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Climate change facilitates a parasite's host exploitation via temperature-mediated immunometabolic processes

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Abstract

Global climate change can influence organismic interactions like those between hosts and parasites. Rising temperatures may exacerbate the exploitation of hosts by parasites, especially in ectothermic systems. The metabolic activity of ectotherms is strongly linked to temperature and generally increases when temperatures rise. We hypothesized that temperature change in combination with parasite infection interferes with the host's immunometabolism. We used a parasite, the avian cestode *Schistocephalus solidus*, which taps most of its resources from the metabolism of an ectothermic intermediate host, the three-spined stickleback. We experimentally exposed sticklebacks to this parasite, and studied liver transcriptomes 50 days after infection at 13°C and 24°C, to assess their immunometabolic responses. Furthermore, we monitored fitness parameters of the parasite and examined immunity and body condition of the sticklebacks at 13°C, 18°C and 24°C after 36, 50 and 64 days of infection. At low temperatures (13°C), *S. solidus* growth was constrained, presumably also by the more active stickleback's immune system, thus delaying its infectivity for the final host to 64 days. Warmer temperature (18°C and 24°C) enhanced *S. solidus* growth, and it became infective to the final host already after 36 days. Overall, *S. solidus* produced many more viable offspring after development at elevated temperatures. In contrast, stickleback hosts had lower body conditions, and their immune system was less active at warm temperature. The stickleback's liver transcriptome revealed that mainly metabolic processes were differentially regulated between temperatures, whereas immune genes were not strongly affected. Temperature effects on gene expression were strongly enhanced in infected sticklebacks, and even in exposed-but-not-infected hosts. These data suggest that the parasite exposure in concert with rising temperature, as to be expected with global climate change, shifted the host's immunometabolism, thus providing nutrients for the enormous growth of the parasite and, at the same time suppressing immune defence.

KEYWORDS

Gasterosteus aculeatus, immunometabolism, liver transcriptomics, parasite, *Schistocephalus solidus*, temperature

1 | INTRODUCTION

Host–parasite interactions are fundamental parts of ecosystems, and their dynamics change in response to environmental variables (Lafferty & Holt, 2003; Lafferty & Kuris, 1999; Lazzaro & Little, 2009; Wolinska & King, 2009; Young & Maccoll, 2017). Accordingly, climate change is predicted to have prominent effects on interactions between hosts and parasites (Altizer et al., 2013; Brunner & Eizaguirre, 2016; Budria & Candolin, 2014; Cohen et al., 2019; Lafferty & Shaw, 2013; Morley & Lewis, 2014; Poulin, 2020; Stensgaard et al., 2019; Thieltges et al., 2013). Climate models illustrate that global climate change coincides with more frequent events of temperature extremes and an increase of average temperatures of 1°C–4°C by 2100 (IPCC, 2015; Masson-Delmotte et al., 2018).

Evidence accumulates that temperature variation has prominent effects on host–parasite interactions, in particular when involving ectothermic hosts (Hakalahti et al., 2006; Hance et al., 2007; Landis et al., 2012; Lazzaro & Little, 2009; Lohmus & Bjorklund, 2015; Marcogliese, 2008; Poulin, 2007; Poulin & Fitzgerald, 1988; Poulin & Mouritsen, 2006; Schade et al., 2016; Studer et al., 2010).

In ectotherms, such as teleost fish, it is well established that metabolic activity is strongly temperature-dependent and generally rises, when temperatures increase (Guderley et al., 2001; Guderley & St-Pierre, 2002; Portner et al., 2008; Roman et al., 2019). However, how the fundamental temperature dependence of ectotherms interferes with metabolic requirements of their parasites has received only marginal attention (Morley & Lewis, 2014). Furthermore, the activity of the immune system of ectotherms depends on temperature (Makrinos & Bowden, 2016; Morley & Lewis, 2014; Scharsack et al., 2016). Accordingly, depending on the ambient temperature, immunity and metabolism of ectotherms in concert might constrain or assist the metabolic requirements of their parasites.

To disentangle between temperature, host immunometabolism and the fitness of a parasite, we used a host–parasite system in which the cestode, *Schistocephalus solidus*, grows enormously in cold-blooded hosts, before it switches to the warm-blooded final host (Arme & Owen, 1967). Its life cycle starts with cyclopoid copepods as first hosts (Figure S1), in which the parasites start to grow (approx. 100-fold) in the body cavity. Upon ingestion by the next host, the three-spined stickleback (*Gasterosteus aculeatus*), *S. solidus*, penetrates through the gut wall and grows about 10,000-fold in the hosts' body cavity. Here it can reach up to 20%–30% of a sticklebacks' body mass (Scharsack et al., 2007). Final hosts of the parasite are fish-eating birds, and the parasite starts to reproduce after a couple of days in the bird gut. In the laboratory, parasite reproduction can be initiated with an in vitro system run at 40°C (Smyth, 1946; Wedekind, 1997).

It was observed that the infection of sticklebacks with *S. solidus* is influenced by environmental temperature. Sticklebacks from the Islandic lake Mývatn with cold and warm springs showed higher levels of infection with the cestode at the warm sites (Karvonen et al., 2013). Experimental infections showed that *S. solidus* grew larger at warmer temperatures (Franke et al., 2017, 2019; Macnab & Barber, 2012). Growth performance of *S. solidus* is closely linked to its fitness since (a) it has to reach a threshold weight of about 50 mg to become infective to the final bird host (Tierney & Crompton, 1992) and (b) the weight of *S. solidus* at the time of reproduction in the bird gut is positively correlated with the number of eggs it produces (Luscher & Milinski, 2003). From the parasites' perspective, temperature-accelerated growth in its stickleback host will increase its reproductive output. From the sticklebacks' perspective, temperature-accelerated parasite growth must be detrimental due to elevated host exploitation.

Experimental exposure of *S. solidus*-infected sticklebacks, originating from habitats with different temperature regimes to temperature variation, revealed a consistent pattern of host–parasite–temperature interactions. Namely, *S. solidus* growth is constrained at relatively low temperatures when host growth and immune activity are high. Vice versa, rising temperature facilitates *S. solidus* growth while it deteriorates stickleback immune functions (Dittmar et al., 2014; Franke et al., 2017, 2019). These findings suggest that also in cold-blooded vertebrates, immunity and metabolism are connected and that temperature mediates a parasite's interaction with the host's immunometabolism.

The link between immunity and metabolism (immunometabolism) was first described in mammals and proposed to become a new frontier in immunology, less than 10 years ago (Mathis & Shoelson, 2011). Since then, it has come more and more into the focus of immune-medical research due to its potential implications for many auto-immune disorders (e.g. Katsiougiannis et al., 2019; Weyand & Goronzy, 2020; Zezina et al., 2020) while also discussing the evolutionary perspective of immunometabolism (Wang, Luan, et al., 2019; Wang, Ping, et al., 2019).

