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

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11

12   **Abstract**

13   Understanding why certain host species harbor more parasites is an important question in parasite and

14   fish ecology. Parasite infection among sympatric species may integrate various processes affecting

15   parasite assembly, including the species-specific ecology and the success of invaders. We focused on

16   spatial, environmental, and host-related determinants of parasite assembly in three riverine fish species:

17   the native three-spined stickleback and stone loach, and the invasive topmouth gudgeon. Parasite

18   community structure differed significantly between the three host species. Three-spined stickleback

19   harbored the most ectoparasites. Host density and nutrients were the strongest predictors of its parasite

20   communities. Topmouth gudgeon harbored the lowest number of parasites, both in terms of abundance

21   and species richness. None of the parasites identified originated from its expanded range, suggesting

22   that enemy release occurred during invasion. Temperature and host density predicted parasite

23   assemblages of topmouth gudgeon. Stone loach hosted the most endoparasites, likely resulting from its

24   varied diet, benthic lifestyle, and larger size. Additionally, host size and condition were positively

25   correlated with parasite infection, which was similar among the three host species. The results suggest

26   that host-related factors have a similar effect among host species, while the importance of environmental

27   and spatial factors differs according to host ecology.

Key words

Host density, parasite communities, physico-chemical quality, riverine fish, sympatric species

31

Introduction

Parasites cover a significant portion of worldwide biodiversity, playing essential roles in both ecological and evolutionary processes (Dobson et al., 2008; Gómez & Nichols, 2013; Poulin & Morand, 2000). 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 (Behnke, 2008; Krasnov et al., 1997; 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 (Brouat et al., 2007; Krasnov et al., 1997; 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 (Craft et al., 2008; Kilpatrick et al., 2006). Variation in parasite infection of sympatric fish species has been attributed to differences in both parasite exposure and defence mechanisms (e.g. variation in immunity, stress, and physiology among species; Dallas & Presley, 2014; Lima et al., 2012; Poulin, 2010; Poulin & Forbes, 2012). Factors influencing parasite exposure include diet, habitat use, behavior, life history strategy, population density, and dispersal capacity (Borer et al., 2009; Cronin et al., 2010; Lee, 2006; Takemoto et al., 2005). Host diet has been identified as a main determinant because

55   many parasites are transmitted via ingestion. Additionally, species sharing food sources, generally use

56   similar habitat types (Benesh et al., 2014; Marcogliese, 2002; Poulin & Leung, 2011; Zander, 2005).

57   Consequently, ecological convergence seems to play a key role in variation of parasite infection

58   (Carrassón et al., 2019). Other studies report significant effects of variation in host density (Takemoto

59   et al., 2005) and life history strategies (Poulin, 2010). However, the relative importance of these factors

60   may also differ, depending on parasite traits. The influence of host diet, for example, is expected to be

61   stronger for heteroxenous endoparasites, which depend on trophic transmission to complete the life

62   cycle (Poulin, 2010), while monoxenous ectoparasites, infecting their host actively, may benefit more

63   from dense host populations (Pariselle et al., 2011).

64   As human activities are triggering a sixth global mass extinction event, understanding the influence of

65   anthropogenic changes on biodiversity patterns and parasite infections is essential for conservation

66   (Barnosky et al., 2011; Postel & Richter, 2003). Freshwater ecosystems provide an ideal study system

67   as they are heavily impacted by human activities (Strayer & Dudgeon, 2010). The impact of

68   environmental pollution on freshwater parasite communities may be significant. Examples include

69   eutrophication, salinity, pH, calcium, chlorophyll a, and dissolved organic carbon (Budria & Candolin,

70   2014; Goater et al., 2005; Hartvigsen & Halvorsen, 1994; Poulin et al., 2011). Another important

71   human-induced threat altering parasite-host interactions includes the introduction of non-native

72   invasive species (Gozlan et al., 2005, 2009; Peeler et al., 2011; Perkins et al., 2009). Examples of

73   disastrous invasions in freshwater systems include the introduction of the nematode *Anguillicoloides*

74   *crassus* Kuwahara, Niimi & Itagaki, 1974, resulting in a decline of the European eel [*Anguilla anguilla*

75   (Linnaeus, 1758); Székely et al., 2009] and whirling disease caused by the myxosporean *Myxobolus*

76   *cerebralis* (Hofer, 1903; Gozlan et al., 2006). Invasive fish significantly impact native parasite fauna

77   following two mechanisms (Prenter et al., 2004; Torchin et al., 2003). Invasive species may carry and

78   introduce new parasites which subsequently infect native species (parasite spillover). Occasionally,

79   invasive fish are healthy carriers of parasites which pose a serious threat to native fauna [e.g., rosette-

80   like agent in *Pseudorasbora parva* (Temminck & Schlegel, 1846)]. Invasive hosts can be responsible

81   for spreading endemic parasites among native hosts and alter the abundance of parasites that infect

82 native species (parasite spillback, Kelly et al., 2009; Poulin et al., 2011; Telfer et al., 2005). In absence

83 of native predators and parasites, the fitness of invasive species is enhanced, potentially increasing

84 infection among native hosts (enemy release hypothesis; Colautti et al., 2004).

85 We focus on the parasite communities of three riverine fish species with contrasting ecology and life

86 history: three-spined stickleback (*Gasterosteus aculeatus* Linnaeus 1758; Gasterosteidae), stone loach

87 [*Barbatula barbatula* (Linnaeus 1758); Nemacheilidae] and topmouth gudgeon (*Pseudorasbora parva*;

88 Cyprinidae). Three-spined stickleback is an important model species, often used for studying parasite

89 community dynamics (Barber, 2013). The species inhabits a broad geographic and environmental range,

90 occupying a wide variety of habitats, and has a maximum lifespan of two years. Due to their central

91 position in the food web, sticklebacks are hosts in many parasite life cycles with trophically transmitted

92 stages. Additionally, sticklebacks are relatively tolerant to disturbance and their response to pollution

93 is well documented (Barber, 2013; Budria & Candolin, 2014). Stone loach is a benthic species,

94 commonly occurring in fast flowing streams and rivers with an expected lifespan of approximately five

95 years (Wheeler, 1992). In contrast to three-spined stickleback, stone loach does not tolerate pollution

96 and low levels of oxygen (Wheeler, 1992). Additionally, stone loach is a poor disperser (Barluenga &

97 Meyer, 2005) and limited data are available on its parasite fauna (Jarkovský et al., 2004; Šimková et

98 al., 2005; Přikrylová et al., 2008; Shershneva & Zhokhov, 2013). Topmouth gudgeon is a highly

99 successful invasive cyprinid, originating from East Asia, and has invaded at least 32 countries in less

100 than 50 years (Gozlan, 2002). Individuals reach maturity within a year and females are able to spawn

101 up to four times in one season, spawning up to a few thousand eggs each season. Individuals can reach

102 a maximum age of five years (Beyer et al., 2007; Kapusta et al., 2008). Additionally, topmouth gudgeon

103 has a large environmental tolerance, and occupies a wide variety of habitats, ranging from small rivers

104 to ponds and large lakes (Beyer et al., 2007). Importantly, Declerck et al. (2002) reported the presence

105 of interspecific competition for food with other native fish (e.g. three-spined stickleback) in Belgium.

106 None of the parasites of topmouth gudgeon occurring in its native range, have been reported in Western

107 Europe yet (Gozlan et al., 2010). However, most studies focus on topmouth gudgeon as a carrier of the

108 intracellular parasite *Sphaerothecum destruens* Arkush, Mendoza, Adkison & Hedrick, 2003 (e.g.

109 Andreou et al., 2011; Andreou & Gozlan, 2016; Spikmans et al., 2013) and none have performed a

110 community-level analysis in the invasive range of the host.

111 In this study we compare parasite community structure between two native and one non-native riverine

112 fish species. We expect significant variation in parasite infection among the three host species as they

113 differ in many ecological and life history traits, factors that strongly contribute to differences in parasite

114 infection between species. Moreover, we expect that topmouth gudgeon harbors fewer parasites, as

115 previous studies have suggested that enemy release occurred during its introduction. First, we

116 investigate whether the abundance of individual parasite species, infracommunity richness, and

117 community composition differ across species and sampling locations. In addition, we investigate the

118 influence of host-related factors (host size, condition, and sex) on parasite abundance and community

119 structure, and whether these effects are similar across species. Next, we assess the relative importance

120 of environmental (host-community related and water quality) and spatial (network centrality, upstream

121 and downstream distance) variables on parasite communities of the three host species separately to

122 investigate whether environmental and spatial factors affect parasite communities of different species

123 in a similar way.

