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PRIMARY RESEARCH PAPER

Environmental and spatial determinants of parasite communities in invasive and native freshwater fishes

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Abstract Understanding why certain host species harbor more parasites is an important question in parasite and fish ecology. Parasite infection among sympatric species may integrate various processes affecting parasite assembly, including the species-specific ecology and the success of invaders. We focused on spatial, environmental, and host-related determinants of parasite assembly in three riverine fish species: the native three-spined stickleback and stone loach, and the invasive topmouth gudgeon. Parasite community structure differed significantly between the three host species. Three-spined stickleback harbored the most ectoparasites. Host density and nutrients were the strongest predictors of its parasite communities. Topmouth gudgeon harbored the lowest number of parasites, both in terms of abundance and species richness. None of the parasites identified originated from its expanded range, suggesting that enemy release occurred during invasion. Temperature and host density predicted parasite assemblages of topmouth gudgeon. Stone loach hosted the most endoparasites, likely resulting from its varied diet, benthic lifestyle, and larger size. Additionally, host size and condition were positively correlated with parasite infection, which was similar among the three host species. The results suggest that host-related factors have a similar effect among host species, while the importance of environmental and spatial factors differs according to host ecology.

Keywords Host density · Parasite communities · Physico-chemical quality · Riverine fish · Sympatric species

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Introduction

Parasites cover a significant portion of worldwide biodiversity, playing essential roles in both ecological and evolutionary processes (Poulin & Morand, 2000a, b; Dobson et al., 2008; Gómez & Nichols, 2013). Yet, parasite diversity is not often considered in conservation planning and factors determining parasite distribution in freshwater systems remain poorly understood. Generally, individuals, populations, and species differ significantly in the intensity, abundance, and diversity of parasite infection (Krasnov et al., 1997; Behnke, 2008; Thieltges et al., 2009). Variation across populations of one host species has been attributed to environmental and spatial factors acting at both regional (e.g., species pool, dispersal limitation, the effect of distance and colonization) and local scales (e.g., biotic and abiotic differences across habitats). Variation within one host population has been attributed to differences in host-related factors such as sex, age, size, host genetic factors, and immune level responses, which in turn are affected by environmental variation, resulting in a complex interplay between environmental and host-related factors (Krasnov et al., 1997; Brouat et al., 2007; Randhawa & Poulin, 2010).

Studying variation in parasite infection across multiple hosts rather than in a single species may provide a better understanding of the underlying processes affecting parasite community structure (Carrassón et al., 2019). Studies focusing on parasite infections among host species report remarkable variation, even when species co-occur under identical environmental conditions, indicating the importance of host-level factors (Kilpatrick et al., 2006; Craft et al., 2008). Variation in parasite infection of sympatric fish species has been attributed to differences in both parasite exposure and defense mechanisms (e.g., variation in immunity, stress, and physiology among species; Poulin, 2010; Lima et al., 2012; Poulin & Forbes, 2012; Dallas & Presley, 2014). Factors influencing parasite exposure include diet, habitat use, behavior, life history strategy, population density, and dispersal capacity (Takemoto et al., 2005; Lee, 2006; Borer et al., 2009; Cronin et al., 2010). Host diet has been identified as a main determinant because many parasites are transmitted via ingestion. Additionally, species sharing food sources, generally use similar habitat types (Benesh et al., 2014; Marcogliese, 2002; Zander, 2005; Poulin & Leung, 2011). Consequently, ecological convergence seems to play a key role in variation of parasite infection (Carrassón et al., 2019). Other studies report significant effects of variation in host density (Takemoto et al., 2005) and life history strategies (Poulin, 2010). However, the relative importance of these factors may also differ, depending on parasite traits. The influence of host diet, for example, is expected to be stronger for heteroxenous endoparasites, which depend on trophic transmission to complete the life cycle (Poulin, 2010), while monoxenous ectoparasites, infecting their host actively, may benefit more from dense host populations (Pariselle et al., 2011).

As human activities are triggering a sixth global mass extinction event, understanding the influence of anthropogenic changes on biodiversity patterns and parasite infections is essential for conservation (Postel & Richter, 2003; Barnosky et al., 2011). Freshwater ecosystems provide an ideal study system as they are heavily impacted by human activities (Strayer & Dudgeon, 2010). The impact of environmental pollution on freshwater parasite communities may be significant. Examples include eutrophication, salinity, pH, calcium, chlorophyll a, and dissolved organic carbon (Hartvigsen & Halvorsen, 1994; Goater et al., 2005; Poulin et al., 2011; Budria & Candolin, 2014). Another important human-induced threat altering parasite-host interactions includes the introduction of non-native invasive species (Gozlan et al., 2005, 2009; Perkins et al., 2009; Peeler et al., 2011). Examples of disastrous invasions in freshwater systems include the introduction of the nematode *Anguillicoloides crassus* Kuwahara, Niimi & Itagaki, 1974, resulting in a decline of the European eel [*Anguilla anguilla* (Linnaeus, 1758); Székely et al., 2009] and whirling disease caused by the myxosporean *Myxobolus cerebralis* (Hofer, 1903) (Gozlan et al., 2006). Invasive fish significantly impact native parasite fauna following two mechanisms (Torchin et al., 2003; Prenter et al., 2004). Invasive species may carry and introduce new parasites which subsequently infect native species (parasite spillover). Occasionally, invasive fish are healthy carriers of parasites which pose a serious threat to native fauna [e.g., rosette-like agent in *Pseudorasbora parva* (Temminck & Schlegel, 1846)]. Invasive hosts can be responsible for spreading endemic parasites among native hosts and alter the abundance of parasites that infect native species (parasite spillback; Telfer et al., 2005; Kelly et al., 2009; Poulin et al., 2011). In absence of native predators and parasites, the fitness of invasive species is enhanced, potentially increasing infection among native hosts (enemy release hypothesis; Colautti et al., 2004).

