Parasite transmission in aquatic ecosystems under temperature change: effects of host activity and elimination of parasite larvae by filter-feeders

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Abstract

A moderate raise in temperature was suggested to enhance the impact of parasites on aquatic ecosystems. Under higher temperatures, poikilothermic animals (e.g. fish) increase their activity, which can result in a more frequent encounter with parasites. However, temperature increase may also trigger processes counteracting an increased risk of parasitic infections. Thus, the removal of free-living stages of parasites by filter-feeding organisms can increase with temperature and potentially mitigate disease risk in ecosystems under climate change. We aimed to study whether an increased infection transmission under higher temperatures can be compensated by the increased removal of parasitic larvae by aquatic predators. In addition, we planned to reveal the behavioral mechanism underlying the more successful transmission of the parasite at higher temperatures. We studied experimentally how temperature, the behavior of fish hosts (rainbow trout), and the presence of filter-feeding mussels in the environment influence the infection success of trematode larvae *Diplostomum pseudospathaceum* cercariae. We found that temperature raise increased while the presence of filter-feeding mussels in the environment decreased infection intensities in fish. However, the effect of mussel's presence was constant within the tested range of water temperatures (15–23ºC), which suggests that it cannot compensate for the observed increased transmission of parasites under temperature raise. Fish activity before the exposure to parasites was a substantial factor affecting the host's vulnerability to infection. However, fish activity only weakly correlated with temperature, therefore, it is unlikely to be the only factor responsible for the increased infection success under warmer conditions. After the exposure, fish activity decreased and did not influence the infection's success. This decrease was temperature-dependent and more pronounced in more active fish. In general, we showed that the elimination of trematode larvae by filter-feeders is unlikely to deter the potential effects of global warming on host–parasite interactions in temperate freshwater ecosystems.

Keywords: *Diplostomum pseudospathaceum*, fish behavior, freshwater mussels, global warming, host–parasite interactions, infection intensity, predation on cercariae, rainbow trout

Introduction

Recent studies suggest that the impact of parasites on aquatic ecosystems can be considerably affected by climate change (Studer et al. 2010, Lõhmus and Björklund 2015, Marcogliese 2016, Cable et al. 2017). Since parasites and their hosts may have different temperature optima (Macnab and Barber 2011, Horky et al. 2019), one of these competing sides may get an advantage in the evolutionary arms race (Dawkins and Krebs 1979) in warmer conditions. In general, though it differs from one host-parasite system to another, a moderate increase in water temperature can enhance transmission of the majority of parasitic species, e.g., by increasing the rate and lengthening the period of larval production, increasing parasites' infectivity, and changing the global distribution of parasites (Harvell et al. 2002, Thielges and Rick 2006, Utaaker and Robertson 2015, Lõhmus and Björklund 2015, Barber et al. 2016, Baker et al. 2018, Mouritsen et al. 2018). In addition, increased temperature causes multiple shifts in biology (growth, behavior, abundance, diversity, etc.) of both their hosts and non-host organisms, eliminating parasites (Macnab and Barber 2011, Lõhmus and Björklund 2015, Brunner and Eizaguirre 2016).

Along with the fish-host immunity suppression caused by the temperature increase (Dittmar et al. 2014), changes in the behavior of receptive hosts is one of the potential mechanisms increasing parasite transmission. For instance, increased activity or ventilation rate (Pritchard et al. 2001, Mikheev et al. 2014, Lõhmus and Björklund 2015) can lead to increased exposure of fish to parasites.

However, the temperature rise may also influence ecosystem processes, which can hamper transmission success of parasites, such as removal of parasite infective stages by natural enemies. Free-living stages of parasites comprise a substantial share of the biomass in aquatic ecosystems (Lafferty et al. 2008, Kuris et al. 2008), and many aquatic organisms eliminate parasitic larvae, thus, significantly reducing infection transmission (Thieltges et al. 2008, Johnson et al. 2010, Welsh et al. 2014, Gopko et al. 2017a). Feeding rates of poikilothermic organisms are strongly temperature-dependent like most metabolic processes (Schmidt-Nielsen 1997). For instance, removal of free-living stages of parasites by filter-feeders is suggested to increase with temperature up to a threshold level determined by their physiological characteristics (Burge et al. 2016). However experimental data about the effect of temperature on the elimination of parasites by aquatic predators are still scarce (Goedknegt et al. 2015) and do not include observations of host behavior. To our knowledge, there is only one study reporting that the presence of filter-feeders (barnacles) eliminating infective parasite stages has a stronger effect on infection transmission at higher temperatures (Goedknegt et al. 2015).