124

125 **Methods**

126 *Study area and sampling*

127 Fish were sampled in the Demer basin (Belgium) during autumn of 2017. The Demer, a river branch of

128 the Scheldt, is situated in the eastern part of Flanders, and covers a total surface area of 2,334 km². Fish

129 were caught using standardized electrofishing over a stretch of 100 m with a license from the Agency

130 Nature and Forest (ANB). All fish species were identified and counted. Three-spined stickleback, stone

131 loach, and topmouth gudgeon each were kept, while all other species were released on site. Eleven

132 populations of each species were included. All three species were found and collected at seven sites,

133 two out of three species were collected at four sites, and only one species was present at four sites,

134 resulting in a total of 15 locations sampled (**Fig. 1, Table 1**).

6

135

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 ($\pm$ 0.1 mg) and standard length ($\pm$ 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.

149

Environmental data

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

162   (total number of individuals caught of three-spined stickleback, stone loach, and topmouth gudgeon at

163   each sampling location). Pairwise waterway distances were calculated using the Network Analyst

164   toolbox in ArcGIS. Network centrality was calculated as the average distance of a sampling location to

165   all other sampling locations. Upstream distance was calculated as the maximal upstream distance from

166   a sampling location, and downstream distance as the distance to the Dijle-Demer river confluence

167   (50.96867 N; 4.6928 E).

168

169   *Statistical analysis*

170   Statistical analyses were conducted using R v4.0.2 (R Core Team, 2020). Analyses were organized in

171   three groups as further outlined below: an individual-based analysis on total parasite abundance

172   calculated as the individual parasitation index, an individual-based analysis focusing on

173   infracommunity structure (parasite community infecting a single host), and a population-based analysis

174   on component communities (total number of parasites infecting a host species at a sampling site). For

175   the individual-based analyses, we only included the seven sampling locations where each of the three

176   host species co-occurred (referred to as the small dataset). These analyses were performed on all three

177   host species separately, as well as combined. For the population-based analysis, we included all the

178   locations (referred to as the large dataset), and this analysis was run for each species separately.

179   **Total parasite abundance** - To analyze variation in total parasite species richness and abundance, the

180   individual parasitation index (I_{PI}) was calculated for each host individual as following (Kalbe et al.,

181   2002):

$$I_{PI} = \sum_{i=0}^{i=n_p} (10 s_{mi}^{-1} . n_i s_{ti}^{-1})$$

182

183   where n_i is parasite number i, n_p is the total number of parasite species in the index, s_{ti} is the standard

184   deviation of parasite species i in all host individuals examined, $s_{mi}$is then the maximum of the term

185    $n_i s_{ti}^{-1}$for parasite species i. The distribution of all parasite species varies between 0 and 10 in the I_{PI}.

186   Hence, using this index, we can include species with varying ranges of intensities. Some parasite taxa

187   (e.g. *Trichodina* sp.) are present in high numbers. The I_{PI} gives equal weight to each parasite taxon. The

188 value was calculated for ectoparasites and endoparasites separately as well as for both ecto- and

189 endoparasites combined. The I_{PI} was cube root transformed in order to improve normality. Host

190 condition, as relative weight (W_{rm}) was calculated following Froese (2006) for each species separately

191 as follows, with a and b being based on the parameters of the mean weight-length relationship:

192 $$W_{rm} = 100 \frac{W}{a_m L^{b_m}}$$

193 where a_m is the geometric mean of a, and b_m is the mean of b.

194 A linear model was then constructed with host species, sampling site, sex, host size, host condition, and

195 their interactions as explanatory variables. Sampling site and the interaction between sampling site and

196 host species were included as fixed effects in order to assess the importance of variation between

197 sampling sites and species. A significant site effect suggests parallel infection patterns while a

198 significant interaction of site and species may indicate that the effect of site on parasite infections differs

199 between species. Model selection based on the Akaike Information Criterion (AIC) (AIC = -2(log-

200 likelihood) + 2K, with K being the number of model parameters) was executed to decide which variables

201 and interaction should be included in the final model. Stepwise model selection (both backward and

202 forward) was performed using the stepAIC function in the R package MASS v7.3-54 (Venables &

203 Ripley, 2013). The final model was the model with the lowest AIC. Normality of the residuals was

204 visually assessed using qqplots. All models performed well. In addition, the Variance Inflation Factor

205 (VIF) for each variable was calculated to detect multicollinearity. All VIFs were below 5, suggesting

206 that multicollinearity was not an issue (Borcard et al., 2011).

207 **Infracommunity composition** - The variation in infracommunity composition was analysed using a

208 permutational multivariate analysis of variance (PERMANOVA) using the adonis function in the vegan

209 v2.5-7 package in R (Oksanen et al., 2013). Parasite abundance data was first Hellinger transformed

210 (Legendre & Gallagher 2001). Hellinger distances represent relative variation in parasite community

211 composition as they are based on square-rooted proportional abundances. A dummy species was added

212 to each host individual as PERMANOVA does not allow individuals with no parasites (Locke et al.

213 2012). Sampling site, sex, host size, host condition, and their interactions were included as fixed factors

214 in a PERMANOVA.

215 **Component community composition and environmental analysis** - Redundancy analysis (RDA) on

216 the component communities was implemented for each host species to analyze the effect of

217 environmental and spatial variables on Hellinger transformed parasite abundances (Legendre &

218 Gallagher, 2001). Environmental (both water quality and host environmental factors) and spatial

219 variables were standardized using a log transformation and forward-selected to decide which variables

220 would be included in the final model (Blanchet et al., 2008). Nine variables (temperature, oxygen,

221 conductivity, NO_3-, NH_4+, specific host density, host species richness, upstream and downstream

222 distance) were included in the RDA for each species. NO_2-, total host abundance, and network centrality

223 were discarded due to strong correlations to other variables. The explained variance was corrected for

224 the number of variables and sites as expressed by the adjusted R^2. Significance was tested through 999

225 permutations. RDAs were computed using the rda function in the vegan package (Oksanen et al., 2013).

226

227 **Results**

228 *General characteristics of parasite communities*

229 A total of 15 parasite taxa were observed on 684 individuals of three host species (**Table S1**). Some

230 additional unidentified nematodes and metacercariae were discovered. Three-spined stickleback

231 harbored ten parasite taxa. Stone loach harbored seven parasite taxa. We identified six parasite taxa in

232 topmouth and observed one unidentified cestode in one sampling location, and an unidentified

233 nematode species at eight sampling locations. Only three identified taxa were shared between all three

234 species: *Gyrodactylus* spp., *Trichodina* sp., and *Acanthocephalus* sp. with *Acanthocephalus lucii*

235 (Schrank, 1788) being present in both three-spined stickleback and topmouth gudgeon, and *A. anguillae*

236 (Müller, 1780) being present in all three species (**Table S1**). *Gyrodactylus* spp. was not identified to

237 species level, but given its host-specificity it is highly likely that the three hosts harbored different

238 *Gyrodactylus* species (Přikrylová et al., 2008; Raeymaekers et al., 2008). Additionally, all three fish

239 species harbored unidentified cysts (present in the liver and or/intestines). Nine parasite taxa were

240 shared between at least two host species. *Glugea anomala* (Moniez, 1887), *Schistocephalus solidus*

241 (Müller, 1776), and *Proteocephalus filicollis* (Rudolphi, 1802) were only present in three-spined

242 stickleback*,* and metacercariae and *Proteocephalus sagittus* (Grimm, 1872) were exclusively detected

243 in stone loach.

244

245 *Variation in parasite infection levels*

246 When focusing on prevalence based on all examined individuals, 45.2% of all topmouth gudgeon were

247 not infected by any parasite (50.4% for the small dataset), followed by 17.0% of the three-spined

248 stickleback (18.4% for the small dataset) and 10.9% of the stone loach (3.5% for the small dataset).

249 Host sex, condition, and the interaction between host species and condition, size and sex did not improve

250 model performance for the overall parasitation index and were thus not included in the final model (full

251 model AIC = -448.47, selected model AIC = -459.62). The overall I_{PI} differed between host species (F

252 = 11.051, P < 0.001) and sampling locations (F = 3.345, P = 0.003; **Fig. 2a**). The effect of sampling

253 site, however, differed significantly between species (F = 6.183, P < 0.001; **Fig. 2b**). Host size positively

254 affected the total IPI (F = 26.988, P < 0.001). This effect was similar between species.

255 Host sex, size, and the interaction between host species and condition, size and sex did not improve

256 model performance for the I_{PI} of the ectoparasites and were not included in the final model (full model

257 AIC = -503.44, selected model AIC = -516.17). Similarly, host sex, condition, and the interaction

258 between host species and condition, size and sex did not improve model performance for the I_{PI} of the

259 endoparasites and were not included in the final model (full model AIC = -772.78, selected model AIC

260 = -784.66).