Helminth parasites, such as nematodes and cestodes, were recognized as modulators of immunometabolism since they are strong inducers of T-helper 2 (Th2) lymphocytes, which are important in many auto-immune diseases (Hussaarts et al., 2015; Matthias & Zielinski, 2019; Shea-Donohue et al., 2017; van der Zande et al., 2019). The 'hygiene hypotheses' (or old friends hypotheses) suggest that the decline of macro-parasite infections in western societies favoured the development of autoimmune diseases (Rook et al., 2003; Rook, 2012; Strachan, 1989). Indeed, effects of helminths on the immunometabolism of humans contributed to the maintenance of tissue-specific and whole-body metabolic homeostasis and protection against obesity-associated inflammation leading to diabetes type 2 (Guigas & Molofsky, 2015; van der Zande et al., 2019). Immunometabolic principles are present in teleost fish, for example, zebrafish were used to investigate metabolic responses to vaccination (Guo et al., 2015), and their value as models to gain deeper insight in the basal mechanisms of immunometabolism is discussed (Pereiro et al., 2019; Wentzel et al., 2020). An interesting example is Mexican cavefish (*Astyanax mexicanus*), which has adapted to life in caves with almost no parasites. Compared to conspecifics from surface waterbodies with a number of parasites, cavefish drastically reduced immunopathological phenotypes in visceral adipose tissue. Mexican cavefish are an interesting model to investigate immunometabolic adaptations to environments without

macro-parasite infection pressure (Peuß et al., 2020), to help understanding how human immunometabolism responds to declining macro-parasite infections. With temperature, the present study adds a strong environmental component to investigations of immunometabolic host–parasite interactions. This opens a perspective for laboratory studies to manipulate temperature, to foster or supress parasites' effects on the hosts' immunometabolism.

Given that temperature alters both immunity and metabolism of ectotherms, their parasites are likely to be afflicted. Based on these assumptions, we here investigate physiological correlates of such temperature–host–parasite interactions and asked whether the hosts' immunometabolism is affected by parasites. We wanted to explore how host and parasite respond to long-term exposure to high and low temperature, in a range which may occur in their natural habitats. In the previous study, we investigated effects of temperature change on sticklebacks body condition and immune parameters without parasite exposure (Dittmar et al., 2014). We observed that sticklebacks developed higher body condition and immune activity at 13°C versus 18°C and 24°C. While 18°C was well tolerated by sticklebacks, their body condition and immune activity declined at 24°C. Exposure to heat waves, up to 28°C revealed that temperature above 24°C caused immunological disorders and physiological stress to sticklebacks. This was exacerbated when temperatures were increased relatively faster, we tested temperature ramps of 1°C, 2°C and 4°C per day (Dittmar et al., 2014).

For the present study, we wanted to avoid temperatures that would cause major physiological stress to the sticklebacks and decided for 13°C, 18°C and 24°C and to change temperatures at moderate speed. We suspected that parasite infection rates would be higher at warm temperatures (indicated by a pilot experiment, Figure S2), which we wanted to avoid for the present study, to keep comparability between temperature groups. We decided to start the experiment at 18°C, the permissive, temperature for sticklebacks. We kept 18°C after parasite exposure to allow the parasites to establish in the host for 7 days. Then temperature change was initiated and continued for 5 days at rates of 1°C per day from 18°C to 13°C and 1.2°C/day from 18°C to 24°C (Figure 1). We also explored whether the hosts' reactions might depend on a potential local adaptation of the parasite, and thus used a sympatric and an allopatric parasite population. After 36-, 50- and 64-day post-exposure, we obtained host and parasite fitness parameters and measures of the hosts' immunological status and stored liver samples in RNA later for whole-transcriptome sequencing (RNAseq). We decided to focus on livers from one time point (50 days) and from the low and the high temperature tested, to have useful numbers of biological replicates per treatment group (see Table S1 for detailed numbers). Livers were chosen to get insight into the hosts' immunometabolism response to temperature in combination with parasite infections. Liver transcriptome samples were considered most relevant, as in *S. solidus*-infected sticklebacks, liver weights are negatively correlated to parasite weight, probably an effect of the nutrient drain by the parasite (Barber & Scharsack, 2010; Scharsack et al., 2007). Given the higher metabolic rates of sticklebacks at elevated temperature

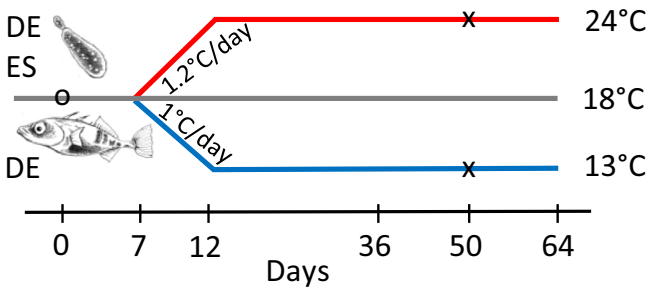

FIGURE 1 Experimental setup. Sticklebacks from Germany (DE, $n = 576$) were exposed to sympatric (DE) or Spanish (ES) *Schistocephalus solidus* ($n = 216$ fish each) or sham-exposed ($n = 144$) on day 0 (o), assigned to each temperature treatment randomly in equal numbers. To avoid temperature effects on infection rates, parasites were allowed to establish at 18°C till day 7. From days 7 to 12, temperatures were changed slowly by 1°C and 1.2°C per day. Sticklebacks were sampled after 36, 50 and 64 days after parasite exposure. Samples for RNA sequencing ($n = 87$) were taken from fish sampled after 50 days (x) only from temperature extremes 13°C and 24°C (see Table S1 for detailed numbers sampled)

(Vezina & Guderley, 1991), we expect that competition of *S. solidus* and its host for resources stored in the liver will be stronger at high versus low temperature. Furthermore, the livers of teleost fish are active in their immune defence (Bayne & Gerwick, 2001).

We expected that immunity and metabolism are closely linked also in cold-blooded vertebrates and modulated by temperature as well as their parasites.

2 | MATERIALS AND METHODS

2.1 | Experimental setup

In total, $n = 576$ sticklebacks at the age of 5–6 months, from eight families were distributed to balanced family groups of 16 in 16 L aquaria. In each tank, six sticklebacks were exposed to sympatric *S. solidus* (DE) and six to allopatric *S. solidus* from Spain (ES) and four were sham-treated controls. Aquaria were installed in blocks of six and two blocks were randomly assigned to the 13°C, 18°C and 24°C temperature treatment. After marking by spine clips and distribution, sticklebacks were given at least 4 weeks to adjust to the new composition in the tanks, before experimentation started.