Changes in fish vulnerability to infection caused by temperature increase could be mediated by fish behavior. Under higher temperatures fish can increase their motor or/and ventilation activity that potentially increases exposure rate, thus increasing parasite's chances to penetrate host skin and gills (Mikheev et al. 2014). In addition, individual behavioral variation can also influence host vulnerability to infection. It was suggested that more risky and exploratory individuals (i.e., individuals with higher motor activity) might be at a higher risk of infection compared with shyer ones (Hoverman and Searle 2016, Buck et al. 2018). Though the correlation between animal behavior traits and the parasitic load was suggested in many studies (Hoverman and Searle 2016, Barber et al. 2017, Cable et al. 2017), an influence of individual behavioral differences on vulnerability to infection has rarely been tested experimentally (Behringer et al. 2018, Koprivnikar et al. 2012, Araujo et al. 2016).

A recent study showed that filter-feeding freshwater mussels *Anodonta anatina* can significantly reduce transmission of the fish trematode *Diplostomum pseudospathaceum* by eliminating its free-living stages, cercariae (Gopko et al. 2017a). This parasite is commonly found in limnetic systems of temperate and boreal zones, infects a plethora of fishes, and can impact fish farming (Valtonen and Gibson 1997, Karvonen et al. 2006).

We investigated the effect of temperature and mussels (*A. anatina*) on the transmission of a common fish trematode (eye fluke, *D. pseudospathaceum*) with a focus on potential interactions between these two factors — temperature and presence of mussels — and fish behavior. Our main hypotheses were: (1) fish (*Oncorhynchus mykiss*) will be more vulnerable to parasitic infection under higher temperature due to increased activity of fish that leads to increased encounter rate between parasites and potential hosts; (2) mussels can remove trematode cercariae from the water in a wide range of temperatures and their impact on the reduction of the infection in fish is temperature-dependent (i.e., they can at least partly compensate for increased vulnerability to parasites caused by a temperature rise).

Material and methods

Study objects

Experiments were conducted at the Konnevesi research station (University of Jyväskylä, Finland) in June-August 2017. We used a common fish trematode *D. pseudospathaceum* as the parasite, rainbow trout *O. mykiss* as the host, and freshwater mussels *A. anatina* as filter-feeders eliminating cercariae.

The eye fluke *D. pseudospathaceum* has a 3-host life-cycle: freshwater snails (the first intermediate host), different fishes (the second intermediate host), and fish-eating birds as definitive hosts (Valtonen and

Gibson 1997, Karvonen et al. 2006). In fish, this parasite localizes in the eye lenses and decreases host fitness by impairing vision (Owen et al. 1993, Karvonen et al. 2004) and manipulating host's behavior (Seppälä et al. 2004, Mikheev et al. 2010, Gopko et al. 2015, 2017b). Young-of-the-year rainbow trout (weight range 3.2–15.3 g) were obtained from a commercial fish farm and acclimated in the laboratory at least two weeks before the experiments. At the fish farm, rainbow trout were maintained in groundwater indoors and, therefore, were free of macroparasites. *A. anatina* mussels were collected from Lake Jyväsjärvi and were acclimated at the lab for a week before the experiments. Each mussel was observed to filter actively (siphons protruded) before the start of the experiment. Infected pond snails *Lymnaea stagnalis* collected from Lake Konnevesi were used as a source of *D. pseudospathaceum* cercariae. The shedding of cercariae (trematode larval stage infective for intermediate hosts) was induced by incubation of snails in glasses with filtered lake water under the bright light at room temperature for several hours. Since in Finland (including Lake Konnevesi) *L. stagnalis* is typically infected with *D. pseudospathaceum* rather than other related diplostomidae species (Louhi et al. 2010, Rellstab et al. 2011), cercariae could be identified under the microscope by their morphology.