261 IPI calculated for eco- and endoparasites differed significantly between species (F = 93.974, P < 0.001

262 and F = 22.041, P < 0.001) and sampling sites (F = 23.2307, P < 0.001 and F= 7.900, P < 0.001; **Fig.**

263 **2c-f**). Three-spined stickleback harboured the most ectoparasites, while stone loach harbored the most

264 endoparasites. For both endo- and ectoparasites the effect of sampling site differed between species (F=

265 8.033, P < 0.001 and F= 4.132, P < 0.001). Ectoparasite infection decreased with host condition (F=

266 5.308, P = 0.022), while endoparasite infection increased with host size (F= 33.444, P < 0.001). The

267 effect of size on endoparasite infection and condition on ectoparasite infection was similar between

268 species.

269

270 *Infracommunity structure*

271 Differences between species accounted for the largest portion of variation in infracommunity structure

272 (F= 115.139, P = 0.001, R^2 = 0.272). Infracommunities differed between sampling sites (F= 13.739, P

273 = 0.001, R^2 = 0.097). However, the effect of site differed per species (F= 8.720, P = 0.001, R^2 = 0.124)

274 and accounted for more variation than sampling site.

275 Host size (F= 27.708, P = 0.001, R^2 = 0.033) and condition (F= 2.457, P = 0.045, R^2 = 0.003)

276 significantly affected parasite community composition and the effect was similar between species (F =

277 0.139, P = 0.998, R^2 = 0.0003 and F = 0.552, P = 0.846, R^2 = 0.001). Moreover, NMDS revealed that

278 parasite infracommunities of topmouth gudgeon overlap more with stone loach than with three-spined

279 stickleback (**Fig. S1**).

280

281 *Component community structure*

282 Environmental and spatial predictors of parasite abundance differed between the three host species (**Fig.**

283 **3**). The environmental variables included in the final model significantly predicted parasite abundance

284 in three-spined stickleback (F = 2.003, P = 0.031, adjusted R^2 = 0.286). Three-spined stickleback density

285 (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

286 the final model, but host species richness was the only significant predictor of parasite abundance (F =

287 2.447, P = 0.040).

288 Five environmental and spatial variables were included in the final model for parasite abundance in

289 stone loach (F = 2.235, P = 0.018, adjusted R^2 = 0.382). Downstream distance (F = 4.081, P = 0.017)

290 and conductivity (F = 3.733, P = 0.036) significantly influenced parasite abundance, while the effect of

291 stone loach density (F = 1.220, P = 0.343), host species richness (F = 1.071, P = 0.386), and NH_4+ was

292 not significant (F = 1.072, P = 0.390).

293   Temperature significantly predicted parasite abundance in topmouth gudgeon (F = 3.287, P = 0.024).

294   Topmouth gudgeon density and host species richness were included in the final model (F = 2.313, P =

295   0.038) but were not significant (F = 1.538, P = 0.186 and F = 2.115, P = 0.110).

296

297   **Discussion**

298   As hypothesized, both parasite abundance and community structure differed between the native and

299   invasive riverine fish species with contrasting ecological and life history traits in Flanders. Three-spined

300   stickleback harbored the highest abundance of ectoparasites, while stone loach harbored the most

301   endoparasites. Parasite prevalence and abundance in the invasive topmouth gudgeon was low and all

302   identified parasite species originated from its invasion range. Environmental and spatial determinants

303   of parasite community structure also varied between the three host species. However, specific host

304   density and host species richness seemed to play a role in determining parasite structure in all three

305   species. Nutrients and host environmental factors were correlated with parasite communities in three-

306   spined stickleback. Parasites of stone loach were associated with physico-chemical pollution

307   (conductivity and ammonium) and downstream distance. Parasite communities of topmouth gudgeon

308   correlated significantly with average water temperature.

309

310   *Variation in parasite community composition among sympatric host species*

311   Understanding why certain fish species are more infected with parasites is an important question in fish

312   ecology. As mentioned before, variation in parasite communities among sympatric species has been

313   attributed to variation in both parasite exposure and available defence mechanisms (Dallas & Presley,

314   2014; Lima et al., 2012; Poulin, 2010; Poulin & Forbes, 2012). The three host species showed clear

315   differences in parasite infection levels and parasite community composition. Yet, *Gyrodacytlus* spp.

316   and *Trichodina* sp. were observed in all host species, although likely include several cryptic host-

317   specific species (Raeymaekers et al., 2008, 2011; Přikrylová et al., 2008; Shershneva & Zhokhov, 2013;

318   Zhokhov et al., 2016).

319 The parasite community of three-spined stickleback was characterized by a high abundance of

320 ectoparasites, mainly related to high *Trichodina* sp. abundance. Moreover, the observation of the

321 ectoparasite *Glugea anomala* was restricted to three-spined stickleback. Fish acquire this

322 microsporidian parasite indirectly by preying on infected invertebrates or by ingesting free spores which

323 produce external tumors (Ward et al., 2005). Furthermore, *Trichodina* sp. and *Gyrodactylus* spp. are

324 transmitted directly through body contact between individuals and thus greatly benefit from dense host

325 populations. We observed a marginally significant correlation between host density and parasites

326 community structure in three-spined stickleback. Three-spined sticklebacks display shoaling behavior,

327 which may explain the high levels of directly transmitted parasites and the importance of host density

328 in explaining parasite community structure. Additionally, some parasites (e.g. *Gyrodactylus* sp. and

329 *Glugea anomala*) alter the behavior of three-spined stickleback, increasing the chances of direct parasite

330 transmission (Petkova et al., 2018; Rahn et al., 2015; Ward et al., 2005). The parasite fauna of three-

331 spined stickleback included some specialist species such as *Proteocephalus filicollis* and

332 *Schistocephalus solidus* (Barber & Scharsack, 2010; Wootten, 2013) and it is highly likely that other

333 *Gyrodactylus* and *Trichodina* species infect all three host species. Three-spined stickleback seems to be

334 infected by more specialist species, compared to the other hosts. This may imply that host specificity

335 and host defense mechanisms are at least partially responsible for determining species' parasite

336 communities (Buchmann & Lindenstrøm, 2002).

337 Stone loach harboured the highest number of parasite taxa among the three host species, as well as the

338 highest number of endoparasites. Two unique parasite taxa observed include *Proteocephalus sagittus,*

339 for which definitive host species are limited to species from the families Balitoridae and Cobitidae

340 (Scholz et al., 2003), and metacercariae of an unidentified trematode species. All other identified species

341 were generalists and were also observed in at least one of the other host species, primarily in topmouth

342 gudgeon. High levels of total parasite infection in stone loach mainly resulted from a high abundance

343 of *Raphidascaris acus* (Boch, 1779), followed by high abundances of *Acanthocephalus anguillae*, *P.*

344 *sagittus,* and metacercariae. High infection levels and species diversity may be explained by host-

345 related ecological and behavioral factors, but also variation in host defense mechanisms. All

346 endoparasites of stone loach are acquired by trophic ingestion. High parasite abundance and species

14

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 (Baia et al., 2018; Luque & Poulin, 2008; Marques et al., 2011). 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, 2000; 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 squaemeus* Gusev, 1955, a parasite specific to topmouth gudgeon, in Kazakhstan, Tajikistan, Uzbekistan, Slovakia, and Czech Republic (Galli et al., 2007; Ondračková et al., 2004).

373 The identified parasite species (*Gyrodactylus* spp., *Trichodina* sp., *Acanthocephalus anguillae*,

374 *Acanthocephalus lucii*, *Anguillicoloides crassus, Raphidascaris acus*) infected at least one of the native

375 hosts and are known generalists (Gozlan et al., 2010). Moreover, topmouth gudgeon is known as a

376 vector for the intracellular parasite, *Sphaerothecum destruens* and the swimbladder nematode,

377 *Anguillicoloides crassus,* posing a serious threat to the native freshwater fauna, confirming the

378 hypothesis of a spillback effect (Andreou et al., 2011; Goedknegth et al., 2016; Gozlan et al., 2009,

379 2010).

380 Introduced hosts often harbor fewer parasites than native ones, providing a competitive advantage

381 (Mitchell & Power, 2003; Torchin et al., 2003). Next to its high plasticity in life history traits and wide

382 environmental tolerance, the low parasite abundance and species richness potentially explains topmouth

383 gudgeon's rapid and successful colonization of Europe. It is often hypothesized that the establishment

384 success of non-native species is higher when invasive species' parasite abundance is reduced, as they

385 experience an increased fitness (Torchin et al., 2003). However, we did not observe a different effect

386 of parasite abundance on host condition between topmouth gudgeon and the native species.

387 Additionally, limited information is available on the abundance and prevalence of the species' native

388 parasite fauna and their effect on its condition, as most studies have focused on topmouth gudgeon as a

389 carrier for trematode parasites affecting human health (Gozlan et al., 2010). Hence, it is difficult to

390 conclude that the lack of parasite infection helped the spread and establishment of topmouth gudgeon.