For parasite exposure, sticklebacks were starved for 2 days and transferred individually to small tanks with 500 ml aquarium water. On the next day, each stickleback received two copepods, containing together three procercoids. Controls were sham-exposed to uninfected copepods. After 24 hr, ingestion of copepods was confirmed by the screening of the filtered tank water and sticklebacks were returned to their experimental tanks. To reduce potential temperature effects on the infection rates (Figure S2), we allowed infections to establish for 1 week at 18°C before the temperature was changed to 13°C and 24°C at a rate of 1°C–1.2°C per

day (Figure 1). Moderate speed of temperature change was used to minimize physiological stress (Dittmar et al., 2014). Sticklebacks were sampled 36-, 50- and 64-day post-exposure (dpe). Samples for whole-liver transcriptome sequencing (RNAseq) were taken from sticklebacks sampled at 50 dpe from sticklebacks kept at 13°C and 24°C (Figure 1).

Details on the numbers of sticklebacks sampled are given in the supplement (Table S1).

2.2 | Stickleback husbandry

Experimental sticklebacks and tapeworms were laboratory-bred offspring (F1) of wild parental specimen collected in Germany (DE; Ibbenbürener Aa; 52°17′31.76″N, 7°36′46.49″E; 2013 temperature average: 14.3°C, min: 5.7°C, max: 24.5°C). Additional tapeworms were obtained from an origin in Spain (ES; Xinzo de Limia channel, 42°8′1.27″N, 7°39′47.801″E, 2013 temperature average: 11.9°C, min: 1.9°C, max: 29.1°C). Sticklebacks were maintained in aquaria blocks with re-circulated tap water and temperature. Each block has six small aquaria (16 L), one large aquarium (80 L) and a separate tank for water filtration and temperature regulation (Vewa Tech®). Sticklebacks were kept at 18°C and a 15/9 hr light/dark cycle in family groups and fed daily ad libitum, initially with live *Artemia salina* nauplii and later with frozen mosquito larvae. Sticklebacks were maintained and treated following the EU Directive 2010/63/EU for animal experiments and the local animal welfare authorities under the project number 87-51.04.2010.A297.

2.3 | Parasite husbandry

S. solidus plerocercoids were removed aseptically from the body cavity of wild-caught three-spined sticklebacks from the populations as above. Parasites were cultured in an in vitro system modified from Smyth (1954), as described by Wedekind (1997). Parasite pairs were matched by body weight to ensure outcrossing (Luscher & Milinski, 2003). Parasite pairs were incubated at 40°C with continuous shaking for 6 days in dark in minimum essential medium (MEM) with Earle's salts, 4 mol/L L-glutamine, 25 mmol/L HEPES-buffer, 1 g/L penicillin/streptomycin and 6.5 g/L D-glucose (all ingredients: PAA, except D-Glucose: Roth). Afterwards, the eggs were washed (5×) with sterilized tap water and stored separated by families at 4°C in the dark.

For hatching, eggs were transferred to 20°C. After 3 weeks, hatching of coracidia was stimulated by light (Dubinina, 1957). Four *S. solidus* families were used per origin. For infection experiments, laboratory-bred copepods (*Macrocyclops albidus*) were exposed individually to three *S. solidus* coracidia each and maintained in 2 ml tap water per well of 24-well plates (Sarstedt) at 20°C and a 16/8 hr light/dark cycle. Copepods were fed with *Artemia salina* larvae. Fourteen days after exposure, the copepods were screened for the presence of procercoids microscopically.

2.4 | Sampling of sticklebacks

Sticklebacks were killed with an overdose of MS222 (Sigma Aldrich). Standard length (to the nearest mm) and wet weight (to the nearest mg) were measured. The head kidneys were dissected out and transferred to cell strainers (40 μm, BD Falcon) in Petri dishes with 1 ml RPMI 1640 medium (PAA) with 10% distilled water (R-90) on ice. Livers were transferred to RNAlater (Qiagen), kept overnight at 4°C, taken out of the RNAlater and stored at −80°C.

S. solidus plerocercoids were removed aseptically weighed (to the nearest mg) and transferred to Petri dishes with minimum essential medium (MEM; PAA) for later breeding. A parasite index was calculated as the percent of total parasite weight from total host weight (incl. parasites). Finally, the stickleback's gonads were weighed to the nearest milligram to calculate a gonadosomatic index (GSI) as percent gonad weight from stickleback weight (excl. parasite).

2.5 | Immune assays

For immunological assays, stickleback head kidney leukocytes (HKL) were used as described by Scharsack et al. (2007). Briefly, suspensions of HKL were prepared by forcing the organ through cell strainers. Isolated HKL were washed twice (5 min 600 *g*) and subjected to differential cell counts using flow cytometry (FACS Canto II, Becton and Dickinson).

2.5.1 | Respiratory burst

As one of the most important effector mechanisms of cell-mediated innate immunity, we quantified the respiratory burst activity of HKL in a lucigenin-enhanced chemiluminescence assay. HKL (10^5 well^{-1} of white 96-well flat bottom microtiter plates, Nunc) were incubated 30 min with lucigenin (250 mg/L, Sigma Aldrich) and respiratory burst was initiated by addition of zymosan (750 mg/L, Sigma Aldrich). Luminescence was recorded for 3 hr at 18°C with a Tecan infinite 200 luminescence reader (Tecan). The activity of HKL was expressed as relative luminescence units (RLU), and results were calculated as the area under the kinetic curve (RLU area) with the Magellan v 6 software (Tecan).

2.5.2 | Lymphocyte proliferation

As a proxy for the activity of the adaptive immune system, the proliferation of head kidney lymphocytes was recorded by flow cytometry. Therefore, 2.5×10^5 HKL from the suspension, as described above, were fixed with 80% ethanol at 4°C overnight. After removal of ethanol, HKL were subjected to RNA digestion (10 min with RNAse 0.3 mg/L, Roth) and their DNA was stained with propidium iodide (5 mg/L, Sigma Aldrich). After flow cytometric measurement (FACS Canto), frequencies of lymphocytes in $G_{0\text{-}1}$ and $S + G_{2\text{-}M}$ phase

of the cell cycle were acquired by DNA content analysis of red fluorescence intensity (propidium iodide labelling) of single cells from the lymphocyte gate with the FACS Diva v 6.1.2 software (Becton and Dickinson).

2.6 | Parasite egg production

Pairs of *S. solidus* were matched within treatment (host/parasite origin, temperature, sampling time point) by body weight and cultured as described above. Total egg output per parasite pair was determined, and from each parasite pair, 5,000 eggs were transferred to Petri dishes (30 mm diameter) with 5 ml of tap water to count the hatching rates. If the egg number per family was below 5,000, the total egg output from the family was used. Hatching was induced using the standardized protocol as above, and hatching rates were determined based on the number of eggs with an open operculum after 7 days at 20°C and 16/8 hr light/dark cycles.