We focus on the parasite communities of three riverine fish species with contrasting ecology and life history: three-spined stickleback (*Gasterosteus aculeatus* Linnaeus 1758; Gasterosteidae), stone loach [*Barbatula barbatula* (Linnaeus 1758); Nemacheilidae], and topmouth gudgeon (*Pseudorasbora parva*; Cyprinidae). Three-spined stickleback is an important model species, often used for studying parasite community dynamics (Barber, 2013). The species inhabits a broad geographic and environmental range, occupying a wide variety of habitats, and has a maximum lifespan of two years. Due to their central position in the food web, sticklebacks are hosts in many parasite life cycles with trophically transmitted stages. Additionally, sticklebacks are relatively tolerant to disturbance and their response to pollution is well documented (Barber, 2013; Budria & Candolin, 2014). Stone loach is a benthic species, commonly occurring in fast flowing streams and rivers with an expected lifespan of approximately five years (Wheeler, 1992). In contrast to three-spined stickleback, stone loach does not tolerate pollution and low levels of oxygen (Wheeler, 1992). Additionally, stone loach is a poor disperser (Barluenga & Meyer, 2005) and limited data are available on its parasite fauna (Jarkovský et al., 2004; Šimková et al., 2005; Přikrylová et al., 2008; Shershneva & Zhokhov, 2013). Topmouth gudgeon is a highly successful invasive cyprinid, originating from East Asia, and has invaded at least 32 countries in less than 50 years (Gozlan, 2002). Individuals reach maturity within a year and females are able to spawn up to four times in one season, spawning up to a few thousand eggs each season. Individuals can reach a maximum age of five years (Beyer et al., 2007; Kapusta et al., 2008). Additionally, topmouth gudgeon has a large environmental tolerance, and occupies a wide variety of habitats, ranging from small rivers to ponds and large lakes (Beyer et al., 2007). Importantly, Declerck et al. (2002) reported the presence of interspecific competition for food with other native fish (e.g., three-spined stickleback) in Belgium. None of the parasites of topmouth gudgeon occurring in its native range, have been reported in Western Europe yet (Gozlan et al., 2010). However, most studies focus on topmouth gudgeon as a carrier of the intracellular parasite *Sphaerothecum destruens* Arkush, Mendoza, Adkison & Hedrick, 2003 (e.g., Andreou et al., 2011; Andreou & Gozlan, 2016; Spikmans et al., 2020) and none have performed a community-level analysis in the invasive range of the host.

In this study, we compare parasite community structure between two native and one non-native riverine fish species. We expect significant variation in parasite infection among the three host species as they differ in many ecological and life history traits, factors that strongly contribute to differences in parasite infection between species. Moreover, we expect that topmouth gudgeon harbors fewer parasites, as previous studies have suggested that enemy release occurred during its introduction. First, we investigate whether the abundance of individual parasite species, infracommunity richness, and community composition differ across species and sampling locations. In addition, we investigate the influence of host-related factors (host size, condition, and sex) on parasite abundance and community structure, and whether these effects are similar across species. Next, we assess the relative importance of environmental (host-community related and water quality) and spatial (network centrality, upstream and downstream distance) variables on parasite communities of the three host species separately to investigate whether environmental and spatial factors affect parasite communities of different species in a similar way.

Methods

Study area and sampling

Fish were sampled in the Demer basin (Belgium) during autumn of 2017. The Demer, a river branch of the Scheldt, is situated in the eastern part of Flanders, and covers a total surface area of 2334 km^2. Fish were caught using standardized electrofishing over a stretch of 100 m with a license from the Agency Nature and Forest (ANB). All fish species were identified and counted. Three-spined stickleback, stone loach, and topmouth gudgeon each were kept, while all other species were released on site. Eleven populations of each species were included. All three species were found and collected at seven sites, two out of three species were collected at four sites, and only one species was present at four sites, resulting in a total of 15 locations sampled (Fig. 1; Table 1).