391

392 *Similar responses to host-related factors*

393 Host size was positively correlated with endoparasite abundance. Such positive correlation has often

394 been attributed to larger individuals consuming more parasitized prey and providing a larger surface

395 area for parasite colonization (Poulin, 2000). Moreover, larger and thus potentially older fish had more

396 time to accumulate parasites. In fact, parasite abundance was only correlated with size when considering

397 endoparasites, suggesting that larger (and older) hosts might collect more parasites, potentially as a

398 function of time. Fish size influenced the composition of parasite communities, which is likely the result

399 of fish of different sizes and ages having a different diet composition (Kangur & Kangur, 1998;

400 Keskinen & Marjomäki, 2004). This interaction between parasite abundance and community structure,

401 and host size was similar for the three host species, suggesting that similar mechanisms drive this

402 correlation (Grutter & Poulin, 1998; Poulin, 2000).

403 Host condition, on the other hand, was positively correlated with the abundance of ectoparasites, while

404 we did not observe this effect for endoparasites. This suggests that ectoparasites benefit more from hosts

405 in good condition. This last hypothesis, however, is contradicted by general findings as parasites are

406 assumed to be more successful when hosts are in poor condition resulting from reduced host immune

407 allocation (Krasnov et al., 2005; Bize et al., 2008; Ponton et al., 2011). Hosts may be in better condition

408 because they are better fed and prey on a variety of food sources, making them more susceptible for

409 parasite infection. Moreover, better fed hosts may provide more resources for parasites. Interestingly,

410 the effect of host condition did not differ among the three host species.

411

412 *Contrasting effects of environmental and spatial variables*

413 Parasite abundance and overall community structure varied strongly among host species and sampling

414 locations. Accordingly, the site and species interaction was significant, suggesting that the effect of

415 local environmental variables on parasite communities differed among the three host species.

416 Temperature and host density were associated with parasite communities of topmouth gudgeon.

417 However, the response was rather weak, suggesting that parasite infection in topmouth gudgeon is more

418 affected by random processes. Host density and nutrients, were correlated with parasite communities of

419 three-spined stickleback. In addition to a response to nutrients, the parasite assemblages of stone loach

420 were associated with downstream distance.

421 Many studies have identified temperature as an important abiotic parameter modifying host-parasite

422 interactions by affecting the immune system and/or metabolic rates and virulence traits of parasites

423 (Karvonen et al., 2013; Landis et al., 2012; Lazzaro & Little, 2009; Lugert et al., 2017; Studer et al.,

424 2010). Similarly, the impact of eutrophication on host-parasite interactions is well described and has

425 previously been observed in three-spined stickleback, but not in stone loach (Budria & Candolin, 2014;

426 Budria, 2017). Nutrient enrichment alters host-parasite interactions by changing the food web and thus

17

427 the presence of intermediate and final hosts, by increasing turbidity, affecting host behavior and diet,

428 and by reducing dissolved oxygen concentration, directly affecting viability of ectoparasites and free-

429 living parasite stages (Budria, 2017). As the parasite communities of topmouth gudgeon and stone loach

430 consist of more endoparasitic and generalist species, which use a wide variety of intermediate hosts for

431 transmission, the effect of nutrient enrichment and pollution is expected to be less pronounced. Specific

432 host density, total host density, and host species richness were also identified as predictors of parasite

433 infection in three-spined stickleback. As mentioned earlier, sticklebacks are hosts of many

434 ectoparasites, which benefit most from shoaling behavior, and thus denser host communities (Arneberg

435 et al., 1998). High overall host density and species richness, on the other hand, may be an indication of

436 high habitat quality, and higher abundance and diversity of other intermediate and final hosts (de

437 Montaudouin & Lanceleur, 2011; Hansen & Poulin, 2006). Although not significant, specific host

438 density and host species richness improved model performance in stone loach and topmouth gudgeon,

439 suggesting that these factors are important and common drivers of parasite community structure.

440 Furthermore, downstream distance was identified as a strong driver of parasite community structure of

441 stone loach. Distance is known as a strong driver of ecological communities (Nekola & White, 1999;

442 Soininen et al., 2007). A strong distance effect is generally expected in host species with limited

443 dispersal abilities (Poulin, 2003), confirming that stone loach is a poor disperser (Barluenga & Meyer,

444 2005; Fourtune et al., 2016). Spatial patterns have been described in parasite communities of freshwater

445 fish, but are usually observed in lakes and/or on a larger spatial scale (Fellis & Esch, 2005; Poulin et

446 al., 2011). The observed correlation with downstream distance, however, can also reflect spatial

447 variation in physical habitat structure of rivers (Blannar et al., 2011; Blasco-Costa et al., 2013). The

448 unidirectional flow of water and upstream-downstream change in habitat structure is known to affect

449 several abiotic and biotic properties of riverine systems. Variation in productivity, temperature, water

450 velocity and habitat diversity may influence the abundance of intermediate and definitive hosts,

451 promoting a gradient in parasite infection levels (Blasco-Costa et al., 2013).

452

Conclusion

Parasite abundance and community structure differed between two native and one invasive riverine fish species. Host size and condition similarly 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.

References

Ahne, W. & I. Thomsen, 1986. Isolation of pike fry rhabdovirus from *Pseudorasbora parva* (Temminck & Schlegel). Journal of Fish Diseases, 9: 555−556.

Andreou, D. & R. E. Gozlan, 2016. Associated disease risk from the introduced generalist pathogen *Sphaerothecum destruens*: management and policy implications. Parasitology, 143: 1204−1210.

Andreou, D., R. E. Gozlan, D. Stone, P. Martin, K. Bateman & S. W. Feist, 2011. *Sphaerothecum destruens* pathology in cyprinids. Diseases of Aquatic Organisms, 95: 145−151.

Arneberg, P., A. Skorping, B. Grenfell & A. F. Read, 1998. Host densities as determinants of abundance in parasite communities. Proceedings of the Royal Society B: Biological Sciences, 265: 1283−1289.

Baia, R. R. J., A. C. Florentino, L. M. A. Silva & M. Tavares-Dias, 2018. Patterns of the parasite communities in a fish assemblage of a river in the Brazilian Amazon region. Acta Parasitologica, 63: 304−316.

Barber, I. & J. P. Scharsack, 2010. The three-spined stickleback-*Schistocephalus solidus* system: An experimental model for investigating host-parasite interactions in fish. Parasitology, 137: 411−424.

Barber, I. (2013). Sticklebacks as model hosts in ecological and evolutionary parasitology. Trends in Parasitology, 29: 556−566.

Barger, M. A. & G. W. Esch, 2001. Downstream changes in the composition of the parasite community of fishes in an Appalachian stream. The Journal of Parasitology, 87: 250−255.

Barluenga, M. & A. Meyer, 2005. Old fish in a young lake: Stone loach (Pisces: *Barbatula barbatula*) populations in Lake Constance are genetically isolated by distance. Molecular Ecology, 14: 1229−1239.

Barnosky, A. D., N. Matzke, S. Tomiya, G. O U. Wogan, B. Swartz, T. B. Quental, C. Marshall, J. L. McGuire, E. L. Lindsey, K. C. Maguire, B. Mersey & E. A. Ferrer, 2011. Has the Earth's sixth mass extinction already arrived? Nature, 471: 51−57.

484 Behnke, J. M. (2008). Structure in parasite component communities in wild rodents: Predictability, stability,
485   associations and interactions .... or pure randomness? Parasitology, 135: 751−766.

486 Benesh, D. P., J. C. Chubb & G. A. Parker, 2014. The trophic vacuum and the evolution of complex life cycles in
487   trophically transmitted helminths. Proceedings of the Royal Society B: Biological Sciences, 281:
488   20141462.

489 Beyer, K., G. H. Copp, & R. E. Gozlan, 2007. Microhabitat use and interspecific associations of introduced
490   topmouth gudgeon *Pseudorasbora parva* and native fishes in a small stream. Journal of Fish Biology, 71:
491   224−238.

492 Bize, P., C. Jeanneret, A. Klopfenstein & A. Roulin, 2008. What makes a host profitable? Parasites balance host
493   nutritive resources against immunity. American Naturalist, 171: 107−118.

494 Blanchet, F. G., P. Legendre & D. Borcard, 2008. Forward selection of explanatory variables. Ecology, 89:
495   2623−2632.

496 Borcard, D., F. Gillet & P. Legendre, 2011. Numerical Ecology with R. Springer.

497 Borer, E. T., C. E. Mitchell, A.G. Power & E. W. Seabloom, 2009. Consumers indirectly increase infection risk
498   in grassland food webs. Proceedings of the National Academy of Sciences of the United States of
499   America, 106: 503−506.

500 Brouat, C., M. Kane, M. Diouf, K. Bâ, R. Sall-Dramé & J. M. Duplantier, 2007. Host ecology and variation in
501   helminth community structure in Mastomys rodents from Senegal. Parasitology, 134: 437−450.

