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Distribution of common stickleback parasites on North Uist, Scotland, in relation to ecology and host traits☆

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ABSTRACT

Analysing spatial differences among macroparasite communities is an important tool in the study of host–parasite interactions. Identifying patterns can shed light on the underlying causes of heterogeneity of parasite distribution and help to better understand ecological constraints and the relative importance of host and parasite adaptations. In the present study, we aimed to find correlational evidence that the macroparasite distribution patterns on the Scottish island of North Uist, which had been described by de Roij and MacColl (2012), are indicative of local processes rather than an unspecific influence of habitat characteristics. We therefore reinvestigated parasite abundances and tested for associations with habitat characteristics and host traits. Distribution patterns of the most common parasites were largely consistent with the observations of de Roij and MacColl (2012). In accordance with the published results, we found that the most obvious abiotic habitat characteristic varying among the lakes on the island, pH, did not statistically explain parasite abundances (except for eye fluke species inside the lens). Instead, we found that genetic differentiation between host populations, measured as pairwise F_{ST} values based on available microsatellite data, was significantly correlated with dissimilarity in parasite community composition. Our results indicate that individual lake characteristics rather than physicochemical variables shape parasite distribution on this island, making it an ideal place to study host–parasite interactions. Furthermore, additionally to geographic distance measures taken from maps, we suggest taking into account connectivity among freshwater habitats, indirectly measured via fish population structure, to analyse spatial distribution patterns.

1. Introduction

Identifying constraints imposed by environmental factors on the spatial distribution of free-living organisms remains a key question in understanding (their) evolution. Parasites (here we consider macroparasites) are also limited in their dispersal by (abiotic) environmental factors, but in addition depend on the availability – and therefore on the spatial distribution – of suitable hosts (see, e.g., Bozick and Real, 2015 for a recent review). Furthermore, the interactions between hosts and parasites themselves can be affected by environmental changes like increase of temperature (global warming) or eutrophication (e.g., Brunner and Eizaguirre, 2016). In the study of host–parasite interactions and host–parasite coevolution in particular, it is therefore important to characterise the biotic and abiotic circumstances that determine the dispersal and infection success of a certain parasite. In addition to the abundance of intermediate hosts (e.g., Sures and Streit, 2001; Sokolow et al., 2015), use of different niches within the same habitat (MacColl, 2009; Eizaguirre et al., 2011) or host genetic factors (Lange et al., 2015) can lead to different parasite communities of one host species. On the other hand, parasites can also act as selective agents and promote local adaptation of their hosts (Stokke et al., 2002; Schmid-Hempel, 2011). Local adaptation requires that hosts and parasites co-occur at a place for long enough so that resident hosts (genotypes) can gain an advantage over non-resident hosts (Williams, 1966; also see Feis et al., 2016, for an example). Numerous studies on three-spined sticklebacks (*Gasterosteus aculeatus* L.),

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a model organism in evolutionary biology and ecology (Wootton, 1976, 1984; Bell and Foster, 1994; Östlund-Nilsson et al., 2007; von Hippel, 2010) and the host species of the present study, have shown that local adaptation can lead to spatial differences between populations in resistance against parasites (e.g., Kalbe and Kurtz, 2006; de Roij et al., 2010; Raeymaekers et al., 2011; Konijnendijk et al., 2013; Scharsack et al., 2016). Further, spatial differences in parasite distribution can be due to factors such as geographic distance (Poulin, 2003) or differences in physicochemical variables (Goater et al., 2005; Thieltges et al., 2010). These abiotic factors can act on parasites either directly or indirectly, e.g. by providing more or less suitable conditions for their (intermediate) hosts. It can be assumed that habitat characteristics that directly affect parasites (e.g., temperature, salinity, pH, pollution) have a greater impact on pathogens that are constantly in contact with the surrounding medium (ectoparasites or free-living stages of endoparasites) than on endoparasites that are 'protected' by their host (Blanar et al., 2009).

Here, we examine the distribution patterns of parasites of three-spined sticklebacks from several lakes on the Scottish island of North Uist. This system is particularly interesting for studying host–parasite interactions, because the numerous isolated lakes on the island comprise a wide range of different habitats. A published survey of the macroparasitic fauna of sticklebacks from North Uist found temporally (over two years) consistent differences in parasite distribution patterns (de Roij and MacColl, 2012). Although five prominent habitat characteristics – lake surface area, pH, the concentration of calcium ions, chlorophyll A concentration, and dissolved organic carbon content – were analysed, none of these factors could explain differences in parasite abundances, leaving individual lake characteristics as the most reasonable explanation.

With the present study, we aimed to reinvestigate the distribution of the most common stickleback parasites on North Uist in relation to abiotic factors and host traits. In detail, we (i) analysed associations of infection with pH in a more balanced choice of lakes (7 alkaline and 12 acidic lakes compared to 2 alkaline and 10 acidic lakes in de Roij and MacColl, 2012) and (ii) compared our data to published infection data to see whether general distribution patterns had been consistent over more than two years, i.e. over several stickleback generations. As fish parasites can be assumed to be directly (ectoparasites) or indirectly (suitability for intermediate host(s)) influenced by the quality of the ambient water, we hypothesised that parasite distribution would not be independent of pH. In addition, we compared differences in parasite community composition with neutral genetic differentiation (measured as pairwise F_{ST} based on available microsatellite data) between host populations and hypothesised that common distribution patterns could be indicative of local host–parasite dynamics.

2. Materials and methods

2.1. Sampling

The island of North Uist is relatively small (about 300 km^2) and covered with more than 180 lakes (Giles, 1983), most of which have been colonised by sticklebacks from the sea since the last deglaciation about 15,000 years ago. The lakes in the western part of the island are characterised by shell sediment, with alkaline, clear water, while the lakes in the central and eastern part are influenced by peat and thus tea-stained and more acidic (Giles, 1983). A population genetic analysis of the sticklebacks of North Uist revealed restricted gene flow and strong genetic differentiation among the fish populations (Rahn et al., unpublished data).

To cover a broad spectrum of different habitats, approximately 20 (20.8 ± 2.3, mean ± standard deviation (sd)) three-spined sticklebacks (*Gasterosteus aculeatus* L.) per sampling location were collected from 19 different freshwater lakes and from 3 different brackish water lagoons (see Fig. 1 and Table 1 for sampling locations and number of dissected fish). During the breeding season resident and anadromous sticklebacks co-occur at those brackish water sites and hence fish of both populations were collected. Adult sticklebacks were caught at the beginning of the breeding season, when most fish were still reproductively inactive. Fish were caught in spring 2010 (April and May) and 2011 (April) using minnow traps (green nylon mesh, 3–4 mm, in 2010–Jenzi, Plüderhausen, Germany; galvanized steel mesh, Gee's G40 M, G48 M, in 2011–Tackle Factory, Fillmore, NY, USA). In 2011, 20 nine-spined sticklebacks were caught in Loch Sanndaraigh (8SAN). Fish were transported individually in their original lake water in 1 litre boxes to a rented cottage where they were either dissected the same day or after an average period of four days.

2.2. Dissection and parasite screening

For every fish, standard length (SL), measured as the distance between the tip of the mouth and the end of the caudal peduncle, was measured using graph paper covered by a plastic film. Sticklebacks were killed by decapitation immediately followed by a cut through the brain. Fish were screened for ectoparasites as well as parasites infecting the lens, vitreous chamber, and retina of the eyes under a microscope (Novex RZ-Range, 6.5–45× magnification; Euromex Microscopen, Arnhem, Netherlands) with a cold light source (Schott KL 1500; Schott AG, Mainz, Germany). Additionally, the presence of *Schistocephalus solidus*, a *G. aculeatus*-specific cestode, was recorded and the sex of the respective fish was determined by gonad inspection. Where possible, parasites were identified to species level.