2.7 | Statistical analysis of phenotypic data

Statistical analyses were performed with the SPSS v 22 software (IBM). Data were tested for normality with the Kolmogorov–Smirnov test and by visual examination of histograms. Homogeneity of variance was tested with the Levene's test. Effects of main factors (temperature, stickleback and parasite origin, exposure treatment, sampling time point) and their interactions on response variables were analysed with generalized linear models (GzLM) with stepwise removal of non-significant terms. Multiple pairwise comparisons were analysed with post hoc Wald chi-square tests with sequential Bonferroni correction for multiple testing. Differences of infection rates between treatment groups (temperature, parasite origin) were tested with chi-square analysis followed by a *z*-test with Bonferroni adjusted *p* values.

2.8 | RNA sequencing

For RNA sequencing, livers from $n = 87$ sticklebacks were used. The samples were taken from the intermediate time point (50 dpi) and the temperature extremes (13°C and 24°C) according to sex and infection ratios as present in the whole samples (for detailed numbers, see Table S1). The RNA was extracted with the RNeasy midi kit (Qiagen) and the library preparation for Illumina sequencing was done with the TruSeq RNA Sample Preparation Kit v2 (Illumina) for single indexed libraries. Sequencing was performed on an Illumina HiScanSQ with 101 cycles in paired-end mode with the single-indexed protocol. Sequences were demultiplexed with the Illumina Casava 1.8 software. Nine of the 96 sequenced samples had too few (<200) reads and were excluded from further analyses. After purification, sequenced reads were aligned to version 69 of the *G. aculeatus* genome from the Ensembl database (www.ensembl.org) using Tophat 2.0.4 (Trapnell et al., 2009).

2.9 | Analysis of differential gene expression

The differentially expressed gene (DEG) analysis for the RNA-Seq data was performed using DESeq2 1.14.0 (Love et al., 2014), which is part of the Bioconductor package in R (Huber et al., 2015). DEGs were analysed between temperatures (13°C vs. 24°C) within infection treatments (sham-exposed, infected, exposed not infected). A threshold for significance of DEGs was set as $q < 0.10$ (q = false discovery rate corrected p value; Benjamini & Hochberg, 1995). Upregulated (log2FoldChange > 0) and downregulated (log2FoldChange < 0) DEGs were pooled for each treatment.

2.10 | Comparison of RNA sequencing and phenotypic data

A principal component analysis (PCA) was performed with DEGs per treatment using the princomp function in R. Markov chain Monte Carlo generalized linear mixed models (MCMCglmm) were used for the comparisons of gene expression and phenotypic data. The MCMCglmm were built with the R package (Hadfield, 2010) based on the results of the PCA and the phenotypic data (respiratory burst activity and lymphocyte proliferation) for each infection treatment within the 13°C versus 24°C comparisons. The most significant principal components (PC) were selected for further gene ontology analysis. The bottom and top 20% of the loading DEGs within a PC were analysed using blast2go (Conesa et al., 2005; $p < .10$, results, see Figure 5).

3 | RESULTS

3.1 | Parasite fitness proxies

We used body mass, numbers of produced eggs and hatched larvae as fitness proxies for parasites grown at 13°C, 18°C and 24°C. At 13°C, plerocercoids had grown much slower and reached the 50 mg threshold weight for reproduction (defined previously; Tierney & Crompton, 1992) only by 64 days after infection (dashed line in Figure 2a). At 13°C, the parasites needed relatively more accumulated thermal units (ATU, or °C days) to reach 50 mg. At 18°C, parasites had grown to 50 mg already at 36 days (36 days × 18°C = 648 ATU) and were reproductive (Figure 2b). In the 13°C treatment, parasites developed 7 days at 18°C (=126 ATU) followed by the temperature ramp and approximately 40 days at 13°C (595 ATU) before they reached 50 mg after 52 days, corresponding to 721 ATU. This suggests that low temperature alone does not fully explain the observed constrained parasite growth at 13°C.

At 18°C and 24°C, *S. solidus* grew much faster and accordingly produced more eggs compared to 13°C (Figure 2, see Table 1 for statistics). As a consequence, the number of hatched larvae obtained from parasites grown at 18°C and 24°C was 12- to 13-fold higher than at 13°C (Figure 2c). That the parasites grew faster at 18°C than at either 24°C or 13°C suggests an optimum temperature for *S. solidus* growth around 18°C (Figure 2a).

Parasite origin and its interaction with a temperature significantly influenced the egg production (Table 1), which was highest with Spanish parasites that had grown in sticklebacks at 18°C (Figure 2b). However, parasite origin did not explain the variation of the tested host parameters, including the transcriptome data and was excluded from the following statistical models.

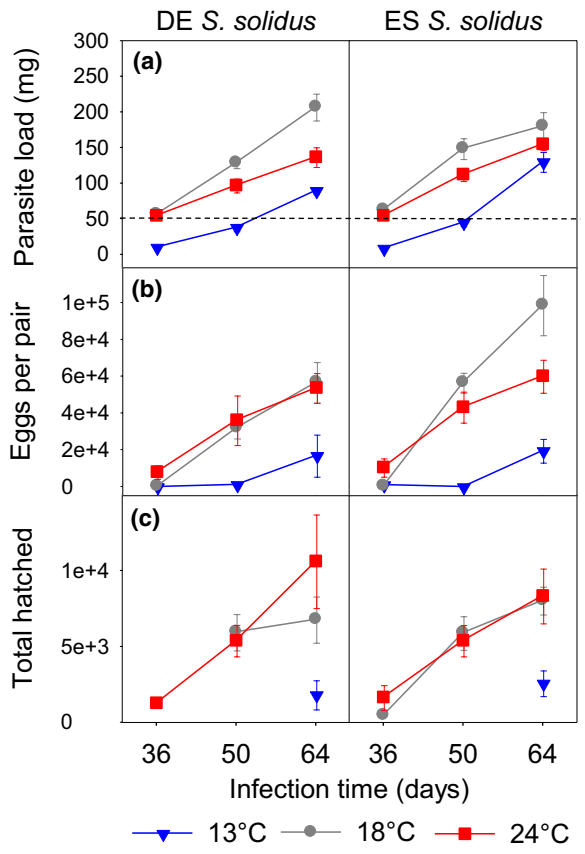

FIGURE 2 *Schistocephalus solidus* fitness. Parasite load per stickleback (a), egg production per parasite pair (b) and the number of parasite larvae hatched per pair (c). *S. solidus* plerocercoids were collected from sympatric (DE) and allopatric (ES), laboratory infections. Dashed line: 50 mg threshold weight for parasite reproduction (Tierney & Crompton, 1992)

3.2 | Host body condition parameters

We acquired body weights (without parasite weight) and the relative gonad weight as GSI to estimate the effects of temperature and *S. solidus* infection on host fitness. The temperature was a strong modulator of host fitness proxies, and sticklebacks grew larger and had higher GSI at the low temperature (13°C) tested here (Figure 3c,d). This result was time-dependent, except for the male GSI (see Table 2 for statistics), and less prominent at the early time point (Figure 3d,e). The effect of parasite infection on host body condition parameters was significant with host weight since infected sticklebacks had lower body mass than their uninfected conspecifics at the same temperature (Figure 3c). In male sticklebacks, *S. solidus* infection reduced the otherwise high GSI at 13°C, resulting in a significant interaction of temperature with infection status (Figure 3d; Table 2).