502 Buchmann, K. & T. Lindenstrøm, 2002. Interactions between monogenean parasites and their fish hosts.
503   International Journal for Parasitology, 32: 309−319.

504 Budria, A. (2017). Beyond troubled waters: the influence of eutrophication on host–parasite interactions.
505   Functional Ecology, 31: 1348−1358.

506 Budria, A. & U. Candolin, 2014. How does human-induced environmental change influence host−parasite
507   interactions? Parasitology, 141: 462−474.

508 Carrassón, M., S. Dallarés, J. E. Cartes, M. Constenla, A. Pérez-del-Olmo, L. Zucca & A. Kostadinova, 2019.
509   Drivers of parasite community structure in fishes of the continental shelf of the Western Mediterranean:
510   the importance of host phylogeny and autecological traits. International Journal for Parasitology, 49:
511   669−683.

512 Colautti, R. I., A. Ricciardi, I. A. Grigorovich & H. J. MacIsaac, 2004. Is invasion success explained by the enemy
513   release hypothesis? Ecology Letters, 7: 721−733.

514 Craft, M. E., P. L. Hawthorne, C. Packer & A. P. Dobson, 2008. Dynamics of a multihost pathogen in a carnivore
515   community. Journal of Animal Ecology, 77: 1257−1264.

516 Cronin, J. P., M. E. Welsh, M. G. Dekkers, S. T. Abercrombie & C. E. Mitchell, 2010. Host physiological
517   phenotype explains pathogen reservoir potential. Ecology Letters, 13: 1221−1232.

518 Czeczuga, B., B. Kiziewicz & Z. Danilkiewicz, 2002. Zoosporic fungi growing on the specimens of certain fish
519   species recently introduced to Polish waters. Acta Ichthyologica et Piscatoria, 2.

520 Dallas, T., & S. J. Presley, 2014. Relative importance of host environment, transmission potential and host
521   phylogeny to the structure of parasite metacommunities. Oikos, 123: 866−874.

522 de Montaudouin, X. & L. Lanceleur (2011). Distribution of parasites in their second intermediate host, the cockle
523   *Cerastoderma edule*: Community heterogeneity and spatial scale. Marine Ecology Progress Series, 428 :
524   187−199.

525 Declerck, S., G. Louette, T. De Bie, & L. De Meester, 2002. Patterns of diet overlap between populations of
526   non−indigenous and native fishes in shallow ponds. Journal of Fish Biology, 61: 1182−1197.

527 Deflem, I. S., E. Bennetsen, Ø. H. Opedal, F.C. Calboli, O. Ovaskainen, G. Van Thuyne, ... & J. A. Raeymaekers,
528   2021. Predicting fish community responses to environmental policy targets. Biodiversity and
529   Conservation, 30: 1457−1478.

530 Dobson, A., K. D. Lafferty, A. M. Kuris, R. F. Hechinger & W. Jetz, 2008. Homage to Linnaeus: How many
531   parasites? How many hosts? Proceedings of the National Academy of Sciences of the United States of
532   America, 105: 11482−11489.

533 Dormann, C. F., J. Elith, S. Bacher, C. Buchmann, G. Carl, G. Carré, J. R. G. Marquéz, B. Gruber, B. Lafourcade,
534   P. J. Leitão, T. Münkemüller, C. Mcclean, P. E. Osborne, B. Reineking, B. Schröder, A. K. Skidmore, D.
535   Zurell & S. Lautenbach, 2013. Collinearity: A review of methods to deal with it and a simulation study
536   evaluating their performance. Ecography, 36: 27−46.

537 Fellis, K. J. & G. W. Esch, 2005. Variation in life cycle affects the distance decay of similarity among bluegill
538   sunfish parasite communities. Journal of Parasitology, 91: 1484−1486.

539 Fourtune, L., I. Paz-Vinas, G. Loot, J. G. Prunier & S. Blanchet, 2016. Lessons from the fish: a multi−species
540   analysis reveals common processes underlying similar species-genetic diversity correlations. Freshwater
541   Biology, 61: 1830−1845.

542 Froese, R. (2006). Cube law, condition factor and weight-length relationships: History, meta−analysis and
543   recommendations. Journal of Applied Ichthyology, 22: 241−253.

544 Galli, P., G. Strona, F. Benzoni, G. Crosa & F. Stefani, 2007. Monogenoids from freshwater fish in Italy, with
545   comments on alien species. Comparative Parasitology, 74: 264−272.

546 Goater, C. P., R. E. Baldwin & G. J. Scrimgeour, 2005. Physico-chemical determinants of helminth component
547   community structure in whitefish (*Coregonus clupeaformes*) from adjacent lakes in Northern Alberta,
548   Canada. Parasitology, 131: 713−722.

549 Gómez, A., & E. Nichols, 2013. Neglected wild life: Parasitic biodiversity as a conservation target. International
550   Journal for Parasitology: Parasites and Wildlife, 2: 222−227.

551 Gozlan, R. E. (2002). Occurrence of the Asiatic cyprinid *Pseudorasbora parva* in England. Journal of Fish
552   Biology, 61: 298−300.

553 Gozlan, R. E., D. Andreou, T. Asaeda, K. Beyer, R. Bouhadad, D. Burnard, N. Caiola, P. Cakic, V. Djikanovic,
554   H. R. Esmaeili, I. Falka, D. Golicher, A. Harka, G. Jeney, V. Kováč, J. Musil, A. Nocita, M. Povz, N.
555   Poulet, … R. J. Britton, 2010. Pan-continental invasion of *Pseudorasbora parva*: Towards a better
556   understanding of freshwater fish invasions. Fish and Fisheries, 11: 315−340.

557 Gozlan, R. E., E.J. Peeler, M. Longshaw, S. St-Hilaire & S. W. Feist, 2006. Effect of microbial pathogens on the
558   diversity of aquatic populations, notably in Europe. Microbes and Infection, 8: 1358−1364.

559 Gozlan, R. E., S. St.-Hilaire, S. W. Feist, P. Martin & M. L. Kent, 2005. Biodiversity: Disease threat to European
560   fish. Nature, 435: 1046−1046.

561 Gozlan, R. E., C. M. Whipps, D. Andreou & K. D. Arkush, 2009. Identification of a rosette−like agent as
562   *Sphaerothecum destruens*, a multi-host fish pathogen. International Journal for Parasitology, 39:
563   1055−1058.

564 Grutter, A. S. & R. Poulin, 1998. Intraspecific and interspecific relationships between host size and the abundance
565   of parasitic larval gnathiid isopods on coral reef fishes. Marine Ecology Progress Series, 164: 263−271.

566 Hablützel, P. I., A. F. Grégoir, M. P. M. Vanhove, F. A. M. Volckaert & J. A. M. Raeymaekers, 2016. Weak link
567   between dispersal and parasite community differentiation or immunogenetic divergence in two sympatric
568   cichlid fishes. Molecular Ecology, 25: 5451−5466.

569 Hablützel, P. I., M. P. Vanhove, P. Deschepper, A. F. Grégoir, A. K. Roose, F. A. Volckaert & J. A. Raeymaekers,
570   2017. Parasite escape through trophic specialization in a species flock. Journal of Evolutionary Biology,
571   30: 1437−1445.

572 Hansen, E., & R. Poulin, 2006. Spatial covariation between infection levels and intermediate host densities in two
573   trematode species. Journal of Helminthology, 80: 255−259.

574 Hartvigsen, R. & O. Halvorsen, 1994. Spatial patterns in the abundance and distribution of parasites of freshwater
575   fish. Parasitology Today, 10: 28−31.

576 Jarkovský, J., B. Koubková, T. Scholz, M. Prokeš & V. Baruš, 2004. Seasonal dynamics of *Proteocephalus*
577   *sagittus* in the stone loach *Barbatula barbatula* from the Haná River, Czech Republic. Journal of
578   Helminthology, 78: 225−229.

579 Kakalova, E., & L. Shonia, 2008. The results of ecological-parasitological study of Pseudorasbora parva populated
580   in Kumisi reservoir and Basaleti lake. Proceedings of the Institute of Zoology, 23: 41−42.

581 Kalbe, M., Wegner, K. M., & Reusch, T. B. H. (2002). Dispersion patterns of parasites in 0+ year three−spined
582   sticklebacks: A cross population comparison. Journal of Fish Biology, 60(6), 1529−1542.

583   Kangur, A., & Kangur, P. (1998). Diet composition and size−related changes in the feeding of pikeperch,
584   *Stizostedion lucioperca* (Percidae) and pike, *Esox lucius* (Esocidae) in the lake Peipsi (Estonia). Italian
585   Journal of Zoology, 65(S1), 255−259.

586   Kapusta, A., E. Bogacka-Kapusta & B. Czarnecki, 2008. The significance of stone moroko, *Pseudorasbora parva*
587   (Temminck and Schlegel), in the small-sized fish assemblages in the littoral zone of the heated lake
588   Licheńskie. Fisheries and Aquatic Life, 16: 49−62.

