this population. A minimum of 10 individual parasites from each of the 3 most common species were required to run the model.

Analyses for each site were repeated using the same model, only swapping IPIsite for each of the following terms individually: IPIsite, and the square-root-transformed abundances of Cyathocotyle sp., Neochinorhynchus sp., Cyathocotyle sp. and Glochidia in L05. Linear mixed-effects models were obtained with the R package lm4 v.1.1 (Bates et al., 2015), and P values were calculated using Satterthwaite’s type II degree of freedom method from the lmerTest package. As each parasite load or abundance fixed factor addressed a discrete hypothesis, we corrected P values for multiple testing for each hypothesis. For instance, does δ15N and δ13C differ among Cyathocotyle sp. infections groups? As a result, each hypothesis had a maximum of 18 linear models, and P values were corrected for multiple testing accordingly using false discovery rate (FDR). However, for Glochidia, only 1 site (L05) had extreme abundances, so P values were not corrected when investigating these patterns.

To test whether parasite infection can specifically mediate host trophic niche constriction or divergence, for each site, fish were grouped by species, and then by parasite infection categories (low, medium and high). The infection categories were created by grouping individuals from each species evenly into 3 groups based upon IPIsite. The groupings were repeated for each of the 3 most important parasite species indicated by the SIMPER analysis and Glochidia in site L05 due to their extreme abundance only in this site. The Kolmogorov–Smirnov test was used to determine whether the low and high infection group distributions differed among the δ15N and δ13C axes within each site. Where appropriate, P values were corrected for multiple testing using FDR. Given the role of stickleback species denoted by previous analyses, this analysis was repeated for all sites for each host species separately. All analyses were conducted in R v4.0.2 (R Development Core Team, 2019).

**Results**

**Parasite infection and the environment**

In total, 19 species of parasites were identified, with Cyathocotyle sp. and Cyathocotyle sp. species being identified in every site (Supplementary Table S1). Notably, Proteocephalus sp., Neochinorhynchus sp., Diplostomum sp. and Anguillicoloides
crassus were all identified in at least 7 of 9 sites. Raphidascaris acus, Schistocephalus solidus and Paradiplopsis scolecina were the rarest parasites in *G. aculeatus*, with the latter being found in 1 site only (Supplementary Table S1); whereas only *Schistocephalus puntitii* was restricted to *P. pungitius*. Parasite diversity significantly differed among species and site, and in general, *G. aculeatus* were infected with a more diverse assemblage of parasites [analysis of variance (ANOVA) parasite diversity, \( F_{17,480} = 16.72, \ P < 0.001 \); Table 1], with mean individual parasite diversity differing among species and site, ranging from 1.50 ± 0.19 (standard error; s.e.) for *P. pungitius* in site L02 to 3.61 ± 0.25 for *G. aculeatus* in site L05. Similarly, parasite load (IPIAll) differed among sites and species, with *G. aculeatus* generally having higher infection loads than *P. pungitius* (ANOVA, \( F_{17,480} = 14.41, \ P < 0.001 \)).

Overall, parasite community composition significantly differed among sites and species (PERMANOVA, individual-based dataset, sites:species, \( F_{8,475} = 3.36, \ P < 0.001 \); aggregated mean parasite abundance dataset, site, \( F_{9,17} = 3.15, \ P < 0.001 \); species, \( F_{1,1} = 2.76, \ P = 0.040 \); pairwise PERMANOVAs are reported in Supplementary Table S2; Fig. 2A). Notably, the parasite community composition among species and site, inferred from PCoA axes (PC1 and PC2), was linked to DO in both stickleback species (3S, \( F_{1,7} = 12.03, \ P = 0.010 \); 9S, \( F_{1,7} = 15.07, \ P = 0.006 \), Fig. 2B). However, when investigating whether parasite diversity or parasite load was correlated with DO, we found no significant correlations in either *G. aculeatus* (parasite diversity, \( F_{1,7} = 1.22, \ P = 0.117 \); IPIAll, \( F_{1,7} = 2.60, \ P = 0.151 \)) or *P. pungitius* (parasite diversity, \( F_{1,7} = 2.57, \ P = 0.153 \); IPIAll, \( F_{1,7} = 2.51, \ P = 0.157 \)).