Experimental design

Fish in our experiments were tested in seven batches (26-28 rainbow trout in each batch). Due to the lack of space in the laboratory batches were tested one after another. The batch ID was included in our statistical models as a random factor (see below). In each batch, fish randomly chosen from the laboratory stock were placed individually in 26-28 white containers (30×40×25 cm) filled with 12L of filtered lake water and were acclimated for an hour before exposure to cercariae. Fish were randomly assigned to four treatments (6-7 replicates in each). During the acclimation period, water in half of the containers was slowly warmed with aquarium heaters, while in another half, similar heaters were placed but switched off. In half of the containers in each heating treatment, we placed live *A. anatina* (one mussel per container), while closed empty *A. anatina* shells served as controls. Empty shells and switched-off heaters were put in containers to minimize the difference in fish behavior between the treatments (Gopko et al. 2017a). Therefore, there were the following treatments: (1) containers with heating

and the presence of live mussel (H+M+), (2) containers with heating and the presence of empty shell, i.e. 'mussel' control (H+M-), (3) containers with switched-off heaters and live mussels (H-M+) and (4) with switched-off heaters and empty shells (H-M-).

All experiments were started at the same time of the day (between 0:30 and 1:30 p.m) to exclude potential effects of the circadian rhythms and lasted two days for each batch (the first day – infection, the second – dissection). In three batches, the temperature in containers with heating was set close to 19.5 °C (mean±SD = 19.6±1.6 °C), while in four others, it was 22.6±1.5 °C. In control containers, the temperature was 16.0±0.7 °C.

These values (around 16 °C) are typical of the surface layer in Lake Konnevesi after wind mixing in summer (mean daily temperature range 11.9–20.0 °C, mean±SE = 16.1±1.2 °C) (Kuha et al. 2016). Thus, the lowest water temperature in our experiment reflected natural conditions in nearshore regions of this lake. These temperatures are also similar to mean summer values in temperate lakes (mean±SE = 16.8±0.5°C), which were calculated using data from the 'laketemps' package (Sharma et al. 2015) (see Supplement, Appendix 1, Methods 1, for details). Therefore, temperatures in containers with heating reflect moderate predictions of water temperature increase (1–5 °C) by the end of the 21st century (IPCC 2014, 2014), being far from the most pessimistic and extreme predictions for temperate lakes in the northern hemisphere (Sharma et al. 2007).

The temperature was measured in each container before the first tracking of fish activity (see below) and at the end of the experiments (after removing fish from containers). It was found that temperature did not change significantly during this period. Temperature values obtained during post-experimental measurements were used in statistical analysis.

However, there was a substantial temperature variation among both controls in different

batches (due to changes in the outside temperature) and heated containers because our heaters cannot be precisely calibrated. Therefore, in statistical analysis, we treated temperature as a continuous predictor; the statistical models, where the temperature was considered as a factor, are presented in the Supplement (Methods 3, Results).

In total, 180 fish were used in the statistical analysis (see, however, *Fish activity tracking* section). Sixteen fish were excluded due to jumping out from containers, death for unknown reasons or obvious signs of sickness. Fish loss never exceeded 3 individuals per test and the resulting numbers of rainbow trout used in all treatments were similar ranging from 43 to 47 fish. Therefore, it is unlikely that an uneven fish loss in different treatments can influence the results of statistical analysis.

Infection protocol and dissections

Fish were exposed to freshly produced *D. pseudospathaceum* cercariae released from five *L. stagnalis* snails in less than 2 h before the exposure. The exposure dose was 300 cercariae per fish, and the exposure time was 2 h.

After each test, rainbow trout were caught and placed individually in 8L flow-through tanks for 24 h to let parasites reach eye lenses of the fish (Lyholt and Buchmann 1996). Then fish were killed with an overdose of MS222, weighed and dissected. The number of *D. pseudospathaceum* metacercariae in the eye lenses of the fish was counted using a dissection microscope (32× magnification).

Fish activity tracking

We video-recorded fish behavior from above the aquaria for 5 minutes before and after exposure to parasites (1 h after the addition of cercariae). A grid (10×10 cm) was drawn on the bottom of each test tank and activity was measured as the number of grid-lines crossed by fish during a 5-minute period. Records were analyzed blindly (i.e. the observer was unaware of the treatment to which an observed fish belonged). Cameras were switched from outside to avoid fish disturbance.