589   Karvonen, A., B. K. Kristjánsson, S. Skúlason, M. Lanki, C. Rellstab & J. Jokela, 2013. Water temperature, not
590   fish morph, determines parasite infections of sympatric Icelandic threespine sticklebacks (*Gasterosteus*
591   *aculeatus*). Ecology and Evolution, 3: 1507−1517.

592   Kelly, D. W., R. A. Paterson, C. R. Townsend, R. Poulin & D. M. Tompkins, 2009. Parasite spillback: A neglected
593   concept in invasion ecology? Ecology, 90: 2047−2056.

594   Keskinen, T. & T. J. Marjomäki, 2004. Diet and prey size spectrum of pikeperch in lakes in central Finland.
595   Journal of Fish Biology, 65: 1147−1153.

596   Kilpatrick, A. M., P. Daszak, M. J. Jones, P. P. Marra & L. D Kramer, 2006. Host heterogeneity dominates West
597   Nile virus transmission. Proceedings of the Royal Society B: Biological Sciences, 273: 2327−2333.

598   Krasnov, B. R., G. I. Shenbrot, S. G. Medvedev, V. S. Vatschenok & I. S Khokhlova, 1997. Host-habitat relations
599   as an important determinant of spatial distribution of flea assemblages (Siphonaptera) on rodents in the
600   Negev Desert. Parasitology, 114: 159−173.

601   Landis, G. A. M., T. L. Mosley, W. R. Haag & J. A. Stoeckel, 2012. Effects of temperature and photoperiod on
602   lure display and glochidial release in a freshwater mussel. Freshwater Science, 31: 775−786.

603   Lazzaro, B. P. & T. J. Little, 2009. Immunity in a variable world. Philosophical Transactions of the Royal Society
604   B: Biological Sciences, 364: 15−26.

605   Lee, K. A., 2006. Linking immune defenses and life history at the levels of the individual and the species.
606   Integrative and Comparative biology, 46: 1000−1015.

607   Legendre, P. & E. D. Gallagher, 2001. Ecologically meaningful transformations for ordination of species data.
608   Oecologia, 129: 271−280.

609   Lima, D. P., H. C. Giacomini, R. M. Takemoto, A. A. Agostinho & L. M. Bini, 2012. Patterns of interactions of
610   a large fish−parasite network in a tropical floodplain. Journal of Animal Ecology, 81: 905−913.

611   Locke, S. A., M. S. Levy, D. J. Marcogliese, S. Ackerman & J. D. Mclaughlin, 2012. The decay of parasite
612   community similarity in ring-billed gulls Larus delawarensis and other hosts. Ecography, 35: 530−538.

613   Loot, G., Y. Reyjol, N. Poulet, A. Simkova, S. Blanchet & S. Lek, 2007. Effects of small weirs on fish parasite
614   communities. Parasitology Research, 101: 1265−1276.

615   Lugert, V., E. I. Meyer, J. Kurtz & J. P. Scharsack, 2017. Effects of an anthropogenic saltwater inlet on
616   three−spined stickleback (*Gasterosteus aculeatus*) (Teleostei: Gasterosteidae) and their parasites in an
617   inland brook. The European Zoological Journal, 84 444−456.

618   Luque, J. L. & R. Poulin, 2008. Linking ecology with parasite diversity in Neotropical fishes. Journal of Fish
619   Biology, 72: 189−204.

620   Marcogliese, D. J., 2002. Food webs and the transmission of parasites to marine fish. Parasitology, 124: 83−99.

621   Marques, J. F., M. J. Santos, C. M. Teixeira, M. I. Batista & H. N. Cabral, 2011. Host-parasite relationships in
622   flatfish (Pleuronectiformes) - The relative importance of host biology, ecology and phylogeny.
623   Parasitology, 138: 107−121.

624   Nekola, J. C. & P. S. White, 1999. The distance decay of similarity in biogeography and ecology. Journal of
625   Biogeography, 26: 867−878.

626   Pariselle, A., W. A. Boeger, J. Snoeks, C. F. Bilong Bilong, S. Morand & M. P. M. Vanhove, 2011. The
627   monogenean parasite fauna of Cichlids: A potential tool for host biogeography. International Journal of
628   Evolutionary Biology, 2011.

629   Peeler, E. J., B. C. Oidtmann, P. J. Midtlyng, L. Miossec & R. E. Gozlan, 2011. Non-native aquatic animals
630   introductions have driven disease emergence in Europe. Biological Invasions, 13: 1291−1303.

631   Perkins, S. L., A. S. Kerwin & A. D. Rothschild, 2009. Patterns of infection of the lizard malaria parasite,
632   Plasmodium floridense, in invasive brown anoles (*Anolis sagrei*) in Southwestern Florida. Parasitology
633   Research, 104: 1191−1196.

634   Petkova, I., R. N. Abbey-Lee & H. Løvlie, 2018. Parasite infection and host personality: *Glugea*-infected
635   three−spined sticklebacks are more social. Behavioral ecology and sociobiology, 72: 1−9.

636   Ponton, F., K. Wilson, S. C. Cotter, D. Raubenheimer & S. J. Simpson (2011). Nutritional immunology: A multi-
637   dimensional approach. PLoS Pathogens, 7: e1002223.

638   Postel, S. & B. Richter, 2003. Rivers for life: managing water for people and nature. Island Press.

639   Poulin, R., 2000. Variation in the intraspecific relationship between fish length and intensity of parasitic infection:
640   Biological and statistical causes. Journal of Fish Biology, 56: 123−137

641   Poulin, R. (2007). Are there general laws in parasite ecology? Parasitology, 134(6), 763−776.

642   Poulin, R. & T. L. F. Leung, 2011. Body size, trophic level, and the use of fish as transmission routes by parasites.
643   Oecologia, 166: 731−738.

644   Poulin, R. & S. Morand, 2000. The diversity of parasites. The quarterly review of biology, 75: 277−293.

645   Poulin, R., 2003. The decay of similarity with geographical distance in parasite communities of vertebrate hosts.
646   Journal of biogeography, 30: 1609−1615.

647 Poulin, R, 2010. Parasite Manipulation of Host Behavior: An Update and Frequently Asked Questions. Advances
648   in the Study of Behavior, 41: 151−186.

649 Poulin, R., C. A. Blanar, D. W. Thieltges & D. J. Marcogliese, 2011. The biogeography of parasitism in
650   sticklebacks: Distance, habitat differences and the similarity in parasite occurrence and abundance.
651   Ecography, 34: 540−551.

652 Poulin, R. & M. R. Forbes, 2012. Meta-analysis and research on host−parasite interactions: Past and future.
653   Evolutionary Ecology, 26: 1169−1185.

654 Poulin, R. & S. Morand, 2000. Testes size, body size and male-male competition in acanthocephalan parasites.
655   Journal of Zoology, 250: 551−558.

656 Prenter, J., C. MacNeil, J. T. A. Dick & A. M. Dunn, 2004. Roles of parasites in animal invasions. Trends in
657   Ecology & Evolution, 19: 385−390.

658 Přikrylová, I., I. Matějusová, J. Jarkovský & M. Gelnar, 2008. Morphometric comparison of three members of the
659   *Gyrodactylus nemachili*-like species group (Monogenea: Gyrodactylidae) on *Barbatula barbatula* L. in
660   the Czech Republic, with a reinstatement of *G. papernai* Ergens & Bychowsky, 1967. Systematic
661   parasitology, 69: 33−44.

662 R Core Team, 2020. R, A language and environment for statistical computing. R Foundation for Statistical
663   Computing, Vienna, Austria. URL https,//www.R−project.org/

664 Raeymaekers, J. A. M., T. Huyse, H. Maelfait, B. Hellemans & F. A. M. Volckaert, 2008. Community structure,
665   population structure and topographical specialisation of Gyrodactylus (Monogenea) ectoparasites living
666   on sympatric stickleback species. Folia Parasitologica 55: 187−196.

667 Rahn, A. K., D. A. Hammer & T. C. M. Bakker, 2015. Experimental infection with the directly transmitted parasite
668   Gyrodactylus influences shoaling behaviour in sticklebacks. Animal Behaviour, 107: 253−261.

669 Randhawa, H. S. & R. Poulin, 2010. Determinants of tapeworm species richness in elasmobranch fishes:
670   Untangling environmental and phylogenetic influences. Ecography, 33: 866−877.

671 Sana, S., E. A. Hardouin, R. E. Gozlan, D. Ercan, A. S. Tarkan, T. Zhang & D. Andreou, 2017. Origin and
672   invasion of the emerging infectious pathogen *Sphaerothecum destruens*: Origin of the emerging infectious
673   pathogen. Emerging microbes & infections, 6: 1−8.

