The association of feeding behaviour with the resistance and tolerance to parasites in recently diverged sticklebacks

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Abstract

Divergent natural selection regimes can contribute to adaptive population divergence, but can be sensitive to human-mediated environmental change. Nutrient loading of aquatic ecosystems, for example, might modify selection pressures by altering the abundance and distribution of resources and the prevalence and infectivity of parasites. Here, we used a mesocosm experiment to test for interactive effects of nutrient loading and parasitism on host condition and feeding ecology. Specifically, we investigated whether the common fish parasite *Gyrodactylus* sp. differentially affected recently diverged lake and stream ecotypes of three-spined stickleback (*Gasterosteus aculeatus*). We found that the stream ecotype had a higher resistance to *Gyrodactylus* sp. infections than the lake ecotype, and that both ecotypes experienced a cost of parasitism, indicated by negative relationships between parasite load and both stomach fullness and body condition. Overall, our results suggest that in the early stages of adaptive population divergence of hosts, parasites can affect host resistance, body condition and diet.

Introduction

Contrasting environmental conditions can generate the divergent selection pressures that drive adaptive population divergence (Schluter, 2000; Nosil, 2012), and during this process, several traits linked to species interactions can evolve rapidly, including those associated with feeding behaviours and defences against predators and parasites (Schluter, 2000; Eizaguirre & Lenz, 2010; Kortet *et al.*, 2010; Karvonen & Seehausen, 2012). The evolution of such traits might influence the build-up of reproductive isolation (Eizaguirre *et al.*, 2009a; Servedio *et al.*, 2011; Nosil, 2012), particularly if they are also involved in mate choice (Maan & Seehausen, 2011) and local adaptation (Bassar *et al.*, 2010; Eizaguirre & Lenz, 2010; Eizaguirre *et al.*, 2011; Konijnendijk *et al.*,

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2013; Arnegard *et al.*, 2014). During the early stages of adaptive population divergence, environmental change can weaken divergent selection regimes, and this might erode ecological and genetic differentiation between populations (Hendry *et al.*, 2009; Vonlanthen *et al.*, 2012; Seehausen *et al.*, 2014). In most cases, however, we lack a mechanistic understanding of how environmental change, be it abiotic or biotic, can influence performance variation among and within diverging populations. This makes it difficult to predict the effect of environmental change on the dynamics of population divergence (MacColl, 2011; Nosil, 2012).

The resistance and tolerance of hosts to parasites are traits that can both evolve rapidly (Murray *et al.*, 1998; Siva-Jothy & Thompson, 2002; Råberg, 2014) and influence host energy acquisition and resource assimilation over a range of environmental conditions (Pianka, 1981; Huey *et al.*, 2001; Howick & Lazzaro, 2014). Resistance is the ability of individuals to limit their parasite load and is measured as the number of parasites acquired per individual host over a given time period (Råberg *et al.*, 2009). Tolerance, on the other

hand, is the ability of individuals to reduce the harmful effects of a given parasite load and can be estimated (at the population level) as the slope of the relationship between parasite load and proxies of fitness (Schneider & Ayres, 2008; Råberg *et al.*, 2009; Kaufmann *et al.*, 2014). The evolution of host resistance and tolerance strongly depends on how parasites affect trade-offs between immune defence and life history traits (Hamilton & Zuk, 1989; Poulin, 2007; Råberg, 2014). For instance, when hosts lack the genetic material to fight off infections, they may increase energy acquisition to compensate for the costs of parasitism (Zuk & Stoehr, 2002; Ponton *et al.*, 2011; Howick & Lazzaro, 2014). If individual hosts are in positive energy balance (e.g. full guts, ample fat reserves), they might be able to either better cope with the physiological demands of the infection or to mount a more effective immune response (Zuk & Stoehr, 2002). In general, host responses to parasites tend to be strongly dependent on environmental conditions that determine host energy balance (Budria & Candolin, 2014; Wong & Candolin, 2015).

In aquatic systems, nutrient loading can have strong effects on host–parasite interactions (Johnson *et al.*, 2007, 2008; Budria & Candolin, 2014), by changing the chemical (e.g. phosphorus and oxygen levels), physical (e.g. light, turbidity) and biological properties of aquatic ecosystems (Smith & Schindler, 2009). Nutrient loading has been shown to alter resource availability and competitive interactions (Leach *et al.*, 1977; Talbot & Hole, 1994), predation risk (Van de Meutter *et al.*, 2005; Cothran *et al.*, 2012), mating behaviour (Jaervenpaeae & Lindstroem, 2004; Cothran *et al.*, 2012), and the prevalence and virulence of parasites (Zuk & Stoehr, 2002; Domenici *et al.*, 2007; Kortet *et al.*, 2010; Johnson *et al.*, 2012; Halstead *et al.*, 2014). Previous experiments in aquatic systems have found strong effects of nutrient loading on the morphology and behaviour of vertebrates (Johnson *et al.*, 2010; Budria & Candolin, 2014). However, less is known about how nutrient loading might affect parasite resistance, tolerance and feeding ecology of hosts in the early stages of adaptive divergence.