Unfortunately, due to a technical problem, all videos of one of the batches from the mild heating treatment were lost. In addition, several records were excluded from the sample because some containers were partly out of camera range. Therefore, activity video records were obtained only for 142 fish.

Statistical analysis

Influence of environmental conditions and fish weight on the infection intensity.

Linear mixed models were used to estimate the influence of temperature and presence/absence of live mussels in the environment. The practical and widely used strategy to determine which variables should be included in the model is an automatic (e.g., backward) model selection. However, when a set of possible models is large (e.g. when the number of predictors or groups is high), the straightforward implementation of the automatic model selection can turn into data-dredging (Bolker 2007, p. 277, Kuznetsova et al. 2017). To avoid this issue, we considered a more accurate and biologically sensible model of interest, where all variables and the interaction purposefully tested in our study were included first. In the case of non-significant predictors or/and interaction, the model was simplified using backward selection tool from the 'lmerTest' package (Kuznetsova et al. 2017).

The model can be defined as the following: log(infection intensity) ~ fish mass (covariate) + temperature (covariate) + live mussel presence/absence (factor) + temperature*live mussel presence/absence + experiment identity (random factor). We included only this double interaction (temperature*live mussel presence/absence) in our model of interest. The response variable (infection intensity, i.e., the number of *D. pseudospathaceum* metacercariae in fish) was log-transformed to meet model assumptions. We also tested the model including all possible interactions using a similar approach to avoid missing important interactions. The resulted models were similar (see results), which suggests that models with higher-order interactions are unlikely to explain the data substantially better than that obtained with the model of interest simplification.

To account for the influence of fish activity on its vulnerability to parasites, we used an abridged dataset, since recordings of rainbow trout behavior were not available for all fish (see the *Fish activity tracking* section). In this case, fish activity was included in the model of interest. In all other respects, the statistical analysis was similar to the described above. We created two separate sets of models for fish activity before and after exposure to cercariae. P-values were calculated using Kenward–Roger's procedure for the approximation of degrees of freedom implemented in the lmerTest package (Kuznetsova et al. 2017).

Since temperature and fish activity could be potentially correlated (multicollinearity problem), it was unclear whether both predictors should be included in the model. To check for multicollinearity, we calculated variance inflation factors (VIFs) for all predictors in the model. None of the VIFs was higher

than 1.1, which is much lower than the common multicollinearity threshold (VIF = 10) (Dormann et al. 2013). Thus, both the temperature and fish activity were included in the model.

The models, where the temperature was considered a categorical variable (three heating treatments), were also fitted (see the Supplement, Appendix 1, Results). Their results were similar to the ones presented in the main text of the article. To present the results of the mixed-effect models graphically, partial regression plots were drawn (see the details in the Supplement, Appendix 1, Methods 2).

Activity

To check whether environmental conditions influence fish activity before and after the addition of cercariae to the containers, we started with linear mixed models where fish activity and differences in activities before and after exposure to parasites served as response variables, and batch ID was a random factor. The presence of live mussel in the container, temperature, fish mass, and interactions between the variables were components of the full model, which was simplified using a backward selection. However, the addition of the random factor did not appear to explain a substantial amount of variance in these models ($p > 0.3$ in both cases). Therefore, the random effect was deleted from the models, and we proceeded with simple general linear models. The variable 'temperature' was centered by subtracting the mean to make the estimates of regression coefficients more biologically sensible.

All statistical tests were performed using R (R Core Team 2018). A package 'lme4' (Bates et al. 2015) was used to fit linear mixed models and get estimates of regression coefficients. 'ggplot2' (Wickham 2009) and 'sjPlot' (Ludecke 2018) packages were utilized to visualize the data.

Results

Infection intensity

Mean±SE fish weight constituted 7.8±0.2 g (total 180 ind.) and 7.5±0.2 g (142 ind. of the abridged "activity dataset"), range 3.2–15.3 g for both datasets. Fish size did not differ between the treatments (live mussel vs control) both for full and abridged datasets (ANOVA: $F_{1, 178} = 0.13$, $p = 0.72$ and $F_{1, 140} = 0.12$, $p = 0.74$ respectively). Mean±SE infection intensity was 46.8±2.4 in the full and 39.2±2.1 metacercariae per fish in the abridged dataset.