Based on site by species comparisons in the individual-based dataset, SIMPER analyses revealed that the abundances of 3 parasites, *Gyrodactylus* sp., *Neochinorhynchus* sp. and *Cyathocotyle* sp., were significantly different in at least 25% of all comparisons (Supplementary Table S3). Next, using the aggregated dataset, *Diplastomum* sp. and *S. solidus* were significantly more abundant in *G. aculeatus* than in *P. pungitius* (Supplementary Table S3). Among the 2 stickleback species, both *Gyrodactylus* sp. and *Neochinorhynchus* sp. were generally more abundant in *G. aculeatus*, whereas *Cyathocotyle* sp. was more abundant in *P. pungitius* (ANOVA; *Gyrodactylus*, \( F_{17,480} = 26.80, \ P < 0.001 \); *Neochinorhynchus*, \( F_{17,480} = 17.17, \ P < 0.001 \); *Cyathocotyle*, \( F_{17,480} = 5.84, \ P < 0.001 \); Supplementary Table S1).

### Linking parasite infection and trophic niche

Across all sites and after model simplification, the best predictor for \( ^{15}N \) was stickleback species, whereby *P. pungitius* had higher \( ^{15}N \) mean than *G. aculeatus* (LMER, random effect variance 4.05 ± 2.01, residual variance 2.91 ± 1.70, stickleback species, \( F_{485} = 24.09, \ P < 0.001 \), Fig. 3A). The same stickleback species effect was detected when replacing IPIAll by the most common parasites, *Gyrodactylus* sp., *Neochinorhynchus* sp. and *Cyathocotyle* sp. Alternatively, \( ^{13}C \) was positively correlated with an interaction between fish species and site, with the slope of *G. aculeatus* being steeper than that of *P. pungitius* (LMER, random effect variance 5.73 ± 2.39, residual variance 1.24 ± 0.12, species:length, \( F_{485} = 8.48, \ P = 0.004 \), Fig. 3B). The same minimum adequate model was reached when replacing IPIAll with *Gyrodactylus* sp. or *Neochinorhynchus* sp. abundances. However, in addition to the species:length term, \( ^{13}C \) increased with *Cyathocotyle* abundance in *G. aculeatus*, but decreased with this parasite in *P. pungitius* (LMER, random effect variance 5.58 ± 2.36, residual variance 1.23 ± 0.11, species:length, \( F_{485} = 10.61 \), \( P = 0.001 \); *Cyathocotyle* sp., \( F_{485} = 6.90 \), \( P = 0.009 \), Fig. 3B and C).

To reiterate, it was hypothesized that by mixing multiple distinct population together it would obscure any context-dependent patterns, which merited investigating sites individually. Using the site-specific parasite index (IPIsite), in 6 of 9 sites stickleback species was the most common predictor of both \( ^{15}N \) (L01, L05, L07, L14) and \( ^{13}C \) variation (L07, L10, L12+; all models reported in Supplementary Table S4), although fish length or a species by length interaction was also significant in some sites (\( ^{15}N \); L05*; \( ^{13}C \); L05*; * indicates a species by length interaction; + indicates length as an additive effect; Supplementary Table S4). Notably, in 2 sites \( ^{15}N \) increases with either IPIsite or the