2.3. Calculation of parasite indices

Prevalence (percentage of infected fish in a lake), abundance (sum of parasite individuals on/in infected fish divided by the number of dissected fish) and mean infection intensity (MI, mean number of parasite individuals on infected fish) were calculated for all parasites and locations sampled in 2010 and 2011. If less than 10 fish were caught in a lake in 2010, that lake was sampled again in 2011 and the 2010 fish were excluded from the analysis (see Table 1). Two indices for comparing the similarity of parasite communities were calculated using the program Past3 (Hammer et al., 2001): the Jaccard index, i.e. the proportion of parasite species shared between two lakes, based on presence/absence data, and the Bray–Curtis similarity index that also takes into account the mean abundance. Calculation of both indices was based on infection data of *Thersitina gasterostei*, *Gyrodactylus* spp., *Schistocephalus solidus*, *Diplostomum* spp. (non-lens), *Apatemon* spp., and *Diplostomum* spp. (lens). As for *S. solidus* only presence/absence data were available, 0 and 1 were included as mean abundance of this parasite.

2.4. Microsatellite genotyping and analysis

Pairwise F_{ST} values calculated from microsatellite data were used as a measure of neutral genetic differentiation between host populations. F_{ST} values were taken from another study (Rahn et al., unpublished data), which largely used tissue samples of the present study as raw material. F_{ST} values were calculated in Arlequin 3.5.1.3 (Excoffier and Lischer, 2010) with 1000 permutations.

In short, a minimum of 20 fish per sampling location (24.2 ± 6.8, mean ± sd; Table 1) was genotyped at nine polymorphic microsatellite loci (genotypes are available at http://dx.doi.org/10.17632/rr434xd2dm.1). Further details on sample sizes and

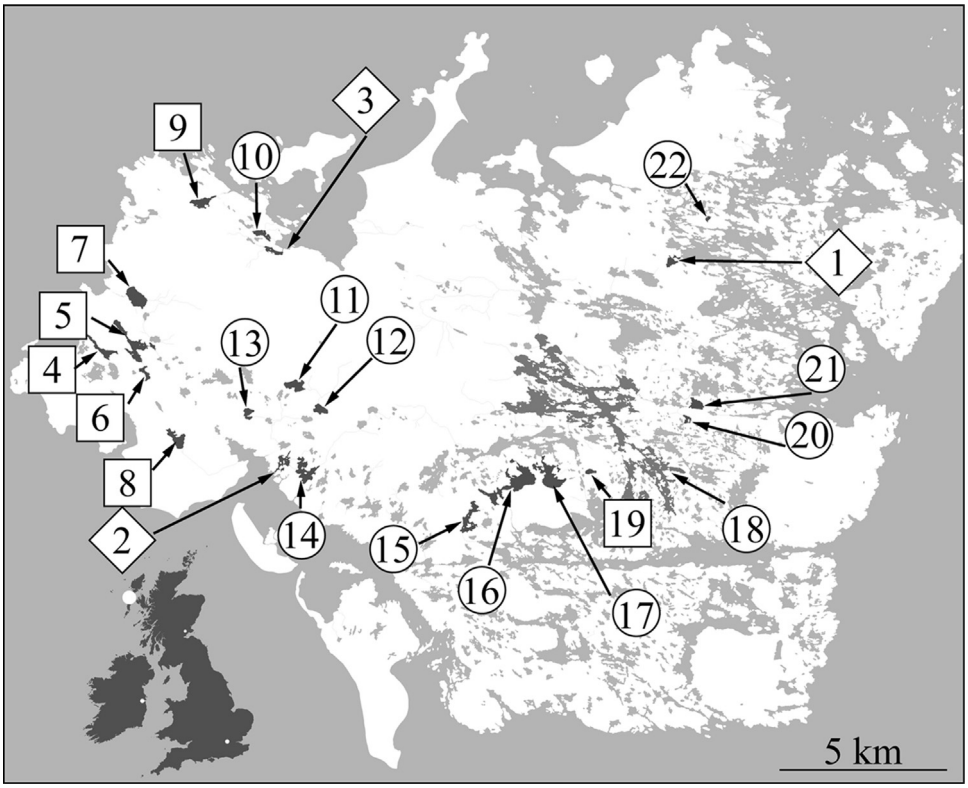

Fig. 1. Distribution of the sampling locations on North Uist. Numbers correspond to numbers in Table 1 (LocID). Squares = alkaline lakes, circles = acidic lakes, diamonds = brackish water sites.

Table 1
Sampling locations (19 freshwater lakes, 3 coastal lagoons with anadromous and resident fish) with three letter codes (LocID), surface area (Area) in km^2, number of dissected fish (N_{dis}), sex ratio (proportion of males), pH value (mean of three measurements), conductivity in µS, habitat type, and absorbance at 400 nm (A400). Fish used for the calculation of pairwise F_{ST} values (N_{ms}, genotyped at nine microsatellite loci, see Section 2 and Supplementary Table S1 for details) were caught in 2010 and 2011 and partly overlap with dissected fish.

Location name	Geographic coordinates	LocID	Area	Year	N_{dis}[c]	N_{ms}	Sex ratio[d]	pH	µS	Habitat	A400
Aileodair *anadromous*	57°38′7″N, 7°12′54”W	1ana	0.069	2011	21	–	0.50	8.32	–	brackish	0.01
Aileodair *resident*		1res		2010	20	–	0.10				
Aird Heisgeir *anadromous*	57°34′48″N, 7°24′48″W	2ana	0.114	2011	19	–	0.84	7.85	–	brackish	0.03
Aird Heisgeir *resident*		2res		2011	20	–	0.33				
nan Clachan *anadromous*	57°38′14″N, 7°24′45″W	3ana	0.109	2011	21	–	0.29	7.52	–	brackish	0.02
nan Clachan *resident*		3res		2011	19	–	0.39				
Croghearraidh	57°36′54″N, 7°30′40″W	4GRO	0.108	2011	21	22	0.45	7.94[e]	375[e]	alkaline	0.03
Eubhal	57°37′6″N, 7°29′42″W	5EUB	0.379	2011	20	20	0.55	7.89	408	alkaline	0.01
nam Magarlan[g]	57°36′10”N, 7°28′54”W	6MAG	0.066	2010	21	22	0.24	7.19	325	alkaline	0.03
Hosta[g]	57°37′40″N, 7°29′18″W	7HOS	0.247	2011	21	20	0.14	8.34	324	alkaline	0.01
Sanndaraigh[a]	57°35′12″N, 7°27′48″W	$8SAN_{10}$	0.157	2010	17	41	0.36	8.10[f]	384[f]	alkaline	0.02
		$8SAN_{11}$		2011	30		0.33				
		8SAN9sp		2011	20	–	0.25				
Olabhat	57°39′8″N, 7°26′48″W	9OLA	0.141	2011	21	20	0.52	7.47	231	alkaline	0.02
na Gearrachun	57°38′34″N, 7°25′18″W	10GEA	0.070	2011	24	33	0.52	6.89	236	acidic	0.02
Mhic Gille-bhride[g]	57°36′6″N, 7°24′36″W	11MGB	0.142	2010	21	21	0.29	6.77	164	acidic	0.03
a’ Charra	57°35′45″N, 7°23′42″W	12ACH	0.093	2010	21	21	0.30	6.62	188	acidic	0.03
Mhic a’ Roin[g]	57°35′42″N, 7°25′48″W	13MOI	0.064	2011	20	20	0.55	6.30	177	acidic	0.04
Dubhasairidh[g]	57°34′54″N, 7°24′12″W	14DUB	0.234	2011	20	25	0.50	6.67	183	acidic	0.05
Tormasad[g]	57°33′45″N, 7°19′W	15TOR	0.213	2010	18	40	0.11	6.87	181	acidic	0.04
a’ Bharpa[g]	57°34′24″N, 7°17′42″W	16BHA	0.482	2011	23	20	0.52	6.10	140	acidic	0.03
na Moracha[g]	57°34′30″N, 7°16′18″W	17MOR	0.367	2011	21	30	0.05	6.53	175	acidic	0.03
Sgadabhagh[b,g]	57°35′6″N, 7°14′10″W	18SCD	5.516	2011	20	20	0.32	6.16	139	acidic	0.03
nan Ceithir Eilean	57°34′24″N, 7°15′30″W	19EIL	0.033	2011	21	21	0.05	7.37	370	alkaline	0.01
an Daimh[g]	57°35′35″N, 7°12′35″W	20DAI	0.034	2011	20	20	0.30	6.87[e]	176[e]	acidic	0.04
na Maighdein[g]	57°35′42″N, 7°12′6″W	21MAI	0.095	2011	21	24	0.35	6.30	187	acidic	0.02
na Buaile[g]	57°38′48″N, 7°11′48″W	22BUA	0.020	2010	20	20	0.65	6.29	247	acidic	0.02