674 Sanchez−Thirion, K., M. Danger, A. Bec, E. Billoir, S. Labaude, T. Rigaud, J. N. Beisel & V. Felten, 2019. High
675   food quality increases infection of *Gammarus pulex* (Crustacea: Amphipoda) by the acanthocephalan
676   parasite *Pomphorhynchus laevis*. International Journal for Parasitology, 49: 805−817.

677 Sheath, D. J., C. F. Williams, A. J. Reading & R. J. Britton, 2015. Parasites of non-native freshwater fishes
678   introduced into England and Wales suggest enemy release and parasite acquisition. Biological
679   invasions, 17: 2235−2246.

25

680 Shershneva, A. V. & A. E. Zhokhov, 2013. Parasites of stone loach *Barbatula barbatula* (L., 1758) (Balitoridae)
681   in small rivers of the upper and Middle Volga Basin. Inland Water Biology, 6: 236−245.

682 Šimková, A., J. Jarkovský, B. Koubková, V. Baruš & M. Prokeš, 2005. Associations between fish reproductive
683   cycle and the dynamics of metazoan parasite infection. Parasitology research, 95: 65−72.

684 Soininen, J., R. McDonald & H. Hillebrand, 2007. The distance decay of similarity in ecological communities.
685   Ecography, 30: 3−12.

686 Spikmans, F., P. Lemmers, H. J. M. op den Camp, E. van Haren, F. Kappen, A. Blaakmeer, G. van der Velde, F.
687   van Langevelde, R. S. E. W. Leuven & T. A. van Alen, 2020. Impact of the invasive alien topmouth
688   gudgeon (*Pseudorasbora parva*) and its associated parasite *Sphaerothecum destruens* on native fish
689   species. Biological Invasions, 22: 587−601.

690 Strayer, D. L. & D. Dudgeon, 2010. Freshwater biodiversity conservation: recent progress and future challenges.
691   Journal of the North American Benthological Society, 29: 344−358.

692 Studer, A., D. W. Thieltges & R. Poulin, 2010. Parasites and global warming: Net effects of temperature on an
693   intertidal host−parasite system. Marine Ecology Progress Series, 415: 11−22.

694 Székely, C., A. Palstra, K. Molnár & G. van den Thillart, 2009. Impact of the swim-bladder parasite on the health
695   and performance of European eels. In Spawning migration of the European eel (pp. 201−226). Springer,
696   Dordrecht.

697 Takemoto, R. M., G. C. Pavanelli, M. A. P. Lizama, J. L. Luque & R. Poulin, 2005. Host population density as
698   the major determinant of endoparasite species richness in floodplain fishes of the upper Paraná River,
699   Brazil. Journal of Helminthology, 79: 75−84.

700 Telfer, S., K. J. Bown, R. Sekules, M. Begon, T. Hayden & R. Birtles, 2005. Disruption of a host-parasite system
701   following the introduction of an exotic host species. Parasitology, 130: 661−668.

702 Thieltges, D. W., B. L. Fredensborg & R. Poulin, 2009. Geographical variation in metacercarial infection levels
703   in marine invertebrate hosts: Parasite species character versus local factors. Marine Biology, 156:
704   983−990.

705 Torchin, M. E., K. D. Lafferty, A. P. Dobson, V. J. McKenzie & A. M. Kuris, 2003. Introduced species and their
706   missing parasites. Nature, 421: 628−630.

707 Ward, A. J. W., A. J. Duff, J. Krause & I. Barber, 2005. Shoaling behaviour of sticklebacks infected with the
708   microsporidian parasite, *Glugea anomala*. Environmental Biology of Fishes, 72: 155−160.

709 Wheeler, A., 1992. Freshwater fishes of Britain and Europe. Rainbow Books.

710 Wootten, Z. I. R., 2013. Population biology of a cestode, *Proteocephalus filicollis* (Rudolphi) from *Gasterosteus*
711   *aculeatus* L. in Scotland. Canadian Journal of Pure and Applied Sciences, 2475.

712 Zander, C. D., 2005. Four-year monitoring of parasite communities in gobiid fishes of the southwest Baltic: III.
713   Parasite species diversity and applicability of monitoring. Parasitology Research, 95: 136−144.

Tables

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

| Code	River	Location	Latitude	Longitude	Three-spined stickleback	Stone loach	Topmouth gudgeon
WingB	Winge	Blauwmolen	4.8000	50.9387	X	X	X
BegB	Begijnenbeek	Bekkevoort	5.0029	50.5494	X	X	X
ZwaP	Zwarte beek	Paal	5.1921	51.0288	X	X	X
SteT	Steenlaak	Thiewinkel	5.2341	50.9651	X	X	X
MelR	Melsterbeek	Runkelen	5.1596	50.8565	X	X	X
KlhS	Kleine Herk	Stevoort	5.2506	50.9178	X		X
VelG	Velpe	Glabbeek	4.9501	50.8563	X	X	X
HerH	Herk	Hoepertingen	5.2973	50.8071	X	X	X
MenT	Mene	Tienen	4.9254	50.8030	X	X	
DemT	Demer	Tongeren	5.5045	50.8163	X		X
KlbS	Kleine beek	Schaffen	5.0483	51.0108		X	X
DorL	Dormaalbeek	Landen	5.0847	50.7368	X		
KlgE	Kleine Gete	Ezemaal	5.0028	50.7751		X	
ZwaB	Zwarte beek	Beringen	5.3175	51.0919		X	
RooZ	Roosterbeek	Kerkom	4.8817	50.8454			X
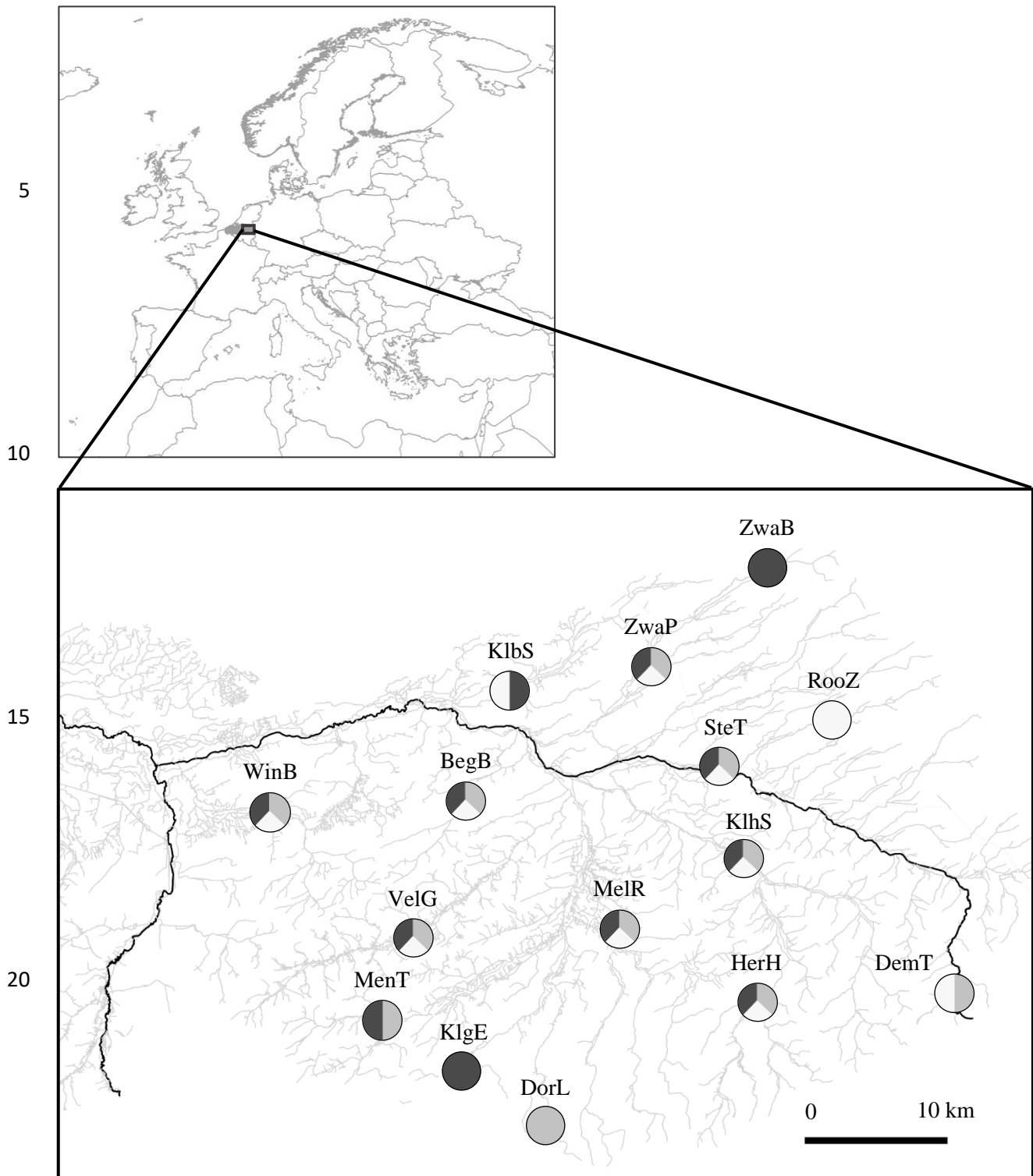
Figure 1 Overview of the 15 sampling locations (Demer basin, Flanders, Belgium). The black lines represent the main rivers (Left = Dijle, right = Demer). See Table 1 for site codes and geographic coordinates. Colors indicate the presence of the three hosts.