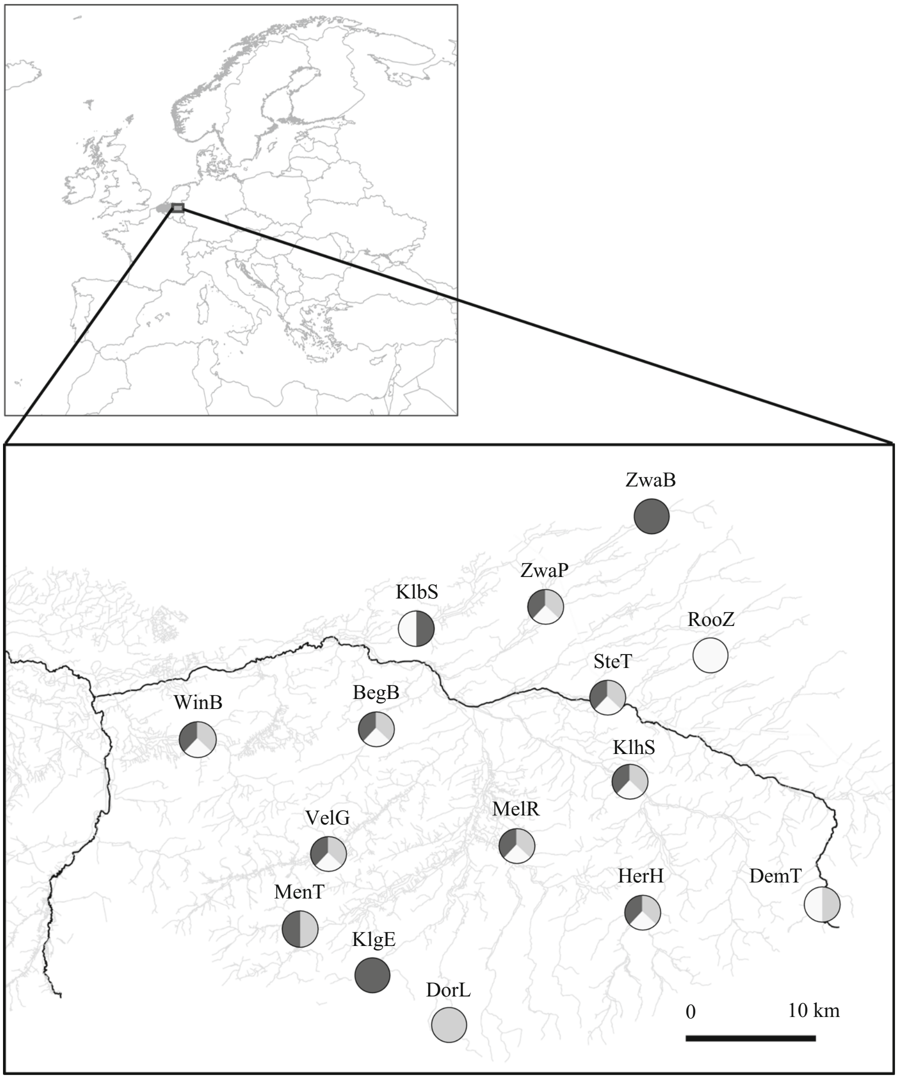


Fig. 1 Overview of the 15 sampling locations (Demer basin, Flanders, Belgium). The black lines represent the main rivers (Left = Dijle, right = Demer). See Table 1 for site codes and geographic coordinates. Colors indicate the presence of the three hosts (Light gray: three-spined stickleback, dark gray: stone loach, white: topmouth gudgeon)

Parasite survey

Fish were euthanized with a lethal dose of MS222 following directions of the KU Leuven Animal Ethics Committee. Euthanized fish were stored individually at − 20 °C. For each species and population, a total of 20 individuals were screened for the presence of endo- and ectoparasites. Prior to dissection and parasite screening, fish were thawed and individually placed in 5 to 50 ml cryo-tubes, depending on the size of the fish. Next, 1 or 2 ml of distilled water was added depending on the size of the tube before shaking it firmly for 10 s. The remaining water was poured into a small petri dish and ectoparasites were identified and counted using a stereomicroscope. Fish were rinsed and checked again for the presence of ectoparasites on the skin and fins. Fish weight (± 0.1 mg) and standard length (± 1 mm) were recorded. Next, fish were dissected and internal organs were checked for the presence of endoparasites under a stereomicroscope using a compressorium. Parasites were counted and identified to the lowest taxonomic level possible. Sex was determined during dissection by inspection of gonad development. A total of 684 fish were examined.

Environmental data

Physico-chemical data were provided by the monitoring network of the Flemish Environmental Agency

Table 1 Overview of the sampling sites and codes used in Fig. 1

Code	River	Location	Latitude	Longitude	Three-spined stickleback	Stone loach	Topmouth gudgeon
WingB	Winge	Blauwmolen	50.9387	4.8000	X	X	X
BegB	Begijnenbeek	Bekkevoort	50.5494	5.0029	X	X	X
ZwaP	Zwarte beek	Paal	51.0288	5.1921	X	X	X
SteT	Steenlaak	Thiewinkel	50.9651	5.2341	X	X	X
MelR	Melsterbeek	Runkelen	50.8565	5.1596	X	X	X
KlhS	Kleine Herk	Stevoort	50.9178	5.2506	X		X
VelG	Velpe	Glabbeek	50.8563	4.9501	X	X	X
HerH	Herk	Hoepertingen	50.8071	5.2973	X	X	X
MenT	Mene	Tienen	50.8030	4.9254	X	X	
DemT	Demer	Tongeren	50.8163	5.5045	X		X
KlbS	Kleine beek	Schaffen	51.0108	5.0483		X	X
DorL	Dormaalbeek	Landen	50.7368	5.0847	X		
KlgE	Kleine Gete	Ezemaal	50.7751	5.0028		X	
ZwaB	Zwarte beek	Beringen	51.0919	5.3175		X	
RooZ	Roosterbeek	Kerkom	50.8454	4.8817			X

(VMM). Each fish sampling site was chosen to closely match one of the environmental monitoring sites of VMM. Environmental variables include year average, based on monthly sampling, for water temperature, pH, conductivity, dissolved oxygen, Biochemical and Chemical Oxygen Demand (BOD and COD). Nutrient related variables include nitrate (NO_3^-), nitrite (NO_2^-), Kjeldahl nitrogen (KjN), total nitrogen (Nt), ammonium (NH_4^+), orthophosphate (oPO_4^{3-}), and total phosphorus (Pt). After removing highly collinear variables ($P < 0.05$, $|r| > 0.6$: Dormann et al., 2013), we retained a physico-chemical dataset which represents the various aspects of water quality and pollution: temperature, conductivity, dissolved oxygen, ammonium, nitrate, nitrite, and orthophosphate. Host environmental factors include host species richness (total number of species caught at each sampling location), total host abundance (total number of individuals caught at each sampling location), and specific host density (total number of individuals caught of three-spined stickleback, stone loach, and topmouth gudgeon at each sampling location). Pairwise waterway distances were calculated using the Network Analyst toolbox in ArcGIS. Network centrality was calculated as the average distance of a sampling location to all other sampling locations. Upstream distance was calculated as the maximal upstream distance from a sampling location, and downstream distance as the distance to the Dijle-Demer river confluence (50.96867 N; 4.6928 E).

Statistical analysis

Statistical analyses were conducted using R v4.0.2 (R Core Team, 2020). Analyses were organized in three groups as further outlined below: an individual-based analysis on total parasite abundance calculated as the individual parasitation index, an individual-based analysis focusing on infracommunity structure (parasite community infecting a single host), and a population-based analysis on component communities (total number of parasites infecting a host species at a sampling site). For the individual-based analyses, we only included the seven sampling locations where each of the three host species co-occurred (referred to as the small dataset). These analyses were performed on all three host species separately, as well as combined. For the population-based analysis, we included all the locations (referred to as the large dataset), and this analysis was run for each species separately.