[a] Three-spined sticklebacks caught in 2010 (8SAN10) and 2011 (8SAN11), and nine-spined sticklebacks (8SAN9sp).
[b] Referred to as “South Sgadabhagh” by Spence et al. (2013).
[c] 2010 samples of lakes 21MAI, 9OLA, 14DUB, 10GEA, and 17MOR were excluded due to low sample sizes (3, 4, 5, 8, and 9 fish, respectively).
[d] Sex not determined for one fish from 1ana, 3res, 4GRO, 10GEA, 12ACH, 18SCD, and 21MAI, two fish from 2res, and six fish from 8SAN10.
[e] One measurement.
[f] Average of four measurements.
[g] Lake also sampled by de Roij and MacColl (2012).

PCR conditions can be found in Table 1 and Table S1 in the supplementary online Appendix.

2.5. Abiotic habitat characteristics

For each freshwater lake, pH and conductivity were measured using a pH meter (HI 98129; Hanna Instruments, Woonsocket, RI, USA). Water samples were taken to the Institute of Cellular and Molecular Botany (IZMB, University of Bonn), where absorbance was measured with a spectrophotometer (range: 300–700 nm, UV mini 1240, program: UVProbe 2.31; Shimadzu Corp., Kyoto, Japan). Absorbance at 400 nm (A400) was used as a measure for turbidity, as differences between water bodies were most pronounced at this wavelength. This measure has proven useful in other studies as well (Reimchen, 1989; Scott, 2001). Lake surface area was used as a proxy for host population size as larger water bodies can be assumed to contain larger populations and expected heterozygosities (H_e) of the stickleback populations on North Uist are significantly positively correlated with lake surface area (Rahn et al., unpublished data). Measures of lake surface area were taken from Rahn et al. (unpublished data). They had been determined from a 1:25000 Ordnance Survey map using ImageJ 1.45 s (Rasband, 1997–2009).

2.6. Statistical analysis

Statistical tests were performed in R 3.0.1 (R Core Team, 2013) except for Mantel tests, which were performed in Arlequin 3.5.1.3 (Excoffier and Lischer, 2010). Significance was determined from Bonferroni-adjusted α levels. Overall sample size was low in 2010 (6 lakes, compared to 14 lakes in 2011) and different lakes were sampled in both years (except for 8SAN). Also, overall parasite abundances might have been different in the two years. We therefore analysed data of 2010 and 2011 separately. First, we tested for associations between the habitat characteristics turbidity (A400), pH, conductivity and lake surface area of all 19 freshwater lakes. Pearson correlations and Spearman rank correlations were used for normally distributed data and data significantly deviating from normal distribution (tested with a Kolmogorov–Smirnov test), respectively.

We then used generalised linear models (GLM) to test whether infections varied significantly among lakes (the only fixed factor; with SL, sex, date of capture as covariates) and generalised linear mixed models (GLMM) with lake as random factor to analyse whether infection status could statistically be explained by host (SL, sex) or habitat characteristics (pH, lake surface area as fixed factors; date of capture as covariate). For this, two different measures of infection were used as a dependent variable in separate models: prevalence, which could take the values 'infected' (with at least one parasite of a given species) and 'uninfected' (respective parasite species not found on/in the fish), and abundance, which was defined as the number of parasites of a given species found on/in the fish. Models with prevalence data were fitted using the glm (GLMs) and glmer function (lme4 package for GLMMs; Bates et al., 2015) with binomial error distribution and logit link function. GLMs with abundance data were fitted using the glm.nb function of the MASS package (Venables and Ripley, 2002), which is specially designed for handling negative binomial data. For GLMMs with abundance data we used the glmmadmb function of the glmmADMB package (Fournier et al., 2012) with negative binomial error distribution and log link function. Changes between full and reduced models were compared to a χ^2 distribution. Model reduction was performed in order of decreasing P values until a minimum model including only terms accounting for significant ($P < 0.05$) changes in model fit was found. All models were calculated for prevalence and abundance data of *Gyrodactylus* spp., *Diplostomum* spp. found in the lens (only 2011 due to low sample sizes in 2010), *Diplostomum* spp. and *Apatemon* spp. from the non-lens region, and *T. gasterostei* as well as for prevalence data of *S. solidus* infections.

Following an approach similar to that in Karvonen et al. (2015), we estimated pairwise differences in parasitic faunas between lakes using three measures: 1-Jaccard dissimilarity, Bray–Curtis dissimilarity (1-Bray–Curtis similarity), and absolute differences in mean abundances of *Gyrodactylus* spp., *Diplostomum* spp. (non-lens), and *Apatemon* spp. We then performed Mantel tests (5000 permutations) to test for significant correlations between dissimilarity in parasitic fauna, absolute differences in pH and pairwise genetic differentiation (F_{ST}).

We also tested for associations between our prevalence and abundance data and those of de Roij and MacColl (2012) using Pearson or Spearman rank correlations. Additionally, we compared our results to the published data by applying similar statistics as used in de Roij and MacColl (2012) to our own data of the lakes sampled in the aforementioned study ($N = 12$) as well as to those sampled in 2010 ($N = 6$), and in 2011 ($N = 14$). In detail, prevalence and mean abundance per lake were regressed against pH and lake surface area.

3. Results

3.1. Parasite abundance

Prevalence and mean infection intensities of 11 common stickleback parasites are summarised in Table S2 and Fig. S1 in the supplementary online Appendix. The distribution of 6 freshwater parasites in relation to pH is displayed in Fig. 2. We detected the ectoparasites *Thersitina gasterostei*, a copepod, the monogenean *Gyrodactylus* spp. (probably *Gyrodactylus arcuatus*; de Roij et al., 2010), and the peritrichs *Trichodina* spp. and *Apiosoma* spp. *Gyrodactylus* spp. was present at nearly all sampling locations, except for two acidic lakes (20DAI and 22BUA). *T. gasterostei* was present only on resident fish from the brackish water sites, on fish from alkaline freshwater lakes (except for 7HOS) and from acidic lakes 10GEA, 11MGB, and 12ACH. This is – regarding the 12 lakes sampled in both studies – nearly the same finding as in de Roij and MacColl (2012) for 2008, when *Gyrodactylus* spp. was absent from lakes 16BHA, 20DAI, and 22BUA and when *T. gasterostei* was only present in lakes 6MAG and 11MGB, but not in lake 7HOS or one of the other more acidic lakes. Metacercariae of the endoparasite *Diplostomum* spp. are notoriously difficult to identify morphologically and species diversity within the stickleback eye is considered higher than previously thought (Locke et al., 2010b; Blasco-Costa et al., 2014; Locke et al., 2015). Molecular identification using the barcode region of the cytochrome *c* oxidase subunit 1 (*cox*1) of the mitochondrial DNA indicates that at least the species *D.* lineage 6 sensu Blasco-Costa et al. (2014) and *D. baeri* 2 sensu Georgieva et al. (2013) are present on North Uist (Rahn et al., unpublished data). As species could not be identified for every metacercaria, we will speak of "*Diplostomum* spp." and only distinguish between *Diplostomum* spp. from the lens or the non-lens region of the eye. *Diplostomum* spp. (lens and non-lens) were not found in resident and anadromous fish caught at the brackish water sites (see Fig. S1) due to a lack of the mollusc intermediate host (the lymnaeid snail *Radix peregra*). Likewise, *Apatemon* spp. (probably *A. gracilis* (Blair, 1976)) and *S. solidus* were, as expected, found almost exclusively in freshwater lakes with the exception of one *Apatemon*- and one *Schistocephalus*-infected fish caught at the north-western brackish water site. Trematodes causing the 'black spot disease' (probably *Cryptocotyle* spp.) and the microsporidian *Glugea anomala* were predominantly found in fish from the brackish water sites (*G. anomala* also in 17MOR, 'black spot' also in nine-spined sticklebacks from lake 8SAN; see Table