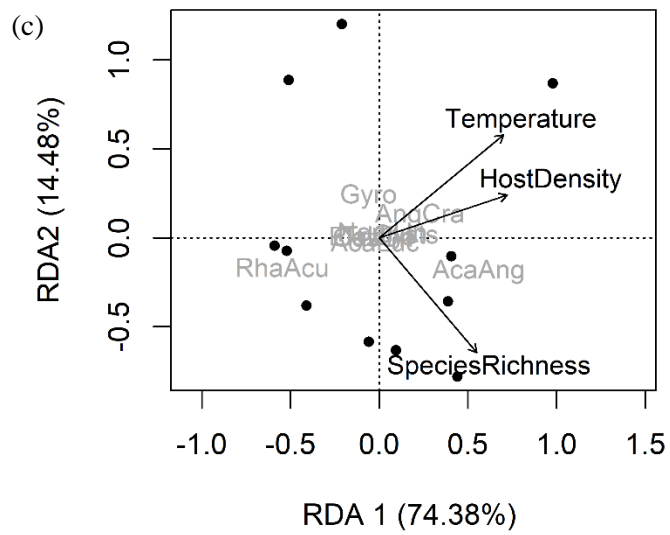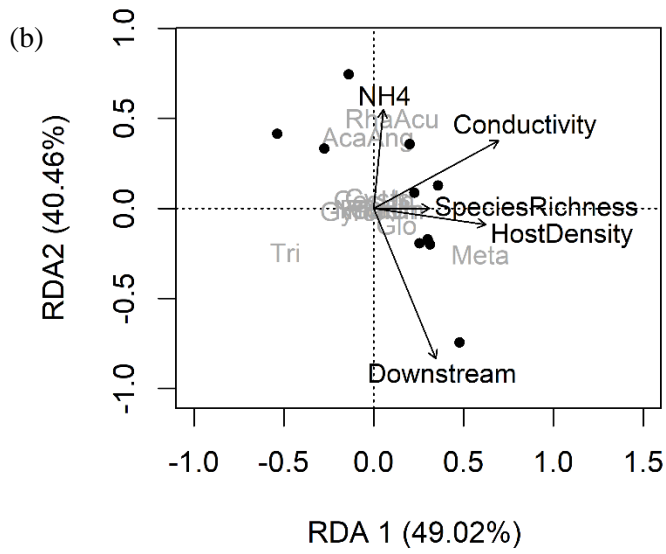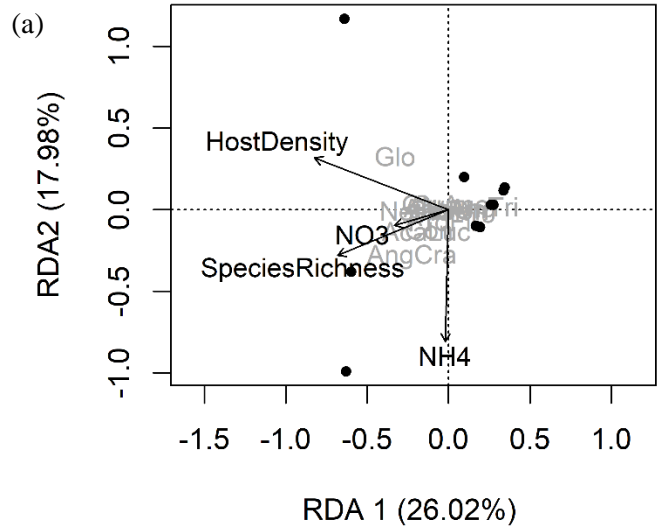◐ three-spined stickleback   ● stone loach   ○ topmouth gudgeon
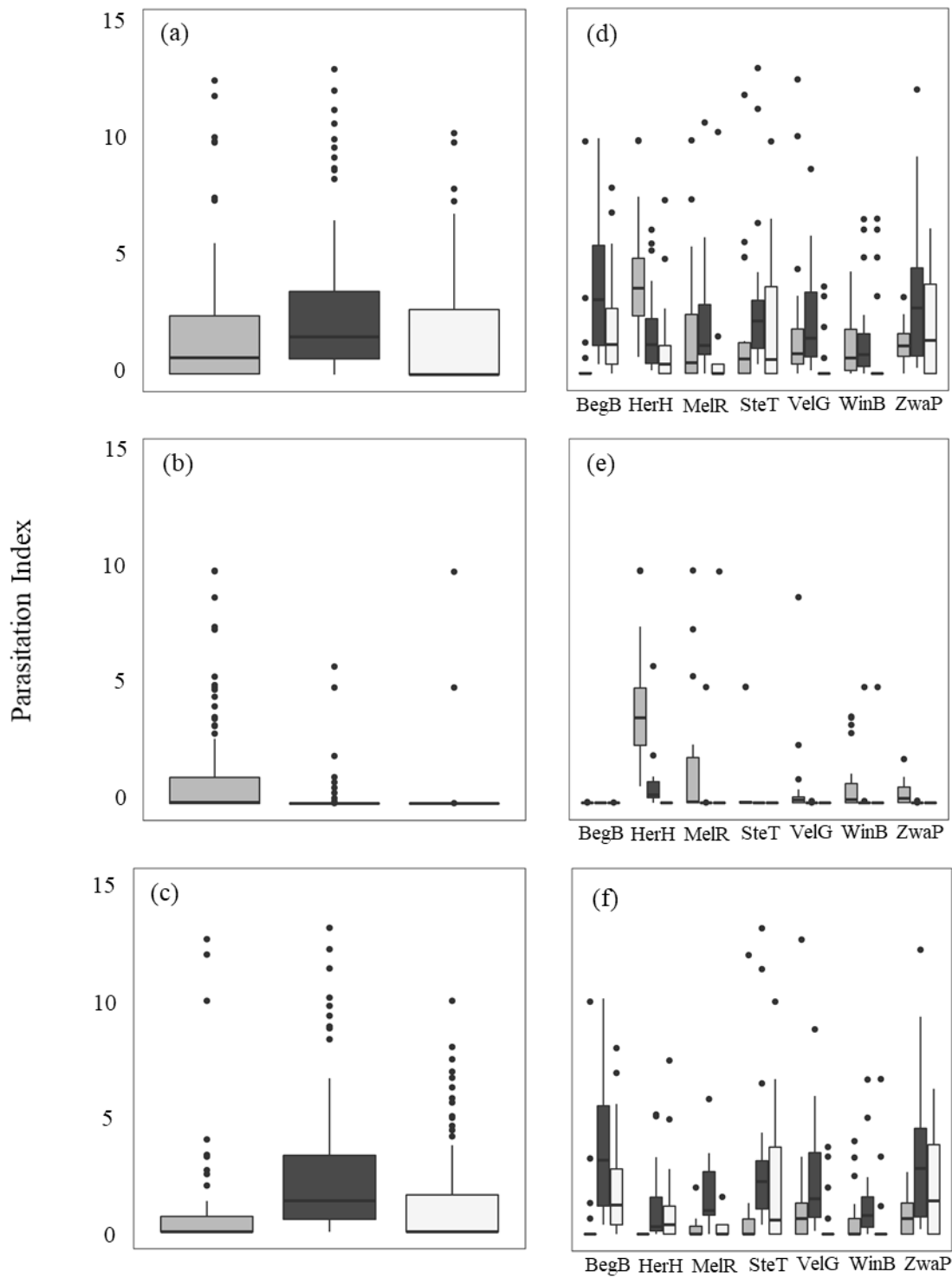
Figure 2 Individual parasitation index (I_{PI}) by species, combining all sampling sites (left) and per species per sampling site (right) for (a, d) all parasites (b, e), ectoparasites, and (d, f) endoparasites.

⬤ three-spined stickleback ⬤ stone loach ◯ topmouth gudgeon

30

35

(a)

(b)

(c)

42   **Figure 3** RDA triplot linking the selected environmental and spatial variables to parasite abundance in

43   three-spined stickleback (a), stone loach (b), and topmouth gudgeon (c). Parasite species are labeled in grey.

44   Percentages reflect the amount of variance explained by axis 1 and axis 2. An explanation for the

45   abbreviations can be found in Table S1. Each dot represents a sampling location.