The stickleback-*Gyrodactylus* model system is useful for investigating the interactive effects of parasitism and environmental change (i.e. nutrient loading) on the evolution of host resistance during adaptive population divergence. Lake and stream three-spined stickleback (hereafter, sticklebacks) populations that are genetically and phenotypically differentiated (i.e. ecotypes) have evolved multiple times in the Northern Hemisphere since the last glaciation ($\simeq$12 000 years) (Hendry *et al.*, 2009; Feulner *et al.*, 2015). For our study, we chose a pair from the Lake Constance region in Central Europe that has diverged very recently (< 150 years) (Lucek *et al.*, 2010), concomitant with dramatic changes in nutrient levels in Lake Constance. Lake Constance has experienced a prolonged phase of eutrophication (1950–1980: total phosphorus, TP increased from $\simeq$ 10 to 80 μg L^{-1}) and re-oligotrophication (1980–2010: TP decreased from $\simeq$ 80 to 10 μg L^{-1}) (Jochimsen *et al.*, 2013), but the ecological and evolutionary effects of these nutrient dynamics on the resident stickleback populations are unknown. The specific lake–stream pair that we used is phenotypically divergent in body size, growth rate (Lucek *et al.*, 2012) and morphology (Berner *et al.*, 2011; Lucek *et al.*, 2013), and is genetically differentiated at several putatively adaptive loci (Marques *et al.*, 2016). For a parasite, we chose the monogean (Platyhelminthes) flatworm, *Gyrodactylus* spp. (hereafter, *Gyrodactylus*), because it is a common ectoparasite of sticklebacks and other fish (Kalbe *et al.*, 2002; Bakke *et al.*, 2007; Raeymaekers *et al.*, 2011; Rahn *et al.*, 2015; Stephenson *et al.*, 2015), and it is known to drive local adaptation in other stickleback populations (Eizaguirre & Lenz, 2010; Konijnendijk *et al.*, 2013). *Gyrodactylus* is a flatworm that reproduces on the skin and gills of fish (Bakke *et al.*, 2007), and can increase host mortality (Bakke *et al.*, 2007), reduce body condition (Eizaguirre *et al.*, 2011) and reduce lifetime reproductive success (Eizaguirre *et al.*, 2009b). It has no intermediate host, and it is generally more common in stream than in lake environments (Kalbe *et al.*, 2002; Bakke *et al.*, 2007; Eizaguirre *et al.*, 2011).

We performed a 7-week mesocosm experiment to investigate how closely related lake and stream ecotypes of stickleback differ in their resistance and tolerance to the monogenean ectoparasite *Gyrodactylus* in aquatic ecosystems with low and high levels of nutrient loading. In this study, we tested the following two predictions. First, we predicted that lake and stream ecotypes would differ in their resistance and tolerance to *Gyrodactylus* infections. This prediction is based on previous work showing rapid parasite-mediated evolution of stickleback immune systems in other populations (Eizaguirre *et al.*, 2012a,b). Second, we predicted that nutrient loading might differentially affect the resistance and tolerance of both ecotypes to parasite exposure, partly because of the potential impacts of nutrient loading on the energy balance (e.g. stomach fullness), diet composition (i.e. gut contents) and body condition (a fitness proxy) of sticklebacks (Schlotz *et al.*, 2013; Budria & Candolin, 2014; Howick & Lazzaro, 2014).

Materials and methods

Mesocosm experiment

Our experimental set-up consisted of 40 outdoor mesocosms and was a complete randomized block design, with factorial combinations of fish ecotype (lake and stream), nutrient level (high and low nutrients, HN and LN) and *Gyrodactylus* exposure (exposed and unexposed, G+ and G−, Fig. 1). The experiment lasted 7 weeks,
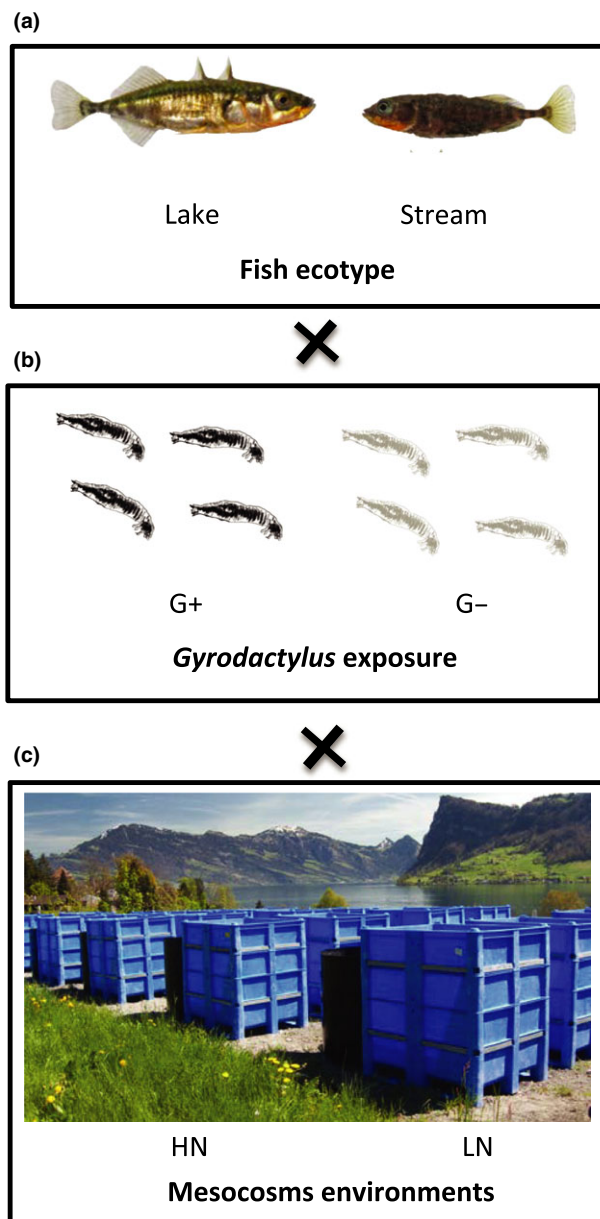
(a)