### Table 1. Summary of parasite diversity and load by site

| Site | Species | Parasite diversity | Mean individual parasite diversity ± s.e. | Mean individual parasite index ± s.e. |
|------|---------|--------------------|------------------------------------------|--------------------------------------|
| L01  | *G. aculeatus* | 8 | 2.160 ± 0.149 | 2.548 ± 0.654 |
| L01  | *P. pungitius* | 8 | 2.120 ± 0.176 | 1.938 ± 0.297 |
| L02  | *G. aculeatus* | 5 | 1.240 ± 0.132 | 0.994 ± 0.443 |
| L02  | *P. pungitius* | 7 | 1.500 ± 0.186 | 2.703 ± 0.806 |
| L03  | *G. aculeatus* | 6 | 1.800 ± 0.152 | 1.363 ± 0.253 |
| L03  | *P. pungitius* | 7 | 1.708 ± 0.185 | 1.310 ± 0.275 |
| L05  | *G. aculeatus* | 11 | 3.613 ± 0.248 | 6.990 ± 0.769 |
| L05  | *P. pungitius* | 9 | 2.867 ± 0.207 | 5.866 ± 0.524 |
| L07  | *G. aculeatus* | 9 | 2.517 ± 0.189 | 2.016 ± 0.260 |
| L07  | *P. pungitius* | 6 | 1.724 ± 0.148 | 0.924 ± 0.123 |
| L09  | *G. aculeatus* | 11 | 2.069 ± 0.191 | 3.470 ± 0.600 |
| L09  | *P. pungitius* | 7 | 2.407 ± 0.179 | 2.728 ± 0.641 |
| L10  | *G. aculeatus* | 8 | 1.815 ± 0.192 | 3.611 ± 0.678 |
| L10  | *P. pungitius* | 8 | 0.963 ± 0.164 | 1.719 ± 0.497 |
| L12  | *G. aculeatus* | 8 | 2.000 ± 0.126 | 6.983 ± 1.035 |
| L12  | *P. pungitius* | 7 | 1.933 ± 0.126 | 2.550 ± 0.537 |
| L14  | *G. aculeatus* | 10 | 3.724 ± 0.209 | 8.636 ± 1.005 |
| L14  | *P. pungitius* | 8 | 2.533 ± 0.164 | 2.750 ± 0.404 |
abundance of Glochidia (LM; L03, IPISite, $F_{2,46} = 4.95$, FDR = 0.025; L05, Glochidia, $F_{1,56} = 6.18$, $P = 0.016$; Supplementary Table S4; Fig. 4A and B). When testing $\delta^{15}N$ variation against Cyathocotyle sp. abundance, a host species and parasite abundance interaction was observed in 1 site, with the correlation for P. pungitius being positive and the correlation for G. aculeatus being negative (L07, $F_{1,54} = 8.47$, FDR < 0.001; Fig. 4C). Furthermore, stable isotope variation was explained by the interaction between parasite load (IPISite) and fish length in 3 sites. Firstly, in site L02 the correlation between $\delta^{15}N$ and IPISite was positive for large fish and negative for small fish (L02, $F_{1,45} = 4.23$, FDR = 0.009; Fig. 4D). Secondly, in site L14 the correlation between $\delta^{13}C$ and Gyrodactylus sp. was negative for large fish and positive for small fish (L14, $F_{1,54} = 7.55$, FDR = 0.001; Fig. 4E). Thirdly, in site L10 the correlation between $\delta^{13}C$ and Cyathocotyle sp. abundance was positive for large fish and negative for small fish ($F_{4,49} = 5.40$, FDR = 0.004, Fig. 4F).

**Trophic niche constriction and divergence**

There were no significant differences in trophic niche distributions when comparing low and high infection groups in each site (Supplementary Table S5, Fig. S1). However, when scrutinizing sites where specific parasites were linked to trophic niche (namely, L05 $\delta^{15}N$-Glochidia, L07 $\delta^{15}N$-Cyathocotyle sp., L10 $\delta^{13}C$-Cyathocotyle sp. and L14 $\delta^{15}N$-Gyrodactylus sp.), it was found that fish in site L05 with high infection of Glochidia had a significantly reduced $\delta^{15}N$ breadth compared to fish with low infection (2-sample Kolmogorov–Smirnov test, $D = 0.5383$, $P = 0.003$; Fig. 5). All other trophic niche distributions were not significantly different (Supplementary Table S5). Yet, when considering trophic niche use as a character, evidence of character displacement among the stickleback species was observed in trophic niche axes in 6 of 9 sites (Supplementary Table S5). Specifically, constricted trophic niches with G. aculeatus having...
the broader niche were observed in 2 sites (2-sample Kolmogorov–Smirnov test; L02, $D_{\delta^{15}N} = 0.408$, FDR$_{\delta^{15}N} = 0.045$; L07, $D_{\delta^{15}N} = 0.483$, FDR$_{\delta^{15}N} = 0.007$, $D_{\delta^{13}C} = 0.690$, FDR$_{\delta^{13}C} < 0.001$; Supplementary Fig. S2) and *P. pungitius* with the broader niche also in 2 sites (L05, $D_{\delta^{15}N} = 0.434$, FDR$_{\delta^{15}N} = 0.012$; L07, $D_{\delta^{13}C} = 0.469$, FDR$_{\delta^{13}C} = 0.007$; L09, $D_{\delta^{15}N} = 0.425$, FDR$_{\delta^{15}N} = 0.019$; Supplementary Fig. S2). Finally, partial trophic niche divergence was observed in 2 sites (L10, $D_{\delta^{15}N} = 0.481$, FDR$_{\delta^{15}N} = 0.010$, $D_{\delta^{13}C} = 0.630$, FDR$_{\delta^{13}C} < 0.001$; L14, $D_{\delta^{15}N} = 0.464$, FDR$_{\delta^{15}N} = 0.007$; Supplementary Fig. S2).