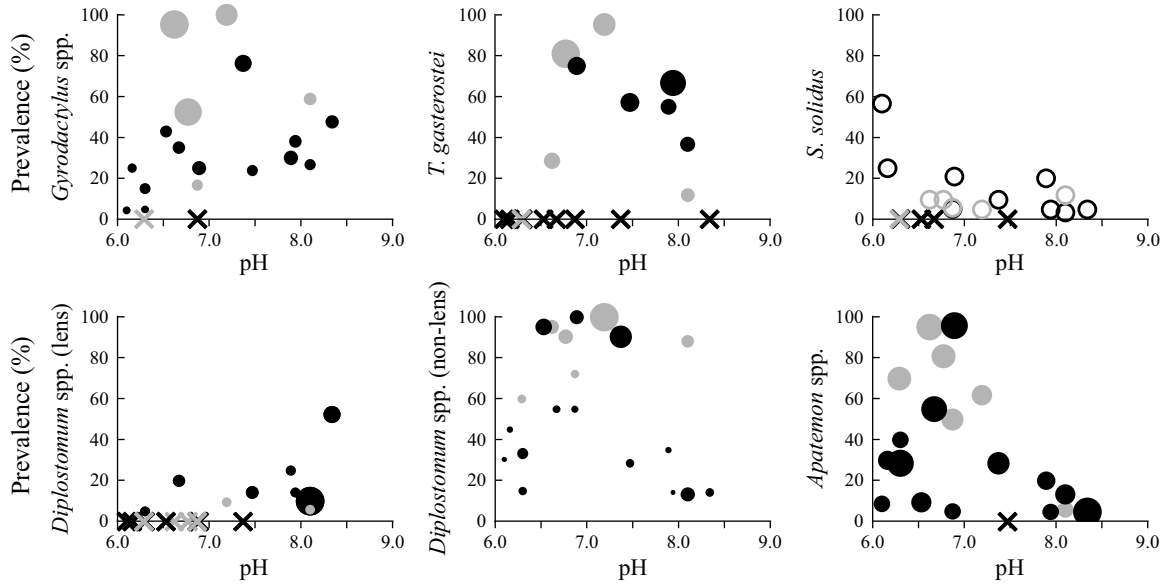

Fig. 2. Prevalence of six stickleback parasites in 19 freshwater lakes on North Uist in relation to pH. Prevalence (% infected) is given as black (2011) and grey (2010) circles or crosses (prevalence = 0%). Circle areas correspond to mean infection intensities (mean number of parasites on infected fish) and are proportional to each other within, but not among, plots. The largest circle in a plot corresponds to an average of 13.2 (*Gyrodactylus* spp.), 6.5 (*T. gasterostei*), 8.3 (*Diplostomum* spp. (lens)), 33.4 (*Diplostomum* spp. (non-lens)), and 3.0 (*Apatemon* spp.) parasites on infected fish. Only prevalence data were available for *S. solidus*.

S2). *Diplostomum* spp. from the lens and from the non-lens region as well as *Apatemon* spp. were also found in nine-spined sticklebacks. As we did not identify these parasites to species level, we cannot say whether they represent the same species as found in the three-spined sticklebacks. However, *Diplostomum* species infesting the eye lens are usually not considered very host-specific (Locke et al., 2010a) and sequencing the barcode region of three *Diplostomum* metacercariae from the non-lens region indicated that at least *Diplostomum* lineage 6 (Blasco-Costa et al., 2014) is present in both stickleback species (Rahn et al., unpublished data).

3.2. Parasite abundance in relation to host and habitat characteristics

Light transmission was reduced in acidic lakes as suggested by the significant and negative correlation between absorbance at 400 nm and pH (Pearson correlation: $r_P = -0.59$, $N = 19$, $P = 0.009$; Fig. S2), which was significantly positively correlated with conductivity (Spearman rank correlation: $r_S = 0.76$, $N = 19$, $P < 0.001$). There was no significant correlation between lake surface area and any of the mentioned habitat characteristics (all $P > 0.2$). Parasitic infections significantly varied among lakes in 2010 and in 2011 ($\chi^2 > 33$, $P < 0.001$; Table S3) except for infections (prevalence) with *Diplostomum* spp. from the non-lens region in 2010 ($\chi^2 = 7.0$, $P = 0.218$; Table S3) and *S. solidus* in 2010 ($\chi^2 = 3.7$, $P = 0.597$; Table S3). Bigger fish were significantly more likely to be infected (with higher burdens) with *Gyrodactylus* spp. (abundance), *T. gasterostei* (abundance), *Diplostomum* spp. (non-lens, prevalence and abundance), and *Apatemon* spp. (abundance) in the lakes sampled in 2010, and with *Diplostomum* spp. (lens, abundance) and *Diplostomum* spp. (non-lens, prevalence and abundance) in the lakes sampled in 2011 (Table 2). For 2011, a female bias of *T. gasterostei* infections was detected ($\chi^2 = 8.2$, $P = 0.004$; Table 2). *Diplostomum* spp. (non-lens) infection (abundance) was significantly positively correlated with pH in the 2010 lake data ($\chi^2 = 7.8$, $P = 0.005$; Table 2), but not in the 2011 data. Infections with *Apatemon* spp. (prevalence and abundance) were significantly negatively associated with pH in the 2010 lake data (both $\chi^2 > 9.7$, $P < 0.003$; Fig. 2 and Table 2). Regarding the lakes sampled in 2011, only infections (prevalence and abundance) with *Diplostomum* spp. (lens) were significantly (positively) correlated with pH (both $\chi^2 > 9.5$, $P < 0.003$; Fig. 2 and Table 2). Lake surface area was never a significant predictor of infection (all $\chi^2 < 5.2$, $P > 0.02$, $\alpha = 0.0056$).

Analysis of dissimilarity in the parasite community revealed that (qualitative) differences in parasite community composition based on presence/absence data (1-Jaccard) were significantly associated with genetic differentiation, but not with the extent of differences in pH in the 2011 data set (Fig. 3 and Table S4). After correcting for multiple tests, no such correlation was found for the 2010 lakes (ibidem). Differences in parasite abundances (Bray–Curtis dissimilarity, *Gyrodactylus* spp., *Diplostomum* spp. (non-lens), *Apatemon* spp.) were not significantly correlated with genetic differentiation (Table S4).

The direct comparison of our data of the lakes sampled in de Roij and MacColl (2012) with the published data revealed positive trends in all cases, but correlations were only significant regarding infection with *Apatemon* spp. (prevalence: 2008, abundance: 2007 and 2008, Spearman rank correlations, $r_S > 0.8$, $P < 0.001$), *Diplostomum* spp. (non-lens, abundance: 2008, Spearman rank correlation, $r_S = 0.8$, $P = 0.003$), and *Gyrodactylus* spp. (abundance: 2008, Spearman rank correlation, $r_S = 0.8$, $P = 0.001$; Table S5). Applying similar statistics to analyse associations with habitat characteristics as in de Roij and MacColl (2012) to our own data yielded (qualitatively) the same result: no significant correlation of infection with pH or lake surface area (all Bonferroni-adjusted $P > 0.05$). The only (positive) trend that remained after correcting for multiple tests was between pH and *Diplostomum* spp. (lens, abundance) in 2011 (Spearman rank correlation, $r_S = 0.7$, $P = 0.005$, $\alpha = 0.0045$; Table S6).