3.3 | Host immunity

As a representative of innate immunity, the production of reactive oxygen species (ROS) of stickleback HKL was analysed. The activity of the adaptive immune system was estimated from the proportion of proliferating lymphocytes ($S + G_2M$ lym) in HKL isolates. Variation of both innate and adaptive immunity depended on time, temperature and infection status (Figure 3a,b; see Table 2 for statistics). For both immune parameters, infection time showed significant interactions with temperature and infection status, and the interaction of temperature and infection status was significant, too (Table 2).

For the respiratory burst, also the three-way interaction of time, temperature and infection status were significant (Table 2). In response to *S. solidus*, the respiratory burst was increased at 18°C and 24°C already 36 days post-infection (dpi) and thereafter decreased, while at 13°C the highest respiratory burst activity was recorded later, at 50 dpi (Figure 3a). The lymphocyte proliferation was generally higher at 13°C and most prominent in *S. solidus*-infected sticklebacks at 13°C (Figure 3b).

TABLE 1 Generalized linear models (GzLM) of parasite data (corresponding to Figure 2), summarizing GzLM after stepwise removal of least significant terms

		Parasite load		Eggs per pair		Total hatched	
	df	X^2	*p*	X^2	*p*	X^2	*p*
Intercept	1	1,490.6	**	159.5	**	13.705	**
Infection time	2	328.3	**	90.8	**	19.908	**
Temperature	2	161.4	**	47.2	**	16.358	**
Parasite origin	1	–	–	5.6	.018	–	–
Inf. time*temperature	4	15.6	.004	25.2	**	–	–
Para. origin*temperature	2	–	–	6.1	.048	–	–

*Interaction; **$p < .001$; ¯$p > .05$.

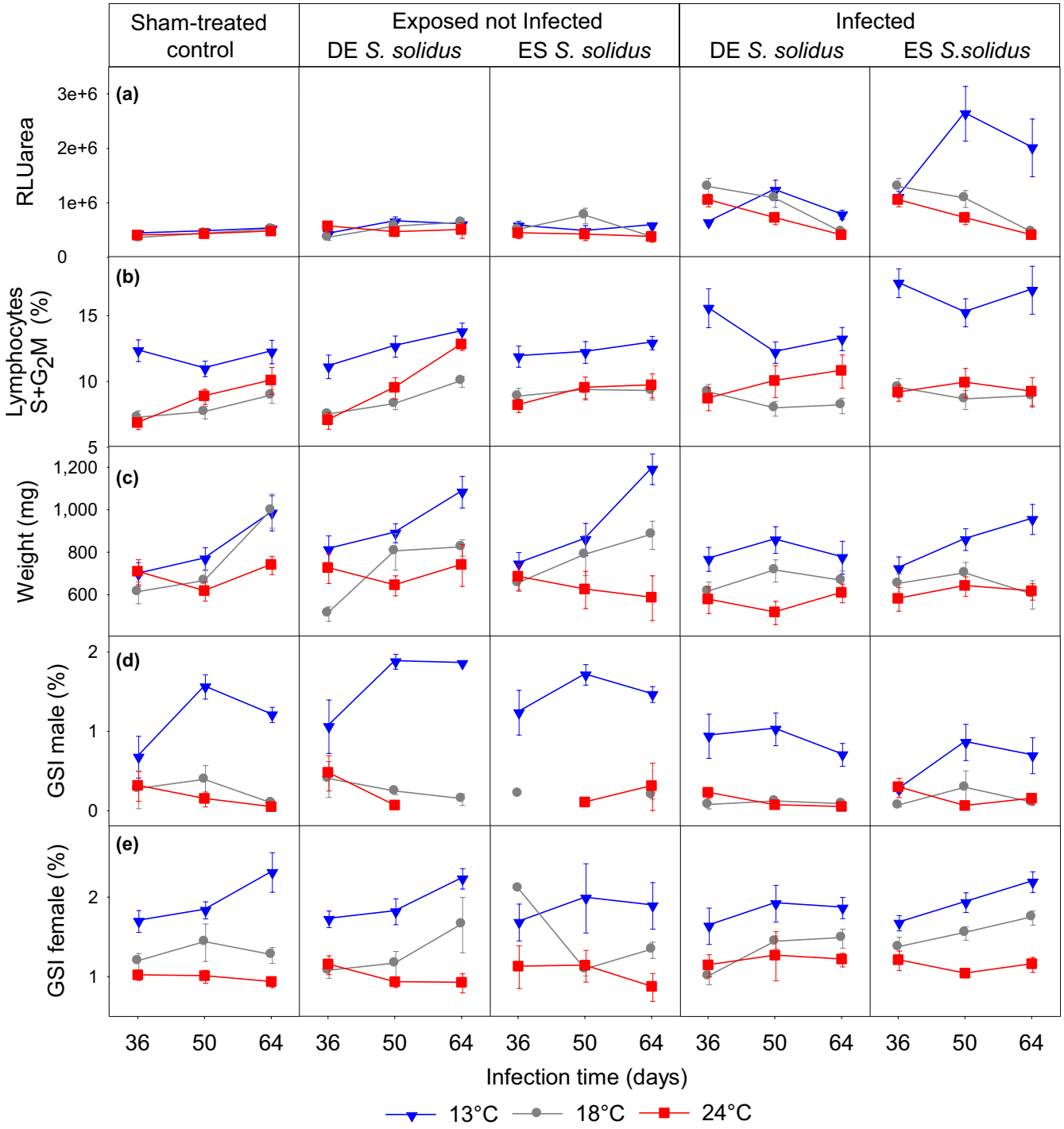

FIGURE 3 Host immune and fitness proxies. Immune activity, (a) respiratory burst, (b) lymphocyte proliferation and body condition (c) weight without parasite and gonadosomatic indices (GSI) of (d) males and (e) females. Sticklebacks originating from a German population were sham-treated or exposed to sympatric (DE) and allopatric (ES) *Schistocephalus solidus*

3.4 | Effects of temperature and *S. solidus* on stickleback liver transcriptomes

To assess the effect of temperature on liver gene expression for control and infected sticklebacks, we quantified the number of genes that were differentially expressed between 13°C and 24°C. In the sham-exposed controls (ctr), 1,213 DEGs were observed, whereas a much higher number of DEGs (5,255) was detected in infected (inf) sticklebacks (Figure 4). Interestingly, exposed but not infected (exp) sticklebacks had the highest numbers of DEGs (5,972). The majority of DEGs between the control groups at 13°C and 24°C were also detected in infected and exposed but not infected sticklebacks, suggesting that basal temperature responses were similar in *S. solidus* exposed and