Total parasite abundance

To analyze variation in total parasite species richness and abundance, the individual parasitation index (I_{PI}) was calculated for each host individual as following (Kalbe et al., 2002):

$$I_{\mathrm{PI}} = \sum_{i=0}^{i=n_{\mathrm{p}}} (10 s_{\mathrm{m}i}^{-1} . n_i s_{\mathrm{t}i}^{-1}),$$

where n_i is parasite number i, n_{p} is the total number of parasite species in the index, $s_{\mathrm{t}i}$ is the standard deviation of parasite species i in all host individuals examined, $s_{\mathrm{m}i}$ is then the maximum of the term $n_i s_{\mathrm{t}i}^{-1}$ for parasite species i. The distribution of all parasite species varies between 0 and 10 in the I_{PI}. Hence, using this index, we can include species with varying ranges of intensities. Some parasite taxa (e.g., *Trichodina* sp.) are present in high numbers. The I_{PI} gives equal weight to each parasite taxon. The value was calculated for ectoparasites and endoparasites separately as well as for both ecto- and endoparasites combined. The I_{PI} was cube root transformed in order to improve normality. Host condition, as relative weight (W_{rm}) was calculated following Froese (2006) for each species separately as follows, with a and b being based on the parameters of the mean weight–length relationship:

$$W_{\mathrm{rm}} = 100 \frac{W}{a_{\mathrm{m}} L^{b_{\mathrm{m}}}},$$

where a_{m} is the geometric mean of a, and b_{m} is the mean of b.

A linear model was then constructed with host species, sampling site, sex, host size, host condition, and their interactions as explanatory variables. Sampling site and the interaction between sampling site and host species were included as fixed effects in order to assess the importance of variation between sampling sites and species. A significant site effect suggests parallel infection patterns while a significant interaction of site and species may indicate that the effect of site on parasite infections differs between species. Model selection based on the Akaike Information Criterion (AIC) (AIC = − 2(log-likelihood) + 2 K, with K being the number of model parameters) was executed to decide which variables and interaction should be included in the final model. Stepwise model selection (both backward and forward) was performed using the stepAIC function in the R package MASS v7.3–54 (Ripley et al., 2013). The final model was the model with the lowest AIC. Normality of the residuals was visually assessed using qqplots. All models performed well. In addition, the Variance Inflation Factor (VIF) for each variable was calculated to detect multicollinearity. All VIFs were below 5, suggesting that multicollinearity was not an issue (Borcard et al., 2011).

Infracommunity composition

The variation in infracommunity composition was analyzed using a permutational multivariate analysis of variance (PERMANOVA) using the adonis function in the vegan v2.5-7 package in R (Oksanen et al., 2013). Parasite abundance data were first Hellinger transformed (Legendre & Gallagher 2001). Hellinger distances represent relative variation in parasite community composition as they are based on square-rooted proportional abundances. A dummy species was added to each host individual as PERMANOVA does not allow individuals with no parasites (Locke et al. 2012). Sampling site, sex, host size, host condition, and their interactions were included as fixed factors in a PERMANOVA.

Component community composition and environmental analysis

Redundancy analysis (RDA) on the component communities was implemented for each host species to analyze the effect of environmental and spatial variables on Hellinger transformed parasite abundances (Legendre & Gallagher, 2001). Environmental (both water quality and host environmental factors) and spatial variables were standardized using a log transformation and forward-selected to decide which variables would be included in the final model (Blanchet et al., 2008). Nine variables (temperature, oxygen, conductivity, NO_3^-, NH_4^+, specific host density, host species richness, upstream and downstream distance) were included in the RDA for each species. NO_2^-, total host abundance, and network centrality were discarded due to strong correlations to other variables. The explained variance was corrected for the number of variables and sites as expressed by the adjusted R^2. Significance was tested through 999 permutations. RDAs were computed using the RDA function in the vegan package (Oksanen et al., 2013).

Results

General characteristics of parasite communities

A total of 15 parasite taxa were observed on 684 individuals of three host species (Table S1). Some additional unidentified nematodes and metacercariae were discovered. Three-spined stickleback harbored ten parasite taxa. Stone loach harbored seven parasite taxa. We identified six parasite taxa in topmouth and observed one unidentified cestode in one sampling location, and an unidentified nematode species at eight sampling locations. Only three identified taxa were shared between all three species: *Gyrodactylus* spp., *Trichodina* sp., and *Acanthocephalus* sp. with *Acanthocephalus lucii* (Schrank, 1788) being present in both three-spined stickleback and topmouth gudgeon, and *A. anguillae* (Müller, 1780) being present in all three species (Table S1). *Gyrodactylus* spp. was not identified to species level, but given its host-specificity it is highly likely that the three hosts harbored different *Gyrodactylus* species (Přikrylová et al., 2008; Raeymaekers et al., 2008). Additionally, all three fish species harbored unidentified cysts (present in the liver and or/ intestines). Nine parasite taxa were shared between at least two host species. *Glugea anomala* (Moniez, 1887), *Schistocephalus solidus* (Müller, 1776), and *Proteocephalus filicollis* (Rudolphi, 1802) were only present in three-spined stickleback, and metacercariae and *Proteocephalus sagittus* (Grimm, 1872) were exclusively detected in stone loach.

Variation in parasite infection levels

When focusing on prevalence based on all examined individuals, 45.2% of all topmouth gudgeon individuals were not infected by any parasite (50.4% for the small dataset), followed by 17.0% of three-spined stickleback individuals (18.4% for the small dataset) and 10.9% of stone loach individuals (3.5% for the small dataset).

Host sex, condition, and the interaction between host species and condition, size and sex did not improve model performance for the overall parasitation index and were thus not included in the final model (full model AIC = − 448.47, selected model AIC = − 459.62). The overall I_{PI} differed between host species ($F = 11.051$, $P < 0.001$) and sampling locations ($F = 3.345$, $P = 0.003$; Fig. 2a). The effect of sampling site, however, differed significantly between species ($F = 6.183$, $P < 0.001$; Fig. 2b). Host size positively affected the total IPI ($F = 26.988$, $P < 0.001$). This effect was similar between species.