4. Discussion

In accordance with the findings published by de Roij and MacColl (2012), we detected significant variation in parasite distribution among lakes. Distribution patterns found in both studies were generally similar regarding presence/absence of the parasites *T. gasterostei*, *Gyrodactylus* spp., *Diplostomum* spp. (non-lens), *Apatemon* spp., and *S. solidus*. Simple correlations revealed that relative differences in abundance data were also similar to those in the pre-

Table 2
ANOVA results from generalised linear mixed models (GLMM) with infection status as dependent variable, lake surface area (Area), pH, standard length (SL), and sex as fixed factors (1 degree of freedom each), date of capture as covariate, and lake as random factor. Separate models were fitted for 2010 (a, 6 lakes, 111 fish) and 2011 (b, 14 lakes, 299 fish). Note that *P* values are those that resulted from model reduction, whereas significance (Sig.) was determined from Bonferroni-adjusted (B.ad.) α levels. Significant *P* values are printed in bold. Tendencies (0.1 > B.ad. $P \geq 0.05$) are printed in italics.

(a)		Area			pH			SL			Sex		
		χ^2	*P*	Sig.	χ^2	*P*	Sig.	χ^2	*P*	Sig.	χ^2	*P*	Sig.
Gyrodactylus spp.	prevalence	0.3	0.598	ns	0.5	0.476	ns	8.7	**0.003**	*	0.2	0.685	ns
Gyrodactylus spp.	abundance	0.1	0.803	ns	0.2	0.701	ns	30.0	**<0.001**	***	0.1	0.803	ns
T. gasterostei	prevalence	0.5	0.476	ns	0.6	0.434	ns	6.8	*0.009*	(*)	1.1	0.305	ns
T. gasterostei	abundance	0.3	0.583	ns	0.6	0.451	ns	9.4	**0.002**	*	0.3	0.617	ns
Diplostomum spp. (non-lens)	prevalence	0.1	0.754	ns	1.6	0.211	ns	17.9	**<0.001**	***	0.2	0.626	ns
Diplostomum spp. (non-lens)	abundance	5.1	0.024	ns	7.8	**0.005**	*	52.7	**<0.001**	***	0.2	0.701	ns
Apatemon spp.	prevalence	2.2	0.139	ns	9.8	**0.002**	*	2.2	0.140	ns	4.6	0.032	ns
Apatemon spp.	abundance	0.0	0.920	ns	14.8	**0.0001**	**	8.0	**0.005**	*	0.2	0.626	ns
S. solidus	prevalence	0.5	0.499	ns	0.2	0.675	ns	0.8	0.360	ns	2.4	0.126	ns

(b)		Area			pH			SL			Sex		
		χ^2	*P*	Sig.	χ^2	*P*	Sig.	χ^2	*P*	Sig.	χ^2	*P*	Sig.
Gyrodactylus spp.	prevalence	0.2	0.701	ns	1.6	0.204	ns	0.3	0.613	ns	5.7	0.017	ns
Gyrodactylus spp.	abundance	0.2	0.639	ns	3.1	0.080	ns	0.6	0.426	ns	4.7	0.030	ns
T. gasterostei	prevalence	0.2	0.699	ns	4.0	0.045	ns	0.2	0.667	ns	8.2	**0.004**	*
T. gasterostei	abundance	0.2	0.691	ns	4.0	0.046	ns	6.2	0.013	ns	1.3	0.248	ns
Diplostomum spp. (lens)	prevalence	0.1	0.791	ns	10.9	**0.001**	*	7.9	*0.005*	(*)	1.2	0.271	ns
Diplostomum spp. (lens)	abundance	0.3	0.580	ns	9.6	**0.002**	*	24.2	**<0.001**	***	1.8	0.179	ns
Diplostomum spp. (non-lens)	prevalence	0.3	0.618	ns	1.6	0.206	ns	19.5	**<0.001**	***	1.7	0.190	ns
Diplostomum spp. (non-lens)	abundance	0.5	0.479	ns	1.6	0.208	ns	78.2	**<0.001**	***	0.4	0.508	ns
Apatemon spp.	prevalence	0.1	0.768	ns	2.3	0.133	ns	4.6	0.033	ns	4.7	0.031	ns
Apatemon spp.	abundance	0.0	0.882	ns	1.6	0.201	ns	5.3	0.022	ns	0.5	0.488	ns
S. solidus	prevalence	2.0	0.158	ns	0.0	0.837	ns	1.1	0.301	ns	0.4	0.547	ns

(*) 0.1 > B.ad. $P \geq 0.05$; ns B.ad. $P \geq 0.1$.
* B.ad. $P < 0.05$.
** B.ad. $P < 0.01$.
*** B.ad. $P < 0.001$.

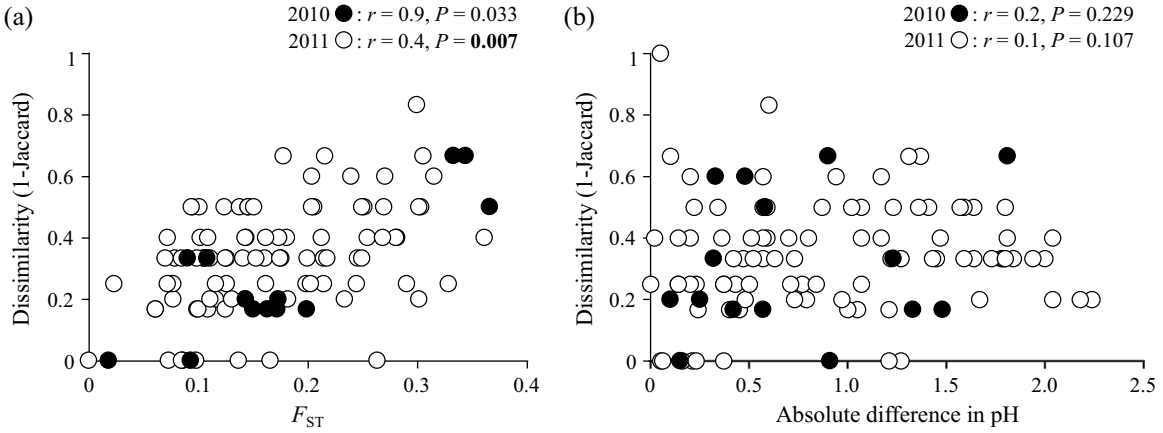

Fig. 3. Dissimilarity in parasite communities given as 1-Jaccard between lakes in relation to (a) pairwise genetic differentiation determined from microsatellite data and (b) absolute differences in pH. Data of 2011 (2010) are shown as empty (filled) circles. Correlation coefficients (*r*) and *P* values of the Mantel tests are given in each plot. The significant *P* value (after Bonferroni correction) is printed in bold.

vious study, at least for the parasites *Apatemon* spp., *Diplostomum* spp. (non-lens), and *Gyrodactylus* spp. Interestingly, the *Diplostomum* species which was found in the eye lens of fish from the western and two of the more central lakes was not analysed in de Roij and MacColl (2012) due to very low abundances in 2007 and 2008. Taken together, the results suggest that the parasites are not randomly distributed on the island and that distribution patterns have been consistent, at least over several host generations.

In the study by de Roij and MacColl (2012) only two of the twelve lakes examined were 'alkaline' (pH > 7) and situated in the western part of the island. In our study six of fourteen lakes (2011, two of six in 2010) had pH values above 7 and five of these were located in the western part of North Uist. Despite a more balanced choice of lakes in terms of pH and geographic location, we did not find convincing evidence that pH was a decisive factor in shaping parasite distribution on North Uist. *T. gasterostei* was absent from acidic lakes (except for 11MGB) and the alkaline lake 7HOS in both studies. The explanation that acidic lakes might be unsuitable for copepods in general seems unlikely given that *S. solidus*, which requires copepods as intermediate hosts, was found in several lakes with pH values below 7. Significant correlations between pH and infection with *Diplostomum* spp. (lens) indicate that pH might play a role in the distribution and/or infection success of this trematode. But, as the eye fluke seemed to be absent from most central and eastern lakes, this correlation cannot be distinguished from geographical distribution and, e.g., preference of the final (bird) host for the Atlantic coast.