Lake   Stream

Fish ecotype

✖

(b)

G+   G−

***Gyrodactylus* exposure**

✖

(c)

HN   LN

Mesocosms environments

Fig. 1 Experimental design. We crossed in a fully factorial design, lake and stream stickleback (a), exposure to *Gyrodactylus* (b), and nutrient loading in mesocosms environments (c).

from 3 May to 21 June 2013. Each mesocosm was filled with 1000 L of filtered water from Lake Lucerne (Switzerland) and seeded with a mixture of sand, gravel, benthic substrate and zooplankton. We amended nutrient levels by adding 20 mL of $NaNO_3$ and HNa_2PO_4 stock solution to achieve the following target concentrations: 640 μg N/L and 40 μg P/L in HN tanks, and 80 μg N/L and 5 μg P/L in LN tanks, which represents approximately the median and the minimum, respectively, of the total phosphorus

concentration in Lake Constance over the past 50 years (Jochimsen *et al.*, 2013). Each mesocosm received either 6 or 7 stickleback so as to standardize biomass among mesocosms (mean lake = 24.55 g ± 0.621 SE, mean stream = 23.15 g ± 0.292 SE).

Fish collection and parasite exposure

We collected stream sticklebacks from two streams (Aubach: 47° 19′ 37.45″ N 9° 34′ 12.82″ E; and Zapfenbachkrummensee Kanal: 47° 21′ 20.65″ N 9° 36′ 11.94″ E) and lake sticklebacks from the shore of an inlet delta of Lake Constance (Staad: 47° 29′ 8.30″ N 9° 32′ 38.25″ E) in early spring 2013. Twenty lake and stream sticklebacks were dissected to estimate the abundance of *Gyrodactylus* parasites in these natural populations. For the experiment, we initially removed *Gyrodactylus* by treating wild-caught fish with a 1 : 4000 diluted solution of formalin (Buchmann & Kristensson, 2003; Raeymaekers *et al.*, 2011). A visual inspection of all fish under stereomicroscope revealed no living parasites after disinfection. After 1 week, we then performed a standardized infection of the fish that we used for the mesocosm experiment. Fish were anesthetized ($N = 278$) with 0.1% MS222 (Gilderhus & Marking, 1987) and placed on a Petri dish filled with saline solution (6.4 g L^{-1} NaCl). With a fine brush, we manually added exactly four individual parasites onto each individual that was part of the infection treatment (G+). We used two parasites that originated from either naturally infected lake fish or stream fish, so as to account for possible differences in host–parasite coevolution (Eizaguirre *et al.*, 2011; Konijnendijk *et al.*, 2013). Unexposed fish (G−) used in the experiment were handled in the same way but received no parasites. After this procedure, the fish were kept in 5 L aquaria for a week prior to their introduction into the mesocosms.

Parasite levels, fish condition and diet

At the end of the experiment, we caught the surviving fish ($N = 199$ fish of $N = 278$), euthanized them using an overdose of MS222 and counted the number of *Gyrodactylus* per fish (i.e. parasite load). Three tanks were excluded from the experiment because of low survival (see Fig. S1 and Table S3). Additionally, one randomly chosen fish from each mesocosm was excluded from all analyses ($N = 40$), because it was needed for another experiment (F. S. Brunner *et al.* submitted). For the remaining 159 fish used in the current study, we measured standard length (mm, ±1 mm), body weight (g, ±0.01 g), liver weight (mg, ±0.001 mg) and wet gut mass (mg, ±0.001 mg) (see Table S3 for descriptive statistics). Fish guts were stored in saline solution (6.4 g L^{-1} NaCl) and frozen at −20 °C in separate vials for later dietary analyses.