Host sex, size, and the interaction between host species and condition, size and sex did not improve model performance for the I_{PI} of the ectoparasites and were not included in the final model (full model AIC = − 503.44, selected model AIC = − 516.17). Similarly, host sex, condition, and the interaction between host species and condition, size and sex did not improve model performance for the I_{PI} of the endoparasites and were not included in the final model (full model AIC = − 772.78, selected model AIC = − 784.66).

IPI calculated for eco- and endoparasites differed significantly between species ($F = 93.974$, $P < 0.001$ and $F = 22.041$, $P < 0.001$) and sampling sites ($F = 23.2307$, $P < 0.001$ and $F = 7.900$, $P < 0.001$; Fig. 2c–f). Three-spined stickleback harbored the most ectoparasites, while stone loach harbored the most endoparasites. For both endo- and ectoparasites the effect of sampling site differed between species ($F = 8.033$, $P < 0.001$ and $F = 4.132$, $P < 0.001$). Ectoparasite infection decreased with host condition ($F = 5.308$, $P = 0.022$), while endoparasite infection increased with host size ($F = 33.444$, $P < 0.001$). The effect of size on endoparasite infection and condition on ectoparasite infection was similar between species.

Infracommunity structure

Differences between species accounted for the largest portion of variation in infracommunity structure ($F = 115.139$, $P = 0.001$, $R^2 = 0.272$). Infracommunities differed between sampling sites ($F = 13.739$, $P = 0.001$, $R^2 = 0.097$). However, the effect of site differed per species ($F = 8.720$, $P = 0.001$, $R^2 = 0.124$) and accounted for more variation than sampling site.

Host size ($F = 27.708$, $P = 0.001$, $R^2 = 0.033$) and condition ($F = 2.457$, $P = 0.045$, $R^2 = 0.003$) significantly affected parasite community composition and the effect was similar between species ($F = 0.139$, $P = 0.998$, $R^2 = 0.0003$ and $F = 0.552$, $P = 0.846$, $R^2 = 0.001$). Moreover, NMDS revealed that parasite