It might appear counterintuitive at first that we did not find evidence for lower *Diplostomum* spp. (non-lens) or *Apatemon* spp. prevalence in more acidic lakes. Both eye flukes depend on a snail as intermediate host and therefore the distribution of these parasites might be expected to be indirectly associated with calcium availability as was indeed found for the distribution of *Diplostomum* infections originating from *Lymnaea arctica* snails in Canada (Curtis and Rau, 1980). Like *Lymnaea stagnalis*, *L. arctica* requires much higher calcium concentrations than those found on North Uist. This might be the reason why *R. peregra,* which can cope with low calcium concentrations, is the predominant species on this island while *L. stagnalis* is absent (Briers, 2003a,b). As both eye flukes were present in fish from almost all lakes examined in this study, we can assume that either snail prevalence was not significantly affected by spatial differences in pH or that lower snail prevalence in more acidic lakes was compensated by higher infection rates.

Neutral genetic differentiation between host populations was significantly positively correlated with dissimilarity of parasite community composition between lakes based on presence/absence data (1-Jaccard). Considering the distribution of *Diplostomum* spp. (lens) and *T. gasterostei*, which were both included in the calculation of the similarity indices and occurred mostly in western, alkaline lakes, this effect is likely to be driven by those two parasites. The result could suggest that parasite distribution patterns have been shaped by the connectivity among lakes. De Roij and MacColl (2012) had tested for distance decay in similarity using Jaccard similarity and the shortest geographical distance between lakes as distance measure. Their negative result was interpreted as evidence that they "could (. . .) rule out 'isolation by distance' as an explanation for spatial variation in parasite communities" (de Roij and MacColl, 2012). We argue that our results suggest that pairwise F_{ST} values between host populations might be a better proxy for 'geographical distance', especially since several of the numerous lakes on North Uist (>180 according to Giles, 1983) are connected by streams (also underground streams). Unlike qualitative dissimilarity in parasite communities, quantitative differences in mean abundances, like Bray–Curtis dissimilarity, or abundances of *Gyrodactylus* spp., *Apatemon* spp., and *Diplostomum* spp. (non-lens) were not significantly correlated with genetic differentiation between host populations. This might be due to the use of neutral genetic markers (microsatellites) for estimating genetic differentiation and indicate that parasite abundances are not determined by geographical position on the island, i.e. neighbouring, but isolated lakes can have very different abundances, but the result of local dynamics.

One further explanation for the different abundances among lakes could be local adaptation of either hosts or parasites or both. Several studies on *D. pseudospathaceum*, which infests the eye lens of sticklebacks, have shown that sticklebacks are able to locally adapt to parasites like eye flukes that quickly evade the immune system of the host before an adaptive response can be elicited (Kalbe and Kurtz, 2006; Rauch et al., 2006; Scharsack and Kalbe, 2014). Also, experimental infections with *G. gasterostei* have shown that the stickleback populations on North Uist differ in their resistance to this monogenean (de Roij et al., 2010). Experimental infections with a fully crossed design could help to find out whether the patterns observed on this island can be the result of host and/or parasite local (co)adaptation.

Although we disagree that parasite abundance is completely independent of pH (at least not for all parasites), our results generally confirm the results and conclusion of the study conducted by de Roij and MacColl (2012) that found that individual lake characteristics such as local host/parasite adaptations rather than general physicochemical variables must be responsible for the different patterns of parasite distribution across North Uist. Further work will be necessary to disentangle the mechanisms behind the consistent parasite distribution patterns, but we conclude that connectivity among habitats, water quality, and host traits contribute to the differences in parasite abundance. It might also be possible that certain abiotic habitat characteristics indirectly affect host local adaptation by providing better or worse conditions for the parasites. Likewise, it also remains to be tested whether physical connectivity among water bodies shapes distribution patterns of hosts and of parasites, whether parasites have 'followed' their hosts during colonisation, or whether parasites have contributed to population divergence of their fish host.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.zool.2016.05.009.

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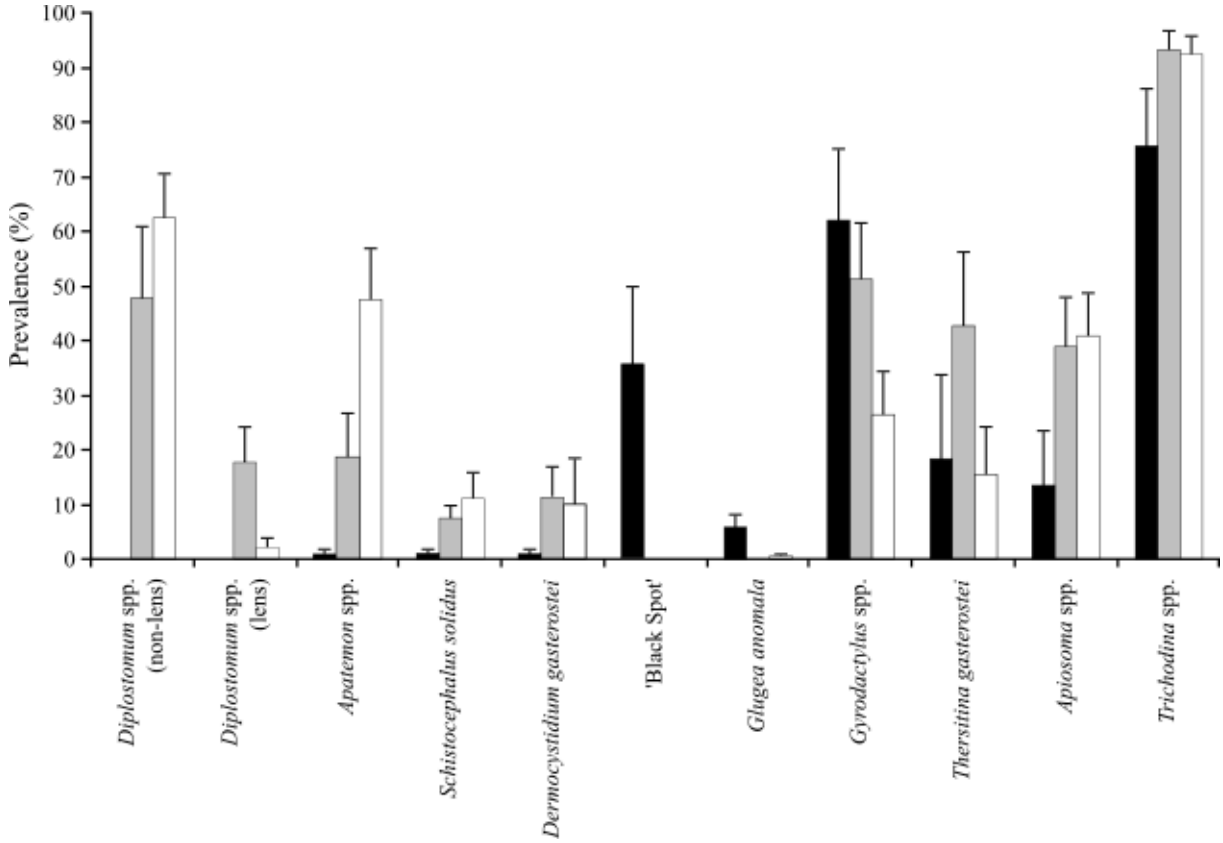

Fig. S1. Mean prevalence (+ standard error) of eleven common stickleback parasites on fish from freshwater lakes with pH > 7 ("alkaline", grey, $N = 7$), pH < 7 ("acidic", white, $N = 12$), and three brackish water sites with anadromous and resident fish ("brackish", black, $N = 6$). Data of fish caught in 2010 and 2011 were combined. Mean values of the years 2010 and 2011 were calculated for lake 8SAN (Sanndaraigh).