The hepatosomatic index (HSI) was used as a proxy of individual variation in body condition, where HSI = [LM/(BM − FSM)] × 100, and LM is the wet liver mass (g), FSM full stomach mass (mg) and BM wet body mass (mg) (Chellappa *et al.*, 1995; Kurtz *et al.*, 2004; Hammerschmidt & Kurtz, 2005). We estimated energy balance based on the relative fullness index of the fish guts (FI), where FI = [SCM/BM] × 100, and SCM is the difference between the mass of the full stomach and empty stomach (Magnusson *et al.*, 2003). To quantify diet composition, the whole content of each gut was flushed into Petri dishes and the prey items were identified to the lowest taxonomic level possible (Tachet *et al.*, 2000; Streble & Krauter, 2006). Individual prey were counted on a 2.5 × 2.5 mm grid under a stereomicroscope, and in our analyses, we focused on the six most abundant prey items, namely Collembola, Nymphs, Chironomidae, Ostracoda, Chydoridae and Cyclopoida (Table S1).

Statistical analyses

The effects on fish mortality were tested using generalized linear mixed effect models (GLMMs), with a binomial family (logit link); fish ecotype, nutrient levels, parasite exposure and their interactions were fixed effects and block was used as random effect. To analyse variation in parasite load (number of *Gyrodactylus* per fish), we used a GLMM with the number of parasites as the response variable, body length as an offset, a negative binomial error family (square root link) and tanks nested within blocks as random effects. Parameters were estimated by penalized quasi-maximum likelihood (PQL) with the R package MASS, which produced a better dispersion of the fitted and predicted values compared to other R packages (e.g. LME4 and GLMMADMB, Zuur *et al.*, 2009; Bolker *et al.*, 2009).

We tested for the effects of our treatments on body condition (HSI) and the fullness index (FI) with linear mixed effect models (LMMs) with tank nested within block as random effects and parasite load as a continuous variable. To test whether nutrient loading altered the resistance and tolerance of the ecotypes to *Gyrodactylus*, we performed mixed effects ANCOVA with either body condition (HSI) or diet (FI) as responses variables, with the interaction of ecotype × nutrient × parasite load as explanatory variables (Råberg *et al.*, 2009), and with tanks nested within block as a random effect. Differences between slopes were tested using likelihood ratio tests and the χ^2 test statistic following (Hayward *et al.*, 2014). To test whether slopes were significantly different from 0 (i.e. tolerant or intolerant), we used a *t*-test with the R package LMERTEST.

To analyse variation in diet composition, we focused on changes in diet community composition among treatments within experimental blocks. To this end, we used a distance-based redundancy analysis (db-RDA)

framework with the function CAPSCALE () in the R package VEGAN in which we (i) Hellinger-transformed the abundance diet matrix by block (Borcard *et al.*, 2011), (ii) averaged the Hellinger-transformed matrix by tank and used it as our response matrix in the db-RDA and (iii) tested for the significance (RDA's *F* statistic) of our experimental treatments on the transformed matrix by permuting all treatments within a block 999 times. We also tested for the effects of the experimental treatments on each individual prey item from the Hellinger-transformed species matrix (159 × 6 matrix) in a binomial GLMM model, with tank nested within block as a random effect (Table S3). For all LMM and GLMM, we estimated χ^2 and *P*-values in an ANOVA type II analysis with the function ANOVA () from the CAR R package. All statistical analyses were performed in R version 3.2.3 (R Core Team, 2015).

Results

In this experiment, 79 of 278 fish died during the 7 weeks of the experiment. Overall, lake sticklebacks died more than stream sticklebacks (ecotype: χ^2 = 4.164, *P* = 0.041, Fig. S1), and mortality was highest for lake sticklebacks that were exposed to *Gyrodactylus* (G+) (ecotype-by-exposure interaction: χ^2 = 6.912, *P* = 0.008, Fig. S1). We did not find a significant main effect of nutrients (χ^2 = 0.033, *P* = 0.854) on mortality.

Prediction 1 (ecotype differences in host resistance and tolerance)

We found that parasite load was highest for lake fish, both in the wild (lake wild = 30.4 ± 5.23 SE, stream wild = 4.68 ± 1.75 SE, $\chi^2_{1,40}$ = 30.22, *P* < 0.001) and in the experiment (lake experiment = 36.27 ± 13.33 SE, stream experiment = 3.01 ± 5.65 SE; $\chi^2_{1,159}$ = 20.262, *P* < 0.001, Fig. 2a). In the experiment, parasite load was negatively related to the body condition ($\chi^2_{1,159}$ = 12.136 *P* < 0.001), and there was no effect of ecotype on the slope of this relationship (Table S2), implying that both ecotypes were equally intolerant to parasite load (Fig. 3a). We also found that stomach fullness (FI) was negatively related to parasite load ($\chi^2_{1,159}$ = 4.838, *P* = 0.028) and that there was no effect of ecotype on the slope of this relationship (Table S2). However, when we split this analysis by ecotype, we found that the relationship between parasite load and gut fullness was significantly negative for lake fish ($\chi^2_{1,72}$ = 6.197, *P* < 0.05) but not for stream fish ($\chi^2_{1,87}$ = 0.264, *P* > 0.1, Fig. 3d).