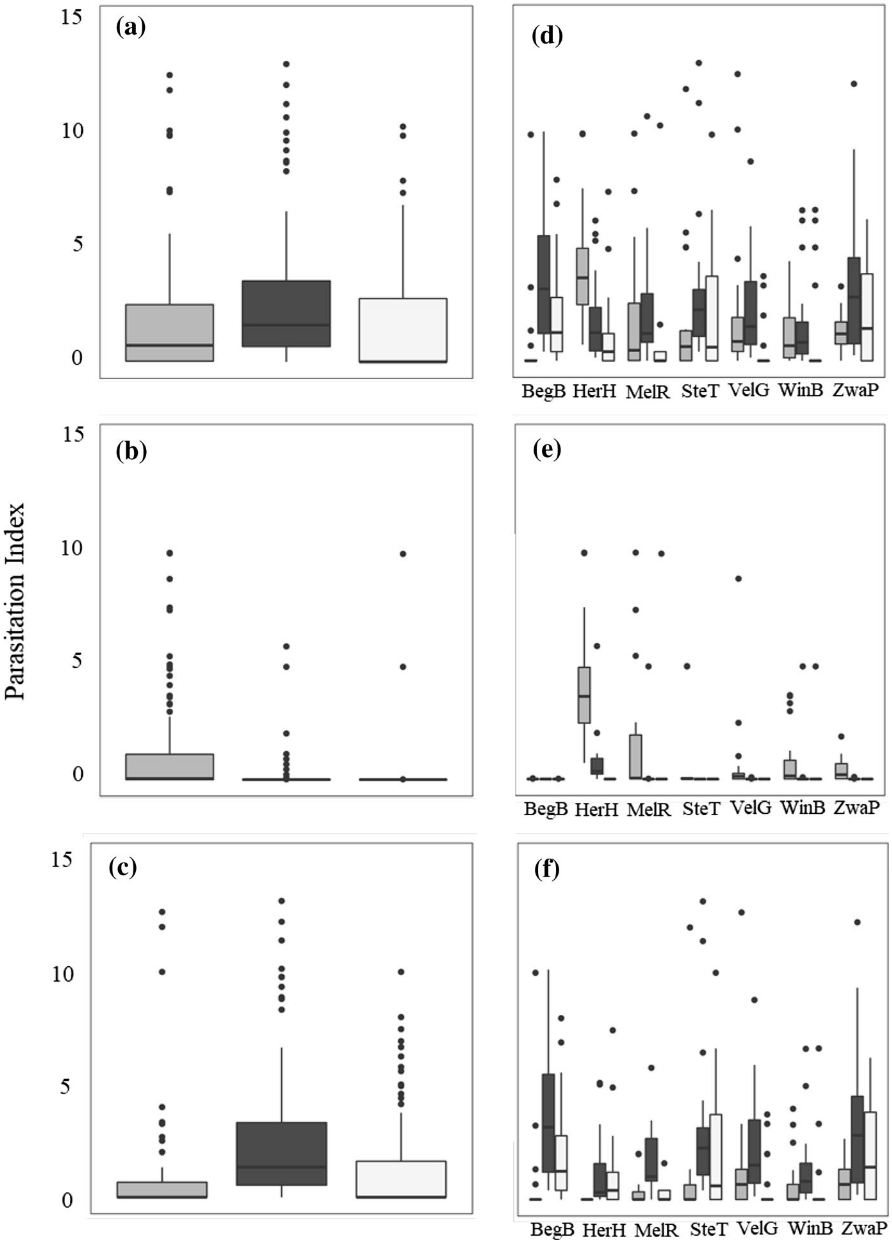


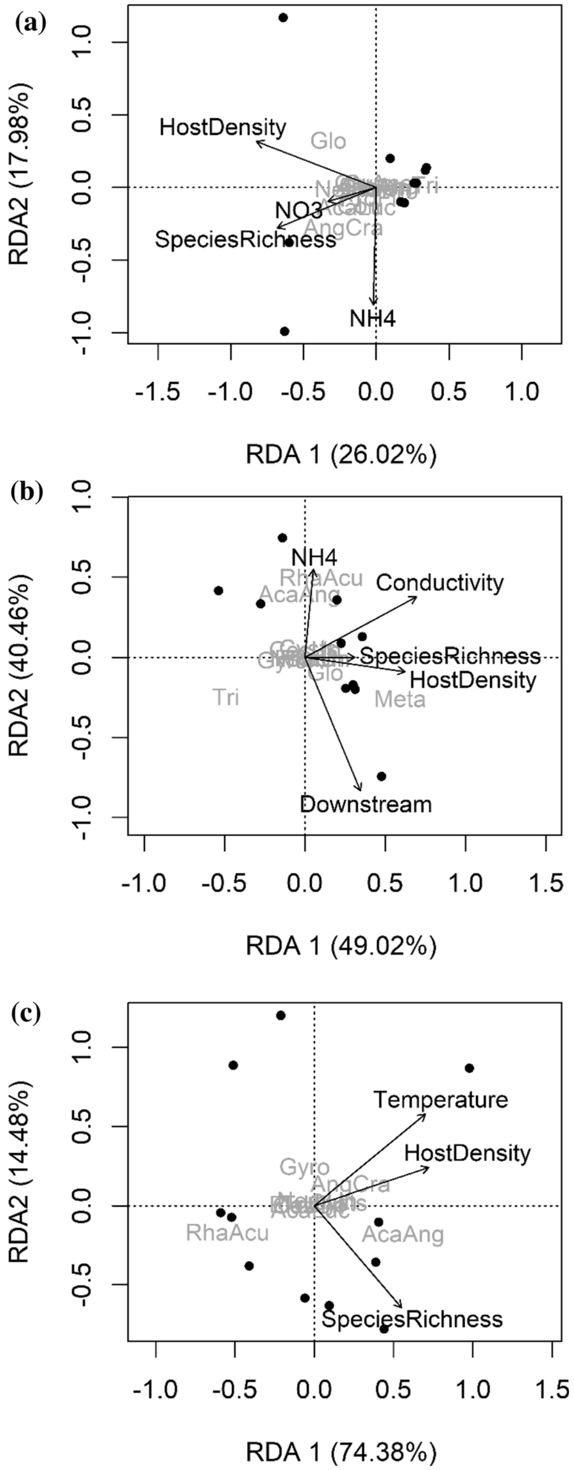
Fig. 2 Individual parasitation index (I_{PI}) by species, combining all sampling sites (left) and per species per sampling site (right) for **a**, **d** all parasites, **b**, **e** ectoparasites, and **d**, **f** endoparasites. Light gray: three-spined stickleback, dark grey: stone loach, white: topmouth gudgeon

infracommunities of topmouth gudgeon overlap more with stone loach than with three-spined stickleback (Fig. S1).

Component community structure

Environmental and spatial predictors of parasite abundance differed between the three host species (Fig. 3). The environmental variables included in the final model significantly predicted parasite abundance in three-spined stickleback (F = 2.003, P = 0.031, adjusted R^2 = 0.286). Three-spined stickleback density (F = 2.404, P = 0.058), NO_3^- (F = 1.409, P = 0.258) and NH_4^+ (F = 1.752, P = 0.154) were included in the final model, but host species richness was the only significant predictor of parasite abundance (F = 2.447, P = 0.040).

Five environmental and spatial variables were included in the final model for parasite abundance in stone loach (F = 2.235, P = 0.018, adjusted R^2 =



◀**Fig. 3** RDA triplot linking the selected environmental and spatial variables to parasite abundance in three-spined stickleback (**a**), stone loach (**b**), and topmouth gudgeon (**c**). Parasite species are labeled in gray. Percentages reflect the amount of variance explained by axis 1 and axis 2. An explanation for the abbreviations can be found in Table S1. Each dot represents a sampling location

0.382). Downstream distance (F = 4.081, P = 0.017) and conductivity (F = 3.733, P = 0.036) significantly influenced parasite abundance, while the effect of stone loach density ($F = 1.220$, $P = 0.343$), host species richness (F = 1.071, P = 0.386), and NH_4^+ was not significant (F = 1.072, P = 0.390).

Temperature significantly predicted parasite abundance in topmouth gudgeon (F = 3.287, P = 0.024). Topmouth gudgeon density and host species richness were included in the final model ($F = 2.313$, $P = 0.038$) but were not significant ($F = 1.538$, $P = 0.186$ and $F = 2.115$, $P = 0.110$).

Discussion

As hypothesized, both parasite abundance and community structure differed between the native and invasive riverine fish species with contrasting ecological and life history traits in Flanders. Three-spined stickleback harbored the highest abundance of ectoparasites, while stone loach harbored the most endoparasites. Parasite prevalence and abundance in the invasive topmouth gudgeon was low and all identified parasite species originated from its invasion range. Environmental and spatial determinants of parasite community structure also varied between the three host species. However, specific host density and host species richness seemed to play a role in determining parasite structure in all three species. Nutrients and host environmental factors were correlated with parasite communities in three-spined stickleback. Parasites of stone loach were associated with physico-chemical pollution (conductivity and ammonium) and downstream distance. Parasite communities of topmouth gudgeon correlated significantly with average water temperature.

Variation in parasite community composition among sympatric host species

Understanding why certain fish species are more infected with parasites is an important question in fish ecology. As mentioned before, variation in parasite communities among sympatric species has been attributed to variation in both parasite exposure and available defense mechanisms (Poulin, 2010; Lima et al., 2012; Poulin & Forbes, 2012; Dallas & Presley, 2014). The three host species showed clear differences in parasite infection levels and parasite community composition. Yet, *Gyrodacytlus* spp. and *Trichodina* sp. were observed in all host species, although likely include several cryptic host-specific species (Přikrylová et al., 2008; Raeymaekers et al., 2008, 2011; Shershneva & Zhokhov, 2013; Zhokhov et al., 2016).