TABLE 2 Summary of GzLM of host data (corresponding to Figure 3), summarizing GzLM after stepwise removal of least significant terms

		RLU area		S + G_2M lym.		Host weight		GSI male		GSI female	
	df	X^2	*p*	X^2	*p*	X^2	*p*	X^2	*p*	X^2	*p*
Intercept	1	802.8	**	6,154.3	**	6,171.5	**	376.0	**	2,181.7	**
Time	2	8.9	.012	11.6	.003	39.2	**	–	–	13.3	.001
Temperature	2	35.4	**	262.5	**	104.8	**	296.4	**	115.3	**
Inf. status	4	166.8	**	38.9	**	19.5	.001	–	–	–	–
Time*temp.	4	23.6	**	16.8	.002	23.0	**	29.7	**	–	–
Time*inf. status	8	18.2	.020	22.8	.004	24.6	.002	–	–	–	–
Temp.*inf. status	8	82.4	**	33.9	**	–	–	29.3	**	–	–
Time*temp.*inf. status	16	51.8	**	–	–	40.3	.020	–	–	–	–

*Interaction; **$p < .001$; ¯$p > .05$.

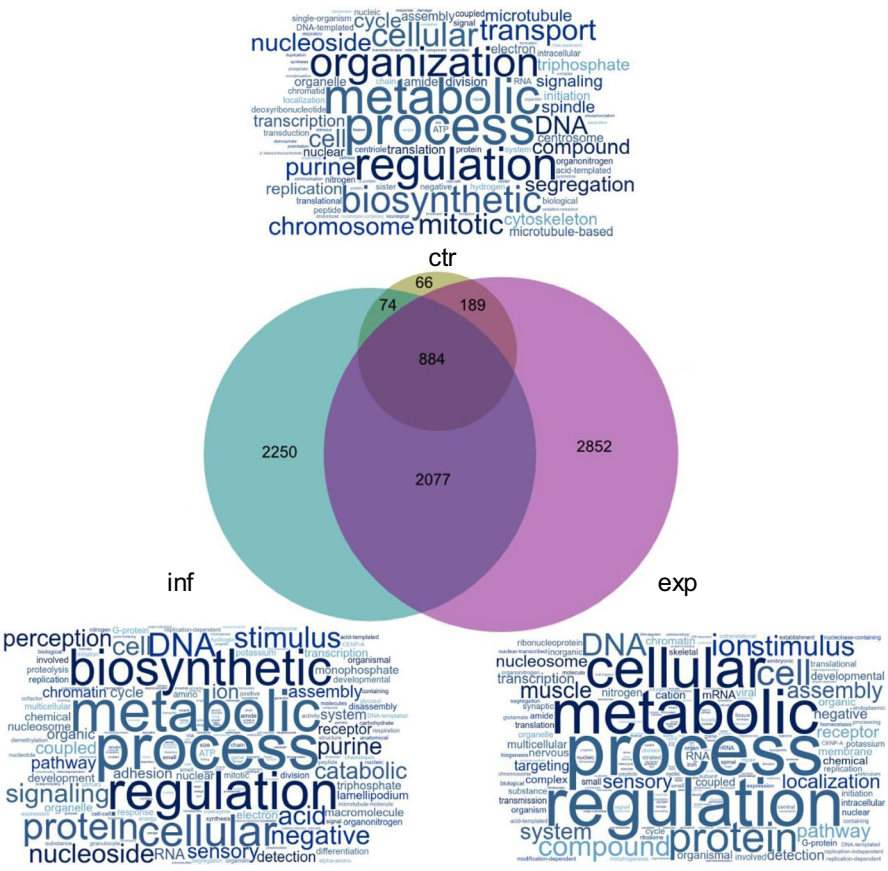

FIGURE 4 Liver transcriptomic responses to temperature and *Schistocephalus solidus*. Venn diagram and tag clouds of differentially expressed genes of the 13°C versus 24°C comparisons of sham-exposed control (ctr), infected (inf) and exposed (exp) but not infected sticklebacks

sham-exposed sticklebacks (Figure 4). Exposed sticklebacks with and without parasite shared only about half of their DEGs (Figure 4). Overall, both temperature change and *S. solidus* infection predominantly changed expression profiles of genes involved in cellular turnover and metabolic processes.

We further focussed on the top 100 most significant DEGs (between 13°C and 24°C) per treatment. Of these, 52 were unique for the sham-exposed controls, 55 for the infected and 40 for the exposed but not infected sticklebacks (Figure S3). In each infection treatment, the majority of the top 100 DEGs were downregulated genes (72 ctr, 76 inf, 87 exp; Figure S4). Functionally, genes involved in general metabolic and mitotic processes dominated the top 100 DEGs in the three infection treatments (Figures S5–S7, see gene list with functional descriptions therein). Occurrences of some DEGs indicated oxidation processes among top 100 DEGs, this was most prominent in livers from infected sticklebacks (marked with yellow in Figures S5–S7).

3.5 | Gene ontology analysis

Gene ontology (GO) analyses revealed that the majority of DEGs in the 13°C versus 24°C comparison in each of the treatments were associated with cellular activity and metabolic processes (Figures 4 and 5). A prominent immune activity was not detected