Prediction 2 (interactive effects of nutrient)

Overall, fish of both ecotypes were in better condition in HN tanks ($\chi^2_{1,159}$ = 7.406, *P* < 0.01, Fig. 2b, Table 1). Although we found no effects of nutrients on the parasite load of stream fish (stream HN = 2.7 ± 0.777 SE,

Fig. 2 Effects of the experimental treatments on (a) the individual number of parasites, (b) fish condition measured as the hepatosomatic index (HSI), (c) stomach fullness measured as the fullness index (FI) and (d) the relationship between HSI and FI. Symbols show mean values, shapes and colours represent ecotypes (circles = lake and squares = stream) and *Gyrodactylus* exposure treatments (red = G+ and blue = G−). *X*-axis shows the nutrient effects (HN = high and LN = low). Bars in panels a–c represent ± SE).
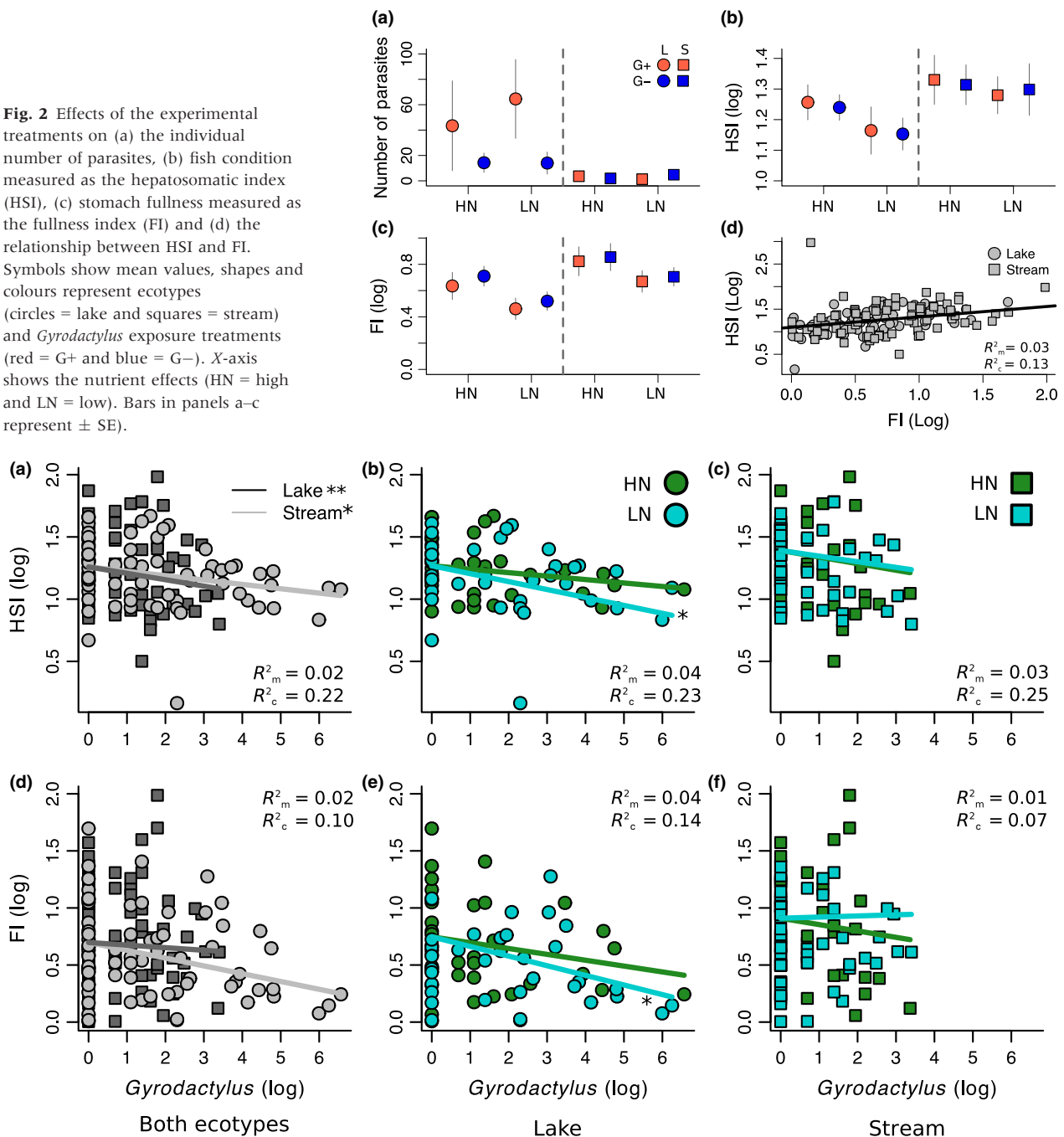