The parasite community of three-spined stickleback was characterized by a high abundance of ectoparasites, mainly related to high *Trichodina* sp. abundance. Moreover, the observation of the ectoparasite *Glugea anomala* was restricted to three-spined stickleback. Fish acquire this microsporidian parasite indirectly by preying on infected invertebrates or by ingesting free spores which produce external tumors (Ward et al., 2005). Furthermore, *Trichodina* sp. and *Gyrodactylus* spp. are transmitted directly through body contact between individuals and thus greatly benefit from dense host populations. We observed a marginally significant correlation between host density and parasites community structure in three-spined stickleback. Three-spined sticklebacks display shoaling behavior, which may explain the high levels of directly transmitted parasites and the importance of host density in explaining parasite community structure. Additionally, some parasites (e.g., *Gyrodactylus* sp. and *Glugea anomala*) alter the behavior of three-spined stickleback, increasing the chances of direct parasite transmission (Ward et al., 2005; Rahn et al., 2015; Petkova et al., 2018). The parasite fauna of three-spined stickleback included some specialist species such as *Proteocephalus filicollis* and *Schistocephalus solidus* (Barber & Scharsack, 2010; Wootten, 2013) and it is highly likely that other *Gyrodactylus* and *Trichodina* species infect all three host species. Three-spined stickleback seems to be infected by more specialist species, compared to the other hosts. This may imply that host specificity and host defense mechanisms are at least partially responsible for determining species' parasite communities (Buchmann & Lindenstrøm, 2002).

Stone loach harbored the highest number of parasite taxa among the three host species, as well as the highest number of endoparasites. Two unique parasite taxa observed include *Proteocephalus sagittus,* for which definitive host species are limited to species from the families Balitoridae and Cobitidae (Scholz et al., 2003), and metacercariae of an unidentified trematode species. All other identified species were generalists and were also observed in at least one of the other host species, primarily in topmouth gudgeon. High levels of total parasite infection in stone loach mainly resulted from a high abundance of *Raphidascaris acus* (Boch, 1779), followed by high abundances of *Acanthocephalus anguillae*, *P. sagittus,* and metacercariae. High infection levels and species diversity may be explained by host-related ecological and behavioral factors, but also variation in host defense mechanisms. All endoparasites of stone loach are acquired by trophic ingestion. High parasite abundance and species richness is often associated with a more diverse diet, suggesting a broader niche for stone loach, which potentially results from a more diverse prey community in the benthic zone of rivers (Luque & Poulin, 2008; Marques et al., 2011; Baia et al., 2018). Sanchez-Thirion et al. (2019), for example, observed higher infection rates of an acanthocephalan species in crustacean hosts provided with higher quality food. In marine ecosystems, benthic fish generally harbor high levels of parasites as the sea bottom is an exchange zone of parasites (Marcogliese, 2002). However, this has not yet been noticed in freshwater habitats. Stone loach are on average larger than three-spined stickleback (56.89 ± 15.48 mm and 37.78 ± 5.62 mm respectively). Many studies report a positive correlation between host body size, species richness, and parasite abundance as larger hosts provide a greater number of habitats and total surface area for parasite colonization (Poulin & Morand, 2000a, b; Poulin, 2007).

Overall prevalence was low in the invasive topmouth gudgeon and species richness and intensity were generally lower in infected individuals compared to the two native hosts. This may suggest that topmouth gudgeon has been released from its native parasites during invasion, as indicated before (enemy release, Gozlan et al., 2010; Sheath et al., 2015). Moreover, all parasite species identified originated from its invasive range, suggesting that a parasite spillback effect occurred (Goedkneght et al., 2016). When invasive species act a host for native parasites, parasite abundance in native hosts may be indirectly affected (Goedknegt et al., 2016). However, next to the six species mentioned, we observed one unidentified cestode and one unidentified nematode. These species, together with *Gyrodactylus* spp. and *Trichodina* sp., may indeed originate from topmouth gudgeon's native range. However, no sign of co-introduction of topmouth gudgeon's native parasites has been reported so far in Western Europe. Most studies focusing on the parasites of topmouth gudgeon show similar results and have observed the exclusive presence of both native and generalist parasites (Ahne & Thomsen, 1986; Czeczuga et al., 2002; Kakalova & Shonia 2008; Sheath et al., 2015). Exceptions are the intracellular parasite, *Sphaerothecum destruens* (Sana et al., 2017) and *Dactylogyrus squameus* Gusev, 1955, a parasite specific to topmouth gudgeon, in Kazakhstan, Tajikistan, Uzbekistan, Slovakia, and Czech Republic (Galli et al., 2007; Ondračková et al., 2004).

The identified parasite species (*Gyrodactylus* spp., *Trichodina* sp., *Acanthocephalus anguillae*, *Acanthocephalus lucii*, *Anguillicoloides crassus, Raphidascaris acus*) infected at least one of the native hosts and are known generalists (Gozlan et al., 2010). Moreover, topmouth gudgeon is known as a vector for the intracellular parasite, *Sphaerothecum destruens* and the swimbladder nematode, *Anguillicoloides crassus,* posing a serious threat to the native freshwater fauna, confirming the hypothesis of a spillback effect (Gozlan et al., 2009, 2010; Andreou et al., 2011; Goedknegth et al., 2016).