**Discussion**

Focusing on 2 coexisting and phylogenetically related species and their parasites, an environmental determinant of parasite infection was identified and to what extent parasites affect trophic niche specialization was tested. Firstly, parasite community composition was correlated with DO. Secondly, parasite load and the abundance of specific parasite species were regularly negatively correlated with hosts’ stable isotope values ($\delta^{15}N$ or $\delta^{13}C$). These patterns were often site-specific, highlighting the context-dependent nature of parasite-mediated selection on niche use. Of the predicted patterns of parasite-mediated trophic niche specialization (Britton and Andreou, 2016), one case of trophic niche constriction was observed, where the trophic niche of the highly infected fish was entirely nested within trophic niche of the lowly infected fish. Thirdly, both species differed in trophic niche use at the majority of the study sites, albeit not consistently or in the same direction.

Together, the results suggest that both host species and parasite infection simultaneously affect niche specialization and the directions of these effects are context-dependent.

**Parasite community composition and the environment**

Many parasite species spend the vast majority of their lifetime, and for some species their entire lifetime, inside their hosts.
Therefore, whether environmental conditions directly affect parasite abundance and diversity is still debated (e.g. Sweeney et al., 2021). Here, a correlation between parasite community composition and DO was observed in both species. Such correlations can independently or jointly shape host–parasite interactions (Johnson et al., 2007; Brunner et al., 2017; Ojwala et al., 2018). Indeed, variation in assemblages of parasites has been shown to correlate with DO in other aquatic systems (Lacerda et al., 2018; Ojwala et al., 2018; Abdel-Tawwab et al., 2019). However, when investigating whether specific measures of parasite community composition — namely parasite diversity and load — were also correlated with DO (and the other environmental variables: pH, temperature, conductivity and total stickleback density) we found no significant correlations. Such a result highlights the complex nature of the interaction between parasite communities and their environments over a spatial scale.

**Linking parasite infection and trophic niche**

The effects of parasite infection on host physiology and behaviour are well documented (Barber and Dingemanse, 2010; Abdel-Tawwab et al., 2019). However, relatively little is known about the role of parasite infection on host trophic niche use in nature (Britton and Andreou, 2016; Brunner et al., 2017). There are a few empirical examples of an association between parasite infection and host trophic niche constriction and divergence (Pegg et al., 2015a, 2017; Villalobos et al., 2019), but whether environmental conditions, such as abiotic factors, interspecific competition or host density, affect these patterns remain elusive.

Across the entire dataset, a correlation between *Cyathocotyle* sp. abundance and δ¹³C was observed, with a host-specific direction of this correlation being observed. *Cyathocotyle* sp. is a trematode parasite that uses snails as intermediate hosts before actively infecting fish hosts, including both stickleback species (Lenz et al., 2013; Kvach et al., 2016). Trematode parasites are known to affect host locomotion and trigger host immune responses (Khan et al., 2003; Goodman and Johnson, 2011), including in fish hosts (Barber et al., 2000; Binning et al., 2017). Such effects are consistent with species-specific shifts in δ¹³C, which suggest increasing *Cyathocotyle* sp. infection load is associated with change in host diet and may be a proxy or driver of displacement in niche use among host species. While both species share a similar landscape, the different responses to *Cyathocotyle* sp. infections likely stem from species-specific immune response and may be an example of parasite-mediated trophic niche divergence.