Fig. 3 The Association of the number of parasites with fish condition (a–c) and stomach fullness (d–f). Panels a and d show the effects of parasite load on body condition (HIS) and relative stomach fullness (FI) for each ecotype. Panels b, c, e and d show the effects of nutrient levels on the relationship between parasites and conditions on lake (b and e) and stream (c and f) separately. Symbols' shapes and colours represent ecotypes (circles = lake and squares = stream) and nutrient treatments (dark green = HN and cyan = LN). The lengths of the regression lines represent the range of infection (resistance) and slope (tolerance) the degree of damage produced by increasing parasite infections. Slopes significantly different from 0 are marked with (*). Marginal (fix) and conditional (random) R^2 are included in each panel.

stream LN = 3.28 ± 0.919 SE, $\chi^2_{1,87}$ = 0.011, P = 0.916), the parasite load of lake stickleback was lower in HN compared to LN tanks (lake HN = 29.60 ± 18.9 SE,

lake LN = 43.74 ± 18.94 SE, $\chi^2_{1,72}$ = 7.47, P < 0.01). In addition, we found that the lake fish in the LN tanks had higher parasite loads and there were significant

Table 1 Response of sticklebacks for parasite load, metabolic condition (HSI) and stomach fullness (FI) to fish ecotype, nutrient levels, parasite exposure, their interactions and parasite load.

	Parasite load						Metabolic Condition (HSI)						Stomach fullness (FI)					
	Overall		Lake		Stream		Overall		Lake		Stream		Overall		Lake		Stream	
	χ^2	P	χ^2	P	χ^2	P	χ^2	P	χ^2	P	χ^2	P	χ^2	P	χ^2	P	χ^2	P
Gyrodactylus N	20.262	**<0.001**					12.136	**<0.001**	8.295	**0.004**	3.668	0.055	4.838	**0.028**	6.197	**0.013**	0.264	0.608
Ecotype	0.023	**<0.001**					1.855	0.173					2.981	0.084				
Nutrient	0.008	0.929	7.47	**0.006**		**0.011**	7.406	**0.006**	0.365	0.546	5.546	**0.019**	4.463	**0.035**	1.063	0.302	3.281	**0.070**
Exposure	0.015	0.903	6.276	**0.012**	1.19	0.275	1.778	0.182	1.225	0.268	0.445	0.505	0.407	0.524	0.144	0.704	0.162	0.687
Ecotype : Nutrient	5.626	**0.018**					1.093	0.296					0.195	0.659				
Ecotype : Exposure	1.918	0.166					0.053	0.819					0.002	0.968				
Nutrient : Exposure	1.229	0.268	0.354	0.552	2.08	0.149	0.226	0.635	0.348	0.555	0.056	0.813	0.165	0.685	0.001	0.974	0.017	0.897
Ecotype : Nutrient : Exposure							0.358	0.55					0.007	0.935				
N	159		72		87		159		72		87		159		72		87	

Significant *P*-values (*P* < 0.05) are highlighted in bold.

negative relationships between parasite load and body condition (lake HN: slope = −0.027 ± 0.024 SE, $t\text{-test}_{60.23}$ = −1.158, $P = 0.251$; lake LN: slope = −0.063 ± 0.021 SE, $t\text{-test}_{61.03}$ = −2.942, $P < 0.01$, Fig. 3b) and stomach fullness (lake HN: slope = −0.051 ± 0.039 SE, $t\text{-test}_{58.71}$ = −1.198, $P = 0.199$; lake LN: slope = −0.084 ± 0.036 SE, $t\text{-test}_{63.21}$ = −2.307, $P = 0.024$, see Fig. 3e).

Ecotypes did not differ in their relative gut fullness (Table 1 and Fig. 2c) or in their diet composition (Table 2, Fig. 4). However, initial parasite exposure significantly altered diet composition ($F = 2.776$, $P < 0.05$; Table 2 and Fig. 4), such that parasite-exposed fish ate more copepods ($\chi^2_{1,159}$ = 4.152, $P = 0.042$) and fewer nymphs ($\chi^2_{1,159}$ = 4.703, $P = 0.03$) than nonexposed fish (see Fig. S2 and Tables S1 and S3).

Discussion

Understanding how environmental changes affect species evolution is an ongoing challenge. Here, we predicted that lake and stream stickleback ecotypes would differ in their resistance and tolerance to *Gyrodactylus* and that nutrient loading would differentially affect the host–parasite interactions for each ecotype. Overall, our results provide partial support for both predictions. First, we found that lake sticklebacks had higher mortality and lower resistance to infection than stream sticklebacks when exposed to *Gyrodactylus* (Fig. 2). However, among the surviving fish, both lake and stream ecotypes were equally intolerant to parasite load (i.e. similar slopes in Fig. 3a). Second, we found that nutrient loading increased the resistance of lake but not stream stickleback (Table 1 and Fig. 2a), and, exclusively for lake stickleback, we found some evidence that nutrient loading reduced the cost of parasite load (Fig. 3b).