The comparison of nested linear mixed models showed that addition of interaction terms did not lead to significant improvement in the model fit. Importantly, the interaction between temperature and presence

of live mussels was non-significant ($F_{1, 173.1}$ = 1.50, p = 0.22, see also Table 1a). This suggests that the ability of *A. anatina* to eliminate cercariae did not change substantially under the tested temperatures. Moreover, adding one of the main effects to the model (fish mass) also did not significantly increase the amount of variance explained by the model (Table 1a). However, we kept this predictor in the final model since it seems biologically relevant and important. When mass was excluded from the model, p-values related to other predictor variables and the magnitude of estimated coefficients did not change substantially. Therefore, the final model contained only the main effects and batch ID (random effect) as predictors (Table 1a). It showed that the effect of heating was significant, and there was a 1.094-fold (exp(0.09) = 1.094) increase in parasitic load per each additional 1 °C (Table 1a, Fig. 1A, B). The presence of mussels in the environment decreased the *D. pseudospathaceum* infection intensity in fish by ~28% (Table 1a, Fig. 1A, B).

In the set of models, where the fish activity was included, similar results were obtained. Interactions were also not significant and were excluded from the final model. The effect of mass was again non-significant; however, this predictor was left in the model for its biological relevance. The effect of temperature was still highly significant (see table 1b, Fig. A2A, B in the Supplement, Appendix 1). Fish activity before the exposure varied substantially among fish (with range 0-278 and mean±SE = 117.8±4.75 crossed lines/5 min). The effect of fish pre-exposure activity on the infection intensity was significant but relatively week (about a 1.0015-fold increase of infection intensity per each additional gridline crossed by fish) (table 1b). Interestingly, when fish activity after exposure (range 1-155 and mean±SE = 61.4±3.21 lines/5 min) was added to the model instead of pre-exposure activity, it had no significant effect on infection intensity ($t_{132.2}$ = 0.67, p = 0.51). The difference in fish activity before and after exposure to cercariae also was not a significant predictor of infection intensity ($t_{132.5}$ = -1.77, p = 0.14). When the temperature was added as a factorial variable, the results were similar to the above-presented findings (Supplement, Appendix 1, Fig. A1, and Table A1).

Activity

There was only a weak correlation between fish activity before and after exposure (Spearman's rho: r_s = 0.19, p = 0.03). For the pre-exposure activity, we found that the model, where only the temperature was a predictor, fits our data significantly better than the only intercept model ($F_{1, 141}$ = 8.49, p = 0.004). The addition of other predictors and interactions did not explain the significant additional amount of variance. Fish activity increased with the temperature increase (Fig 2A) by an extra four lines per each additional 1

°C (Estimate±SE = 4.27±1.47). However, when the interaction between temperature and presence of live mussel along with both main effects was included in the model there was a non-significant tendency ($F_{1, 139}$ = 3.63, p = 0.059, Fig. 2A) towards a higher increase in activity in the presence of mussels.

For the post-exposure activity, the model including the presence/absence of live mussel in the container, temperature, and interactions of these effects, was found to be the most parsimonious. There was a significant effect of temperature (Estimate±SE = -3.71±1.45, t = -2.57, p = 0.011) and interaction between the temperature and presence of live mussel in the model (Estimate±SE = 5.95±2.00, t = 2.97, p = 0.004 Fig. 2B) on the post-exposure activity. It means that in containers with mussels, fish post-exposure activity increased with temperature (regression coefficients was -3.71 + 5.95 = 2.24), while in containers with empty shells, fish activity even decreased with temperature increase, and the slopes of the regression lines differ significantly between the treatments. Though we found a significant influence of temperature on fish pre- and post-exposure activity, the amount of variance explained by our predictors was fairly small (6% and 7% respectively).

Temperature influenced the degree of activity change after the exposure to cercariae, i.e. pre-exposure activity minus post-exposure activity (Estimate±SE = 4.95±1.67, t = 2.97, p = 0.004, Fig. 3), while the addition of treatment (live mussel/empty shell) and the interaction in the model did not increase the additional amount of variance. Fish changed their activity more under high temperatures compared with low temperatures.