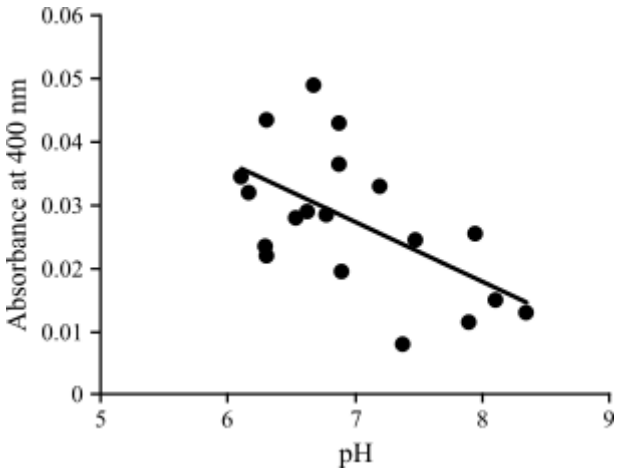

Fig. S2. Relationship between light absorbance and pH in 19 freshwater lakes on North Uist. Light absorbance was significantly higher in less alkaline lakes (Spearman rank correlation: $r_S = –0.59$, $N = 19$, $P = 0.009$).

Table S1. Primer sequences published by Heckel et al. (2002) and Largiadèr et al. (1999) with GenBank accession numbers and PCR conditions used for microsatellite genotyping. The tailed primer method (Schuelke, 2000) was applied. Fragments were analysed on a CEQ^{TM} 8800 capillary sequencer (Beckman Coulter, Brea, CA, USA) with GenomeLabTM GeXP 181 (version 10.2) software. T_A = annealing temperature, Mix = combination of markers within a single PCR reaction, Reverse/Labeled/Forward = amount of primer molecules within a single PCR reaction.

Locus	Tail (dye)	Primer sequences 5'→3'	T_A (°C)[a]	Mix[b]	Reverse (pmol)	Labeled (pmol)	Forward (pmol)
Gac1116PBBE	T7 (D3)	for GGTGTCATGTGGGGGCGAGCAG	60/56	A	4	4	2
AJ010353		rev CCCGAAGCATTGTGGCATCATC					
Gac7033PBBE	M13 (D4)	for AGGTGGATTGGTTTTCTG	60/56	A	0.6/1	0.6/1	0.3/0.5
AJ010360		rev GGACGCTCGCTCTTTC					
Gac3133PBBE	SP6 (D4)	for CGCCCAGTTCCTGAACTTGAACTG	56	B	1	1	0.5
AJ010356		rev CATGGTGGGCTGACTGAC					
Gac4174PBBE	T7 (D3)	for CCGCGATGATGAGAGTG	56	B	2	2	1
AJ010358		rev GTGAAATGCGACAGATGATG					
Gac7010PBBE	M13 (D2)	for CGAGTAAAGACACGGAGTAG	56	B	1.6	1.6	0.8
AJ311863		rev CTGTAGGGAGGGTTGACT					
Gac1097PBBE	M13 (D2)	for AGGAACTCTCTTCTTCTCTG	58	C	3/2.5	3/2.5	1.5/1.25
AJ010352		rev CCCGGGTTAGTCACT					
Gac1125PBBE	M13 (D2)	for CATCACACCCAGCCTCTC	58	C	0.7/0.6	0.7/0.6	0.35/0.3
AJ010354		rev CCTCCCTCCAACTCTTATCA					
Gac4170PBBE	SP6 (D4)	for GCCGAGCCACATAGAGA	58	C	1/1.5	1/1.5	0.5/0.75
AJ010357		rev CCAATATAACAGCCGAGCAG					
Gac5196PBBE	T7 (D3)	for ACTTCTCCCCTCATTATGCT	58	C	4	4	2
AJ010359		rev GGGGTCTGATGGATACAAA					

[a] PCR programme: 15 min at 94 °C, 60 s at 94 °C, 45 s at T_A, 60 s at 72 °C (30 cycles), 60 s at 94 °C, 45 s at 53 °C and 60 s at 72 °C (8 cycles), 30 min at 72 °C.

[b] PCR mixes A, B, and C included primers, 5 µl Multiplex mix (Qiagen), 40 ng DNA and H_2O to adjust reaction volume to 10 µl.

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Table S2. Distribution of common stickleback parasites on North Uist given as prevalence (Prev, percentage of infected fish) and mean infection intensity (MI, mean number of parasites per infected fish, rounded to the nearest integer). For full names of the sampling locations see Table 1 of the main article. ’10 = 2010, ’11 = 2011.

Parasite		1ana ‘11	1res ‘10	2ana ‘11	2res ‘11	3ana ‘11	3res ‘11	4GRO ‘11	5EUB ‘11	6MAG ‘10	7HOS ‘11	8SAN10 ‘10	8SAN11 ‘11	8SAN9sp ‘11	9OLA ‘11	10GEA ‘11	11MGB ‘10	12ACH ‘10	13MOI ‘11	14DUB ‘11	15TOR ‘10	16BHA ‘11	17MOR ‘11	18SCD ‘11	19EIL ‘11	20DAI ‘11	21MAI ‘11	22BUA ‘10
Gyrodactylus spp.	Prev	95	100	63	40	57	16	38	30	100	48	59	27	15	24	25	52	95	15	35	17	4	43	25	76	0	5	0
	MI	33	18	3	2	11	3	3	3	8	3	3	2	2	2	3	12	13	2	3	2	1	2	1	5	–	1	–
Diplostomum spp. (non-lens)	Prev	0	0	0	0	0	0	14	35	100	14	88	13	55	29	100	90	95	15	55	72	30	95	45	90	55	33	60
	MI	–	–	–	–	–	–	1	2	33	3	6	8	7	3	8	9	8	3	3	3	1	11	2	21	2	5	3
Diplostomum spp. (lens)	Prev	0	0	0	0	0	0	14	25	10	52	6	10	30	14	0	0	0	5	20	0	0	0	0	0	0	0	0
	MI	–	–	–	–	–	–	1	1	1	3	1	8	2	2	–	–	–	1	2	–	–	–	–	–	–	–	–
Apatemon spp.	Prev	0	0	0	0	0	5	5	20	62	5	6	13	20	0	96	81	95	40	55	50	9	10	30	29	5	29	70
	MI	–	–	–	–	–	1	1	1	2	3	1	2	2	–	3	2	3	1	2	2	1	2	1	2	1	3	2
Schistocephalus solidus	Prev	0	0	0	0	0	5	5	20	5	5	12	3	0	0	21	10	10	0	0	6	57	0	25	10	5	0	0
Dermocystidium gasterostei[a]	Prev	0	0	5	0	0	0	5	0	14	0	47	33	0	19	21	0	0	0	0	0	0	0	0	0	0	0	100
	MI	–	–	I	–	–	–	I	–	I	–	II	II	–	I	I	–	–	–	–	–	–	–	–	–	–	–	III
Thersitina gasterostei	Prev	0	5	0	10	0	95	67	55	95	0	12	37	35	57	75	81	29	0	0	0	0	0	0	0	0	0	0
	MI	–	3	–	1	–	4	5	2	4	–	2	2	5	3	3	6	2	–	–	–	–	–	–	–	–	–	–
"Black Spot"	Prev	48	90	16	55	0	5	0	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Glugea anomala	Prev	10	0	5	15	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0
Apiosoma spp.[a]	Prev	0	60	0	0	0	21	48	15	81	48	41	43	0	29	29	67	62	25	35	50	9	76	55	10	0	76	5
	MI	–	IV	–	–	–	IV	III	III	IV	IV	IV	III	–	III	III	IV	IV	III	III	III	II	IV	III	IV	–	III	II
Trichodina spp.[a]	Prev	95	95	32	75	62	95	95	75	100	95	100	73	100	100	83	100	100	100	95	100	70	95	70	100	100	100	95
	MI	III	IV	II	II	II	IV	III	II	III	III	III	II	II	III	III	III	III	III	III	III	II	III	III	III	II	III	II

[a] 0 = not infected, I = 1–10, II = 11–50, III = 51–100, IV = more than 100 parasites

Table S3. ANOVA results from generalised linear models (GLM) with infection status as dependent variable, lake as explaining variable, and standard length (SL), sex, and date of capture as covariates. In the separate models for the two sampling years, lake was associated with 5 (13) degrees of freedom for 2010 (2011). Note that *P* values are those that resulted from model reduction, whereas significance (Sig.) was determined from Bonferroni-adjusted (B.ad.) α levels. Significant *P* values are given in bold.