In the wild, lake and stream stickleback ecotypes inhabit different foraging habitats during most of their life cycle, which expose them to contrasting parasite communities (Kalbe *et al.*, 2002; Feulner *et al.*, 2015; Karvonen *et al.*, 2015). Around the Lake Constance basin, stream sticklebacks reside year round in streams, feed primarily on benthic prey items and are exposed to a lower diversity of parasites than lake fish (Moser *et al.*, 2012, 2015b; Lucek *et al.*, 2013; Karvonen *et al.*, 2015). Lake sticklebacks, on the other hand, live most of their life in the open water of the lake and migrate to nearshore environments and stream channels to breed (Moser *et al.*, 2012; Lucek *et al.*, 2013). They primarily feed on planktonic prey and are exposed to a higher diversity of parasites than stream fish (Moser *et al.*, 2012, 2015b; Lucek *et al.*, 2013; Karvonen *et al.*, 2015). Previous work on other stickleback populations has shown that contrasting foraging habitats and parasite communities are important drivers of phenotypic and genetic differentiation (Feulner *et al.*, 2015; Karvonen *et al.*, 2015) and that rapid evolution of host

Table 2 Effects on sticklebacks prey composition at the end of the experiment.

		Prey composition (db-RDA)											
		Overall				Lake				Stream			
	d.f.	SS	F	P	R^2	SS	F	P	R^2	SS	F	P	R^2
Ecotype	1	0.188	1	0.4	0.04								
Nutrients	1	0.283	1.508	0.181	0.06	0.311	1.285	0.277	0.143	0.171	1.227	0.31	0.102
Exposure	1	0.464	2.469	**0.045**	0.099	0.307	1.272	0.302	0.141	0.219	1.574	0.202	0.131
Ecotype : Nutrients	1	0.229	1.217	0.295	0.049								
Ecotype : Exposure	1	0.071	0.379	0.837	0.015								
Nutrients : Exposure	1	0.086	0.458	0.769	0.018	0.046	0.188	0.98	0.021	0.075	0.535	0.711	0.045
Ecotype : Nutrients : Exposure	1	0.079	0.42	0.81	0.017								
Total $SS_{d.f.}$		4.7_{25}				2.176_9				1.671_{12}			

R^2 values in the db-RDA correspond to partial R^2 = explained sum of squares (SS)/Total SS Significant P-values ($P < 0.05$) are highlighted in bold.
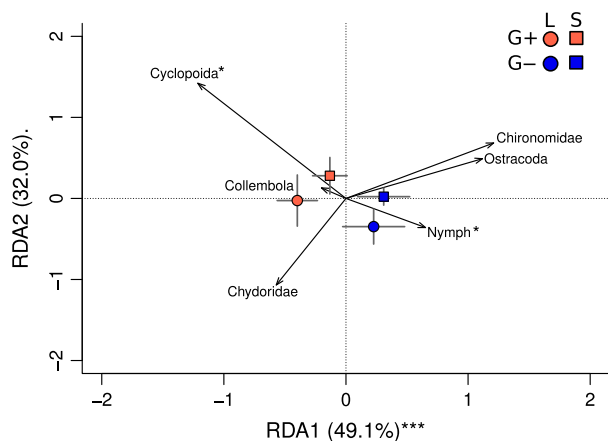

Fig. 4 Effects on diet composition shown in a scaling 3 RDA biplot. Symbols' shapes and colours represent ecotypes (circles = lake and squares = stream) and *Gyrodactylus* exposure treatments (red = G+ and blue = G−). Arrows show the magnitude and direction of change of prey in the constrained multidimensional space (RDA axes). Centroids and bars represent the mean effect of the treatments and the standard errors. RDA-explained variances are shown in brackets. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$ show significant axes and significant effects on prey items (see supporting information).

resistance might be common during the formation of ecotypes (Eizaguirre *et al.*, 2009a, 2011, 2012a,b; Stutz *et al.*, 2014; Feulner *et al.*, 2015; Oke *et al.*, 2016).

Rapid evolution of resistance to contrasting parasite communities is a common outcome of host–parasite interactions (Schmid-Hempel, 2011). The higher level of resistance of stream stickleback could result from coevolution of stream fish with *Gyrodactylus*, one of the most common parasites in stream environments (Kalbe *et al.*, 2002; Eizaguirre *et al.*, 2011). In other stickleback populations, adaptation to contrasting parasite communities in lakes and streams has resulted in divergent

immune responses between ecotypes (Eizaguirre & Lenz, 2010; Karvonen & Seehausen, 2012; Feulner *et al.*, 2015), mainly because contrasting parasite communities are strongly associated with differences in the frequency of alleles of the major histocompatibility complex (MHC) between populations (Eizaguirre *et al.*, 2009a, 2012a; Matthews *et al.*, 2010). Therefore, if host–parasite coevolutionary dynamics in Lake Constance are similar to other systems around Europe, it is possible that resistance alleles, of either the MHC or other relevant genes, have recently increased in frequency in the stream but not in the lake populations Eizaguirre & Lenz, 2010; Eizaguirre *et al.*, 2011, 2012a, b). Although there is recent evidence for adaptive genomic differentiation between the lake and stream ecotypes from in Lake Constance (Moser *et al.*, 2012, 2015a; Lucek *et al.*, 2013; Karvonen *et al.*, 2015; Roesti *et al.*, 2015; Marques *et al.*, 2016), nothing is known about differentiation of MHC genes for this lake–stream pair and this should be addressed in further studies.