Individual trophic niche specialization within an ecosystem can be associated with differences in adaptive and behavioural traits such as body shape, body size, gape size, gill-raker morphology and habitat utilization, differentially exposing hosts to parasites (Cucherousset et al., 2011; Svanbäck and Schluter, 2012; Pegg et al., 2015b; Britton and Andreou, 2016; Barry et al., 2017). Recently, parasites have also been speculated as a driver of trophic niche specialization (Pegg et al., 2015a, 2015b; Britton and Andreou, 2016). Here, parasite load — specifically the abundances of *Gyrodactylus* sp., *Cyathocotyle* sp. or Glochidia — was each associated with alterations of host δ¹⁵N or δ¹³C. Parasite infections can lead to significant phenotypic changes in their hosts (Miura et al., 2006; Britton and Andreou, 2016; Anaya-Rojas et al., 2019). Theory also predicts infected individuals to have different optimal diets due to differences in phenotypic capacity to detect, capture and digest the available prey (Araújo et al., 2011). Hence, the occasionally host size- or host species-dependent nature of the observations may be a consequence of the variation in parasite assemblages and the resultant parasite-induced phenotypes within and among sites. Additionally, it should be noted that previous studies identifying consistent patterns of parasite-mediated trophic niche specialization targeted non-native parasites on native fishes (Pegg et al., 2015a, 2017). In the present study, the effects of both native and non-native parasites were included, where the ecological impact of each may not be in the same magnitude or direction.

Competition within and among species in an environment is another driver of individual niche specialization (Araújo et al., 2011; Evangelista et al., 2014; Newsome et al., 2015). Here, host species was the strongest and most consistent determinant of trophic niche. When considering trophic niche use as a character, these results are suggestive of interspecific competition and character displacement (Schluter and McPhail, 1992; Gray and Robinson, 2002). Given the strength of species interaction, it is difficult to evaluate the role of parasite infection. As parasites contribute to host local adaptation and even to speciation (Eizaguirre et al., 2009; Brunner and Eizaguirre, 2016), it seems likely they also influence these evolutionary mechanisms through processes like parasite spillover and parasite-mediated phenotypic modifications (Britton and Andreou, 2016). Finally, some of the patterns observed may also be summarized by differences in adaptive potential among host species, which is underpinned by differences in demographic histories (Raeymaekers et al., 2017; Bal et al., 2021). Specifically, *G. aculeatus* tends to rapidly adapt to changing local environmental conditions while *P. pungitius* instead has the capacity to tolerate a wide range of conditions (Raeymaekers et al., 2017; Bal et al., 2021).

**Trophic niche constriction and divergence**

After capturing the role of the environment and host species on parasite community and host trophic niche, the main hypotheses of this study can be tested: can parasites drive trophic niche specialization in a consistent and measurable manner (Britton and Andreou, 2016)? Here, the majority of the results showed context-dependent patterns of parasite-mediated shifts in trophic niche, if
detectable at all. Notably, in 1 site (L05), parasite-mediated trophic niche constriction was observed, whereby the trophic niche of highly *Cyathocotyle* sp. infected fish was nested in that of lowly infected fish. A previous study showed similar patterns of trophic niche constriction suggesting it is likely due to infected conspecifics compensating the costs of infection by consuming specific food items already within the population’s dietary breadth (Pegg et al., 2017). Another factor to consider is differences in dietary preferences among life stages have also been shown to differ. Regardless, one explanation for this observation is that parasite-mediated trophic niche constriction is driven by host diet compensation, and matches the only other known study to identify such a pattern (Pegg et al., 2017). Overall, such findings highlight the need for further investigations into the causal mechanisms underlying this ecological phenomenon.

Conclusions

Overall, research on the determinants of trophic niche specialization has primarily identified factors such as competition, predation and resource availability as leading causes (Araújo et al., 2011). Whilst the results principally contribute to evidence supporting the impact of interspecific competition, the role of parasitism in trophic niche specialization remains poorly resolved (Britton and Andreou, 2016). Hence, studies observing an instance of trophic niche constriction, and multiple observations of parasitism putatively affecting the trophic niche of hosts are important. Such results suggest that consequences of parasite infection are an important driver of niche specialization which have been largely overlooked.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S003318202000531.

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