		2010			2011		
		χ^2	*P*	Sig.	χ^2	*P*	Sig.
Gyrodactylus spp.	prevalence	43.4	**<0.001**	***	53.3	**<0.001**	***
Gyrodactylus spp.	abundance	44.7	**<0.001**	***	57.3	**<0001**	***
T. gasterostei	prevalence	55.6	**<0.001**	***	143.4	**<0001**	***
T. gasterostei	abundance	70.1	**<0.001**	***	175.6	**<0.001**	***
Diplostomum spp. (lens)	prevalence	–	–		41.7	**<0.001**	***
Diplostomum spp. (lens)	abundance	–	–		39.3	**0.0002**	**
Diplostomum spp. (non-lens)	prevalence	7.0	0.218	ns	125.8	**<0.001**	***
Diplostomum spp. (non-lens)	abundance	48.0	**<0.001**	***	214.4	**<0.001**	***
Apatemon spp.	prevalence	36.0	**<0.001**	***	103.0	**<0.001**	***
Apatemon spp.	abundance	33.5	**<0.001**	***	96.0	**<0.001**	***
S. solidus	prevalence	3.7	0.597	ns	65.2	**<0.001**	***

*** B.ad. $P < 0.001$; ** B.ad. $P < 0.01$; ns B.ad. $P \geq 0.1$

Table S4. Relationship between dissimilarity of parasite communities, genetic differentiation (pairwise F_{ST} based on microsatellite data), and absolute differences in pH between sampling locations. Dissimilarity of parasite communities is given as 1-Jaccard and 1-Bray-Curtis, and absolute differences in mean abundance for single parasite groups. Separate Mantel tests (5000 permutations) were run for the data of (a) 2011 (14 lakes) and (b) 2010 (6 lakes). Note that *P* values are those from the Mantel tests, but that significance (Sig.) was determined from Bonferroni-adjusted (B.ad.) α values. The significant *P* value is printed in bold.

(a)

	F_{ST}			pH				
	r	*P*	Sig.	*r*	*P*	Sig.	% explained by F_{ST}	% explained by pH
1-Jaccard	0.43	**0.007**	*	0.14	0.107	ns	19.8	3.0
1-Bray-Curtis	0.20	0.111	ns	0.12	0.145	ns	4.6	1.8
Gyrodactylus spp.	0.48	0.070	ns	0.02	0.418	ns	23.8	0.1
Apatemon spp.	0.00	0.392	ns	-0.16	0.950	ns	0.0	2.5
Diplostomum spp. (non-lens)	0.30	0.160	ns	-0.16	0.953	ns	8.4	1.8

(b)

	F_{ST}			pH				
	r	*P*	Sig.	*r*	*P*	Sig.	% explained by F_{ST}	% explained by pH
1-Jaccard	0.86	0.033	ns	0.20	0.229	ns	73.4	3.0
1-Bray-Curtis	0.35	0.072	ns	-0.05	0.518	ns	12.4	0.4
Gyrodactylus spp.	0.10	0.215	ns	-0.27	0.910	ns	1.1	7.6
Apatemon spp.	-0.14	0.433	ns	0.50	0.092	ns	2.3	25.7
Diplostomum spp. (non-lens)	-0.14	0.410	ns	-0.16	0.480	ns	1.9	2.5

* B.ad. $P < 0.05$; ns B.ad. $P \geq 0.1$

Table S5. Correlation of infection data published in de Roij and MacColl (2012) and infection data obtained in the present study of those lakes that were sampled in both studies (N = 12 lakes). Given are correlation coefficients and P values as resulting from Pearson correlations (r_P) and Spearman rank correlations (r_S). Significance (Sig.) was determined from Bonferroni-adjusted (B.ad.) P values. Significant P values are printed in bold.

		All 12 lakes sampled in both studies							
		2008				2007			
			r	*P*	Sig.		*r*	*P*	Sig.
Diplostomum spp. (non-lens)	prevalence	r_P	0.68	0.015	ns	r_P	0.61	0.037	ns
Diplostomum spp. (non-lens)	abundance	r_S	0.78	**0.003**	*	r_S	0.55	0.064	ns
Apatemon spp.	prevalence	r_S	0.94	**<0.001**	***	r_P	0.73	*0.007*	(*)
Apatemon spp.	abundance	r_S	0.88	**0.0001**	***	r_P	0.83	**0.0008**	**
Gyrodactylus spp.	prevalence	r_P	0.54	0.071	ns	r_S	0.51	0.088	ns
Gyrodactylus spp.	abundance	r_S	0.82	**0.001**	**	r_S	0.30	0.341	ns
S. solidus	prevalence	r_S	0.43	0.168	ns	r_S	0.34	0.283	ns

*** B.ad. $P < 0.001$; ** B.ad. $P < 0.01$; * B.ad. $P < 0.05$; (*) 0.1 > B.ad. $P \geq 0.05$; ns B.ad. $P \geq 0.1$.

Table S6. Results of the regression analyses (Pearson correlations (r_P) or Spearman rank correlations (r_S)) based on infection data from the present study of the lakes sampled in de Roij and MacColl (2012), in 2010 and in 2011. Prevalence (% infected) or mean abundance (number of parasites divided by the number of dissected fish) per lake were correlated with either pH or lake surface area (Area). No significant correlation was found after Bonferroni correction. Only tendency (0.1 > Bonferroni-adjusted $P \geq 0.05$) printed in italics (*Diplostomum* spp. (lens) abundance with pH, 2011).

		pH								Area							
		de Roij and MacColl lakes			2010			2011			de Roij and MacColl lakes			2010			2011	
			r	*P*		*r*	*P*		*r*	*P*		*r*	*P*		*r*	*P*		*r*	*P*
Diplostomum spp. (non-lens)	prevalence	r_P	–0.02	0.959	r_P	0.43	0.393	r_P	–0.31	0.281	r_S	–0.18	0.586	r_P	0.08	0.874	r_S	–0.14	0.642
Diplostomum spp. (non-lens)	abundance	r_S	0.34	0.284	r_S	0.37	0.497	r_S	–0.12	0.675	r_S	–0.27	0.391	r_S	–0.09	0.919	r_S	–0.39	0.170
Apatemon spp.	prevalence	r_P	–0.15	0.641	r_P	–0.86	0.030	r_P	–0.32	0.263	r_S	–0.36	0.246	r_P	–0.42	0.408	r_S	–0.13	0.658
Apatemon spp.	abundance	r_P	–0.10	0.750	r_P	–0.79	0.063	r_S	–0.27	0.346	r_S	–0.31	0.329	r_P	–0.37	0.477	r_S	–0.17	0.573
Diplostomum spp. (lens)[a]	prevalence	r_S	0.50	0.095		–	–	r_S	0.67	0.009	r_S	–0.01	0.980		–	–	r_S	0.23	0.428
Diplostomum spp. (lens)[a]	abundance	r_S	0.50	0.095		–	–	r_S	0.70	*0.006*	r_S	–0.01	0.980		–	–	r_S	0.21	0.474
T. gasterostei[a]	prevalence	r_S	0.40	0.192	r_P	0.06	0.916	r_S	0.55	0.043	r_S	–0.20	0.524	r_P	–0.18	0.729	r_S	–0.09	0.761
T. gasterostei[a]	abundance	r_S	0.37	0.234	r_S	0.17	0.742	r_S	0.57	0.035	r_S	–0.18	0.570	r_S	–0.12	0.827	r_S	–0.08	0.787
Gyrodactylus spp.	prevalence	r_P	0.51	0.088	r_P	0.33	0.521	r_P	0.48	0.086	r_S	0.35	0.270	r_P	–0.11	0.837	r_S	0.10	0.725
Gyrodactylus spp.	abundance	r_S	0.54	0.072	r_P	–0.15	0.777	r_S	0.62	0.017	r_S	0.35	0.270	r_P	–0.23	0.664	r_S	0.05	0.863
S. solidus	prevalence	r_S	–0.05	0.875	r_P	0.63	0.177	r_S	–0.09	0.754	r_S	0.45	0.145	r_P	0.57	0.233	r_S	0.26	0.372

[a] Not analysed in de Roij and MacColl (2012)

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