While the evolution of host resistance can influence diversification and maintenance of genetic diversity between and within populations (Buckling & Rainey, 2002; Summers *et al.*, 2003; Eizaguirre *et al.*, 2009b), the evolution of tolerance may favour the spread of parasites (Best *et al.*, 2008; Kaufmann *et al.*, 2014), break down 'Red Queen dynamics' and generate multiple stable states (Best *et al.*, 2014; Råberg, 2014). This is because tolerance can mitigate the negative effects of parasitism, but does not contribute to decreasing parasite load (Best *et al.*, 2014; Råberg, 2014). In our experiment, we did not find significant differences in the degree of tolerance between ecotypes (e.g. slopes in Fig. 3a are not different). However, we did find lower parasite loads in the lake ecotype at high nutrient levels (Tables 1 and S1), and this suggests that lake fish might be able to mitigate the negative effects of parasite load on body condition if they can increase energy intake in more productive environments (Fig. 2b,e). Such

compensatory effects could have significant impacts on host–parasite dynamics if they help maintain susceptible host genotypes in the population (Brunner & Eizaguirre, 2016) or if they allow virulent parasite strains to increase in frequency (Kause & Ødegård, 2012; Best *et al.*, 2014; Råberg, 2014).

Our experiment also revealed a number of links between individual variation in parasite load, body condition and diet (i.e. stomach fullness and composition). First, the positive relationship between body condition (HSI) and stomach fullness (FI) is consistent with an expected positive relationship between food acquisition and overall metabolic state. Second, the negative relationship between stomach fullness and parasite number in the lake ecotype suggests potentially higher costs of parasitism for lake fish. This is also consistent with the higher mortality of lake fish when exposed to parasites. In high nutrient environments, lake fish might be able to improve their capacity to cope with *Gyrodactylus* by increasing feeding rates, or changing their diet (e.g. Fig. 3e) so as to include more profitable prey (Pianka, 1981; Huey *et al.*, 2001; Arrington *et al.*, 2002). Second, despite known ecotype differences in feeding traits (Berner *et al.*, 2011; Lucek *et al.*, 2013; Marques *et al.*, 2016), in a common foraging environment, we found no diet differentiation between ecotypes, but found that stickleback exposed to *Gyrodactylus* ate more cyclopoids and fewer nymphs than nonexposed individuals (Figs 4, S1 and Table S2). More work on the food quality of different prey items and the effect of these parasites on the functional response of sticklebacks would be necessary to determine whether the diet changes associated with parasite exposure were indicative of a compensatory mechanism or not.

Although it is well known that trophically transmitted parasites can affect host feeding behaviour (e.g. *Schistocephalus* and *Diplostomum* parasites on stickleback feeding rate (Jakobsen *et al.*, 1988; Milinski, 1993; Aeschlimann *et al.*, 2000; Lefèvre *et al.*, 2009), our results suggest that directly transmitted parasites can also affect feeding behaviour (e.g. diet preference) and activity (e.g. gut fullness) of their hosts, possibly via effects on host condition. The stickleback populations used in this experiment were introduced in the Lake Constance region approximately 150 years ago (Lucek *et al.*, 2010; Marques *et al.*, 2016), and over this short time period (< 100 generations), they have evolved divergent life history traits such as growth rate, lifespan and time of first reproduction (Lucek *et al.*, 2012, 2013; Moser *et al.*, 2012). Building on these previous studies, we found that parasitism can influence both ecological interactions between stickleback and their prey (e.g. effects of parasite exposure on diet and stomach fullness) and potentially influence selection gradients experienced by hosts (i.e. relationships between parasite load and body condition). However, more work is needed to understand the mechanisms by which parasitism can affect trade-offs between immunity and feeding behaviour, particularly in natural populations.

Authorship

JMAR analysed the data and wrote the first draft of the manuscript. JMAR and FSB performed the experiment, and NS contributed to data collection and writing the manuscript. BM and CE conceived the experiment and contributed substantially to the revisions of the manuscript. OS made substantial contributions to the structure and revisions of the manuscript draft. Here, we confirm that we do not have any conflict of interest.

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Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1 Mortality during the experiment as the percentage of dead fish per tank.

Figure S2 Effects of the experimental treatments on individual prey abundances.

Table S1 Descriptive statistical summary of the variables measured.

Table S2 ANCOVA results for differences in slopes between ecotype and nutrient treatments.

Table S3 Statistical summary of the effects on individual prey types from the Hellinger transformed individual prey items in a binomial GLMM.