Introduced hosts often harbor fewer parasites than native ones, providing a competitive advantage (Mitchell & Power, 2003; Torchin et al., 2003). Next to its high plasticity in life history traits and wide environmental tolerance, the low parasite abundance and species richness potentially explains topmouth gudgeon's rapid and successful colonization of Europe. It is often hypothesized that the establishment success of non-native species is higher when invasive species' parasite abundance is reduced, as they experience an increased fitness (Torchin et al., 2003). However, we did not observe a different effect of parasite abundance on host condition between topmouth gudgeon and the native species. Additionally, limited information is available on the abundance and prevalence of the species' native parasite fauna and their effect on its condition, as most studies have focused on topmouth gudgeon as a carrier for trematode parasites affecting human health (Gozlan et al., 2010). Hence, it is difficult to conclude that the lack of parasite infection helped the spread and establishment of topmouth gudgeon.

Similar responses to host-related factors

Host size was positively correlated with endoparasite abundance. Such positive correlation has often been attributed to larger individuals consuming more parasitized prey and providing a larger surface area for parasite colonization (Poulin, 2000). Moreover, larger and thus potentially older fish had more time to accumulate parasites. In fact, parasite abundance was only correlated with size when considering endoparasites, suggesting that larger (and older) hosts might collect more parasites, potentially as a function of time. Fish size influenced the composition of parasite communities, which is likely the result of fish of different sizes and ages having a different diet composition (Kangur & Kangur, 1998; Keskinen & Marjomäki, 2004). This interaction between parasite abundance and community structure, and host size was similar for the three host species, suggesting that similar mechanisms drive this correlation (Grutter & Poulin, 1998; Poulin, 2000).

Host condition, on the other hand, was positively correlated with the abundance of ectoparasites, while we did not observe this effect for endoparasites. This suggests that ectoparasites benefit more from hosts in good condition. This last hypothesis, however, is contradicted by general findings as parasites are assumed to be more successful when hosts are in poor condition resulting from reduced host immune allocation (Krasnov et al., 2005; Bize et al., 2008; Ponton et al., 2011). Hosts may be in better condition because they are better fed and prey on a variety of food sources, making them more susceptible for parasite infection. Moreover, better fed hosts may provide more resources for parasites. Interestingly, the effect of host condition did not differ among the three host species.

Contrasting effects of environmental and spatial variables

Parasite abundance and overall community structure varied strongly among host species and sampling locations. Accordingly, the site and species interaction was significant, suggesting that the effect of local environmental variables on parasite communities differed among the three host species. Temperature and host density were associated with parasite communities of topmouth gudgeon. However, the response was rather weak, suggesting that parasite infection in topmouth gudgeon is more affected by random processes. Host density and nutrients were correlated with parasite communities of three-spined stickleback. In addition to a response to nutrients, the parasite assemblages of stone loach were associated with downstream distance.

Many studies have identified temperature as an important abiotic parameter modifying host-parasite interactions by affecting the immune system and/or metabolic rates and virulence traits of parasites (Lazzaro & Little, 2009; Studer et al., 2010; Landis et al., 2012; Karvonen et al., 2013; Lugert et al., 2017). Similarly, the impact of eutrophication on host-parasite interactions is well described and has previously been observed in three-spined stickleback, but not in stone loach (Budria & Candolin, 2014; Budria, 2017). Nutrient enrichment alters host-parasite interactions by changing the food web and thus the presence of intermediate and final hosts, by increasing turbidity, affecting host behavior and diet, and by reducing dissolved oxygen concentration, directly affecting viability of ectoparasites and free-living parasite stages (Budria, 2017). As the parasite communities of topmouth gudgeon and stone loach consist of more endoparasitic and generalist species, which use a wide variety of intermediate hosts for transmission, the effect of nutrient enrichment and pollution is expected to be less pronounced. Specific host density, total host density, and host species richness were also identified as predictors of parasite infection in three-spined stickleback. As mentioned earlier, sticklebacks are hosts of many ectoparasites, which benefit most from shoaling behavior, and thus denser host communities (Arneberg et al., 1998). High overall host density and species richness, on the other hand, may be an indication of high habitat quality, and higher abundance and diversity of other intermediate and final hosts (Hansen & Poulin, 2006; de Montaudouin & Lanceleur, 2011). Although not significant, specific host density and host species richness improved model performance in stone loach and topmouth gudgeon, suggesting that these factors are important and common drivers of parasite community structure.

Furthermore, downstream distance was identified as a determinant of parasite community structure of stone loach. Distance is known as a strong driver of ecological communities (Nekola & White, 1999; Soininen et al., 2007). A strong distance effect is generally expected in host species with limited dispersal abilities (Poulin, 2003), confirming that stone loach is a poor disperser (Barluenga & Meyer, 2005; Fourtune et al., 2016). Spatial patterns have been described in parasite communities of freshwater fish, but are usually observed in lakes and/or on a larger spatial scale (Fellis & Esch, 2005; Poulin et al., 2011). The observed correlation with downstream distance, however, can also reflect spatial variation in physical habitat structure of rivers (Blanar et al., 2011; Blasco-Costa et al., 2013). The unidirectional flow of water and upstream–downstream change in habitat structure is known to affect several abiotic and biotic properties of riverine systems. Variation in productivity, temperature, water velocity, and habitat diversity may influence the abundance of intermediate and definitive hosts, promoting a gradient in parasite infection levels (Blasco-Costa et al., 2013).

Conclusion

Parasite abundance and community structure differed between two native and one invasive riverine fish species. Host size and condition were correlated with parasite abundance in the three species. The effect of environmental and spatial variables, however, differed between species. This suggests that variation in parasite infection is mediated by different responses of species to the environment which in turn may be the result of by species-specific differences in defense mechanisms and host ecology. Moreover, topmouth gudgeon was infected with low numbers of generalist and native parasite species, suggesting that enemy release occurred during its invasion, potentially explaining its success.

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Author contributions ISD and FAMV designed the study. ISD and FCFC collected the samples. MV, FVDE, and ISD performed the parasite screening. ISD and JAMR analyzed the data. ISD wrote the manuscript with input from FCFC, JAMR, and FAMV. All authors contributed to the writing and approved the final version for publication. The authors declare that they have no competing interests.

Data availability Data is added as supplementary information.

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