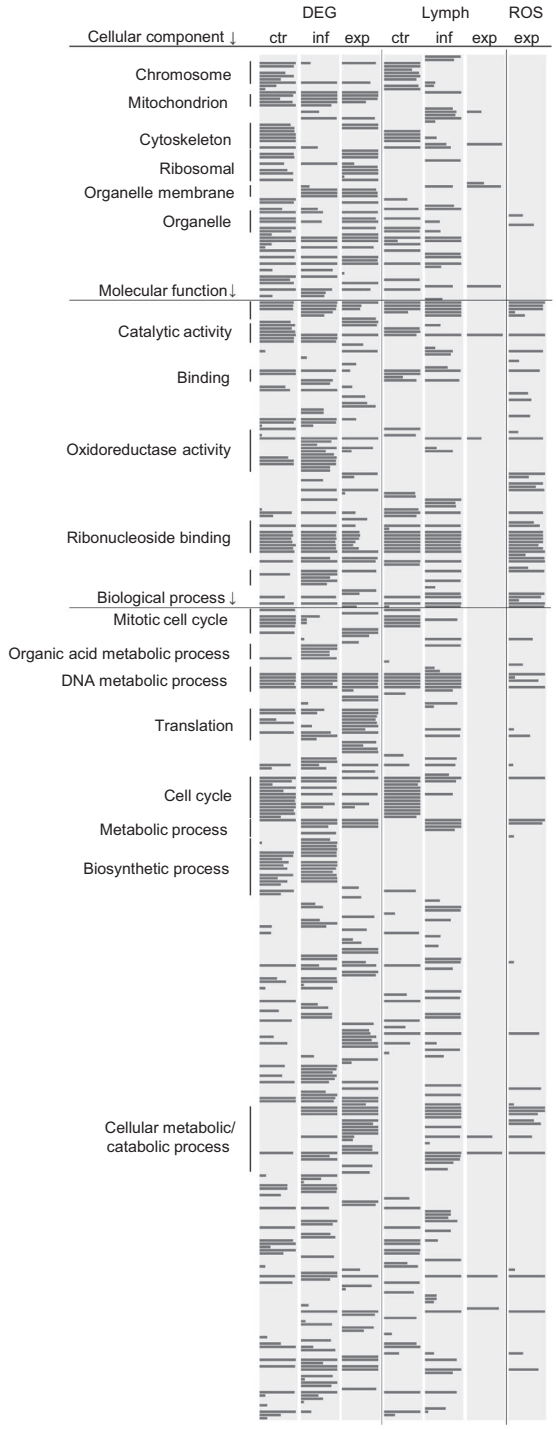

FIGURE 5 Functional categorization of gene ontology (GO) terms. GO terms were computed for differentially expressed genes (DEGs) from the 13°C versus 24°C differentially expressed gene analysis and for the principal component analysis of these DEGs with lymphocyte proliferation (Lymph.) and respiratory burst activity (ROS). Only the GO terms of the most significant principal components (PCs) are shown with *q* (false discovery rate [FDR] adjusted *p* value) <.05. Each bar represents a significant term after GO term enrichment. Bar length corresponds with significance from $p = .049$ to $p < .000001$ with FDR. Bars are grouped by key functions within categories 'cellular component', 'molecular function' and biological process' (ctr = controls, inf = infected, exp = exposed not infected)

by liver transcriptome analysis. Only elevated oxidoreductase activity in infected sticklebacks might be attributed to elevated oxidative burst activity in response to *S. solidus* infection (Figures 4 and 5; see also Figure 3a).

In Figure 5, individual GO terms are presented by treatment. The three left columns summarize the data from the enrichment of DEGs alone. Columns following to the right present GO terms derived from the PCAs of DEGs with the immune parameters 'lymphocyte proliferation' (lymph) and 'production of oxygen radicals' (ROS). The illustration (Figure 5) enables to identify groups of GO terms within and across treatments (ctr, inf, exp) and analysis type (DEGs GO term enrichment and PCAs of DEGs with immune parameters). Accordingly, bars (or groups of bars) significant in the three treatment groups, identify GO terms which are differentially regulated due to temperature differences (Figure 5, e.g. mitochondrion, ribonucleoside binding, DNA metabolic process). Some groups of GO terms were differentially regulated mostly in infected sticklebacks, such as oxidoreductase activity, possibly due to elevated respiratory burst during infection (Figure 5). However, the PCA of DEGs and the functional parameter for respiratory burst (ROS) did not identify significant GO terms in controls and infected sticklebacks and is therefore not presented in Figure 5. Another term, which was dominant in infected sticklebacks, was cofactor binding, possibly also due to elevated immune activity.

Many GO terms that were differentially regulated exclusively in infected sticklebacks represent metabolic processes, presumably a response to the high demand of the parasite for nutrients at elevated temperature. Also, the GO analysis of top 100 DEGs revealed that the majority in each of the treatments was associated with cellular/metabolic processes, differentially regulated immune genes were rather exceptional among the top 100 DEGs (Figures S5–S7).

3.6 | Interrelations between RNA sequencing and immune data

We used PCA and Markov chain Monte Carlo generalized linear mixed models (MCMCglmm) to compare DEGs from livers with lymphocyte proliferation and respiratory burst activity of head-kidney-derived leukocytes. Indeed, lymphocyte proliferation corresponded with several DEGs in the three infection treatments (Figure 5). In particular, lymphocyte proliferation of control and infected sticklebacks was significant with DEGs, commonly regulated in the three infection treatments, thus were presumably caused by temperature difference and not primarily by the parasite infection (e.g. nucleoside binding, ribonucleoside binding and DNA metabolic processes). Cell cycle activity suggested by DEGs in livers of control sticklebacks coincided with lymphocyte proliferation in head kidneys, suggesting a systemic activation of cell proliferation due to temperature increase. For the respiratory burst (ROS), only data from exposed but not infected sticklebacks were correlated with DEGs, in particular with those suggesting ribonucleoside binding (Figure 5).

4 | DISCUSSION

Global warming is expected to strongly impact the metabolism of cold-blooded animals, and potential detrimental effects might be exacerbated under parasite pressure. Here we made use of a vertebrate host—macro-parasite system that allows following host and parasite performance under controlled temperatures and after experimental infection. In the present study, three-spined sticklebacks were experimentally infected with the cestode *S. solidus* and subsequently exposed to high and low temperature. Low temperature, possibly in concert with a more active host immune system, suppressed parasite growth and with that its reproductive capacity. At warmer conditions, the host immune proxies tested here, showed only little responsiveness to the parasite, which drained more nutrients from the accelerated host metabolism.

In central Europe, including the area where the sticklebacks and their sympatric parasites had been collected for the present study, yearly average temperatures increase continuously. In 2020, they were 2°C above the yearlong average (since 1880, German weather service). In mesocosm heated for several months for 2°C above ambient temperature, *S. solidus* were growing bigger as in unheated control mesocosms (Stewart et al., 2018). The present study illustrates that abundant *S. solidus* parasites do not only grow faster, they also produce more eggs and viable offspring under warming conditions. Accordingly, parasite infection pressure will likely increase.

Another mesocosm experiment with sticklebacks and an ectoparasite (*Gyrodactylus* sp.) provided experimental evidence that parasites influence host-mediated effects on ecosystems and, thereby, change the likelihood and strength of eco-evolutionary feedbacks (Brunner et al., 2017). Given the complexity of the live cycle of *S. solidus*, with trophic transmission and behavioural manipulation of its intermediate hosts, rising temperature is likely to change the parasite's impact on its ecosystem.

In the first hosts, planktonic copepods, *S. solidus* at first reduces the copepods activity to hide it from predation. Just when *S. solidus* has grown to infectivity for the next host, it increases the copepods activity, thus increasing the likelihood to be inspected by visual predators, such as sticklebacks (Hammerschmidt et al., 2009). The sticklebacks behaviour is manipulated when parasites have grown to infectivity to the final bird host (Hammerschmidt & Kurtz, 2009). Rising temperature now accelerates the growth of *S. solidus* and consequently reduces the time to reach infectivity to the next host and the behavioural manipulations are triggered earlier.

Taken together, a series of developmental steps in the parasites live cycle are reinforced by rising temperature. This will directly impact the hosts' fitness, indirectly the trophic cascade in the warming habitat will be affected, since there are more parasites which mature faster and trigger more and faster trophic transmissions of their hosts. Accordingly, rising temperature is likely to change the parasites interaction with its environmental niche, and through that reach out to nutritional turnover rates and ecosystem functioning.

In infected sticklebacks, the effect of rising temperature on gene expression in the liver was much more pronounced than in control fish, pointing towards an immunometabolic basis of the interaction of infection and temperature stress. Surprisingly, equally strong effects of temperature on liver gene expression were also observed in exposed, but not infected fish. This observation suggests that the parasite primes its host metabolism upon the first contact. In other words, since the parasite's fitness largely depends on its growth performance in the stickleback host, extracting from the host metabolism and avoiding its immune response might be equally important for *S. solidus*.

The analysis of liver transcriptomes of sticklebacks provided limited evidence for a role of classical indicators of immune activity. This might seem unexpected, since livers of teleost's are immunologically active organs (Bayne & Gerwick, 2001). However, the analysis of DEG renders DEG significant, based on the relative abundance of their transcripts. Thus, possible expression of immune genes might have been overlaid by the more prominent expression response of metabolic genes to temperature and parasite infection. A possible conclusion is that responses of livers of this cold-blooded species to temperature variation and infection are mainly metabolic. However, oxidoreductase activity was differentially regulated most prominently in livers from infected sticklebacks, possibly due to oxidative stress caused by the parasite and high temperature. Comparison of liver transcriptome data with immune data derived from head kidneys revealed correlations between lymphocyte proliferation and differential gene expression. Interestingly, also in control sticklebacks, suggesting that this fundamental trait of immune activity is strongly regulated by temperature alone. Overall, lymphocyte proliferation was mostly correlated with differential regulation of metabolic genes also in infected sticklebacks, which suggests an interaction between the immune response and temperature and parasite-dependent shifts in metabolic activity.

Immune data were derived from head kidneys, a primary immunological organ in teleost fish and correlations with liver gene expression profiles have to be interpreted with caution. Nevertheless, the present data underline that immune and metabolic activity are connected.

At present, interactions between metabolism and the immune system (immunometabolism) are receiving increasing attention in research on human diseases (Boura-Halfon et al., 2019; Caputa et al., 2019; Wang, Luan, et al., 2019).

Immunometabolic research revealed that the activity of the immune system is not possible without the activation of the appropriate metabolic pathways (Wang, Ping, et al., 2019). From a physiological perspective, immunity uses anabolic pathways at the cost of growth and reproduction (Wang, Luan, et al., 2019). Immunometabolic aspects of macro-parasites, such as helminths, have so far received surprisingly little attention, although they can have strong impacts on metabolic tissues (Shea-Donohue et al., 2017).

The present study is an example of a parasite that entirely changes the metabolism of its stickleback host. Under (cold) conditions, which are optimal for the hosts' physiology, the sticklebacks'

immune system seems to have the metabolic support to constrain the parasite while at warmer temperatures *S. solidus* dominates the competition for metabolic resources. In the present study, it is not possible to fully disentangle if temperature alone or the combination with other environmental factors had constrained parasite growth at low temperature. To partially address this point, we have now calculated accumulated thermal units (ATU, or °C days). This showed that, at low temperature, parasites needed relatively more accumulated thermal units to grow, which suggests that low temperature alone is insufficient to fully explain the reduced growth of the parasites. An interesting, but rather unexpected result of the liver transcriptome analysis was that sticklebacks, which defended *S. solidus* (exposed but not infected), exhibited prominent changes in their transcription profiles compared to controls. It is assumed that the clearance of *S. solidus* infection only occurs during the first week(s) of stickleback infection (Scharsack et al., 2007). Accordingly, we did not expect to find prominent transcriptomic responses to the parasite at 50 dpe. About half of the DEGs in exposed and not infected sticklebacks are shared with infected ones. These findings might be explained with initial but persisting activation of differential gene expression by the parasite. Again, the vast majority of these DEGs and the ones not shared between infected and exposed and not infected sticklebacks were metabolic genes and genes needed during cellular turnover, thus provide no evidence towards a long-lasting immune response to the parasite. The functional immune data do not suggest persisting immune activity in sticklebacks, which had defeated the parasite.

A possible explanation for the high numbers of DEGs in exposed and not infected sticklebacks might come from the enormous need of the parasite for nutrients. *S. solidus* must change the metabolism of its stickleback host entirely to provide the nutrients to facilitate the parasites' growth. Possibly, *S. solidus* starts to manipulate the stickleback's metabolism early on after infection, and such initiated manipulation persists, even if the parasite is cleared. We cannot confirm this idea with the present study. However, in sequential *S. solidus* infection, the second intruder was always growing larger than the first (Jager & Schjorring, 2006). The authors explained this observation by the first parasite paying the cost for immune manipulation of the host. An alternative or additional explanation might be that the first parasite (also) paid the cost for manipulation of the stickleback's metabolism, thus providing a laid table (and shelter from an immune response) to the subsequently invading conspecific. This suggests that competition for metabolic resources is a stronger driver in host–parasite coevolution as previously assumed. Consequently, an immune system might have to be seen more as a guard for the allocation of host resources, rather than solely as a defence system to keep parasites from invading their hosts.

A basic assumption was that increased temperature accelerates growth and life cycle completion rates of parasites. Indeed, high mortalities of sticklebacks in a mesocosm experiment during a heat wave were attributed to massive proliferations of infections with parasites (Wegner et al., 2008). Recently, high mortality of fish in the Northern Red Sea was triggered by a rapid onset of warming and the spread of a bacterial infection (*Streptococcus iniea*; Genin et al., 2020). Thus, extreme warming events can have strong effects on infectious diseases in cold-blooded vertebrates. The present study changed temperatures at moderate speed and mortalities of hosts did not occur. However, parasite growth and reproduction increased with temperature, suggesting that parasite proliferation rates may increase with warming and that mortalities may just be the tip of the iceberg, apparent only during extreme warming scenarios. Both temperature extremes and continuous temperature increase seem to benefit parasite proliferation and can be predicted to trigger the co-evolutionary arms race between hosts and parasites (Berkhout et al., 2014).

5 | CONCLUSIONS

Comparison of liver transcriptomes from sticklebacks at low and high temperature revealed a substantial shift in the expression of genes involved in metabolism and cellular turnover. This was much more prominent in *S. solidus* infected sticklebacks, but also in exposed, but not infected ones. Rising temperature alone, and in combinations with *S. solidus*, seems to accelerate metabolic turnover rates of sticklebacks, thus depleting metabolites and preventing their investment in growth and gonad development and immunity. Even if the parasite is lost, it seems to leave a footprint in the host's metabolism, with stronger effects when temperatures rise.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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