Discussion

Temperature, removal of parasitic larvae by non-host organisms, and host behavior can influence parasite transmission (Lõhmus and Björklund 2015, Barber et al. 2016, Barber et al. 2017, Burge et al. 2016, Welsh et al. 2014). However, their joint effect on transmission success has, to our knowledge, never been tested experimentally.

We found that these factors, taken separately, indeed had a marked influence on the parasite's (common eye fluke *D. pseudospathaceum*) infection success. Infection intensity in fish increased with temperature, while the presence of mussels *A. anatina* in the containers led to lower parasitic load in fish. Importantly, there was no interaction between these two factors, which suggests that the effect of the freshwater mussel on infection transmission is constant at least in the temperature range (15-23 °C) tested in our study. Though the increase in filtration rate with temperature increase was demonstrated at least for several bivalve species under laboratory conditions, the slope of regression curves in these studies was

generally gentle (Riisgård and Seerup 2003, Kittner and Riisgård 2005)□□. A review by Cranford et al. (2011)□□ suggested that, in natural conditions, temperature is unlikely to be an important predictor of feeding rate in mussels. Filtration rates of mussels usually decrease under high temperature close to the upper limit of mussel's physiological tolerance (Ehrich and Harris 2015, Burge et al. 2016)□□. However, water temperatures in our experiment were typical for natural nearshore habitats of *A. anatina* and did not exceed comfort values for this species (Pusch et al. 2001, Falfushynska et al. 2014). Consistent with our results, reduction of trematode transmission by marine bivalves (oysters) was not significantly influenced by temperature; however, the infection mitigation effect of another group of filter-feeders (barnacles) increased with temperature (Goedknegt et al. 2015).

Importantly, though cercariae are effectively removed from the water by *Anodonta* mussels, they are probably transformed into pseudofeces and poorly ingested like the similar-sized filamentous cyanobacteria (Bontes et al. 2007). Ingestion of cercariae by mussels and other filter-feeders is questionable and needs further investigation, while there is no doubt that filter-feeders can effectively eliminate parasite free-living stages and reduce their density in water (Gopko et al. 2017a).

Though *D. pseudospathaceum* cercariae are known to become more infective with temperature (Lyholt and Buchmann 1996)□, the mechanism of this phenomenon is unclear. One of the possible explanations is the increase of fish motor and ventilation activity with temperature rise (Krause and Godin 1995, Pritchard et al. 2001)□, which is likely to increase host-parasite encounter probability (Barber et al. 2016)□. Our results showed that the correlation between fish motor activity and temperature was surprisingly weak. However, enhanced ventilation activity, which we did not measure , may be responsible for higher infection success under warmer temperatures found in our study since infection intensity of *D. pseudospathaceum* correlates positively with ventilation rate of fish (Mikheev et al. 2014). An alternative explanation concerns functioning of fish immune system under increased temperature. Since our heatwave was short-term, it is unlikely to have a strong influence on fish immunity, however, a performance of the innate immunity providing a defense against *D. pseudospathaceum* infection (Scharsack and Kalbe 2014)□ deteriorates under warm conditions almost immediately (Dittmar et al. 2014). Another possible explanation is increased activity or/and metabolism of cercariae in warmer water, which can lead to a decrease in cercariae survival (Pechenik and Fried 1995, Morley et al. 2001) but can simultaneously enhance parasite's infectivity (Poulin 2006)□ presumably due to an increase in parasite's host searching activity and penetration success during a short time period.

Only fish activity before the exposure to parasites positively correlated with the parasitic load in fish, while the icorrelation with post-exposure activity was not significant. In contrast, in a study on tadpoles, no significant relationship between pre-exposure activity and infection intensity was found, while post-exposure activity negatively correlated with parasitic load (Koprivnikar et al. 2012)□□. Previously, a decrease in fish activity was reported as a possible defense against a parasitic threat (Stumbo et al. 2012)□, which could explain why we found less active fish with lower infection intensities.

In our study, we did not manipulate fish activity directly, but investigated the natural activity of fish, which theoretically can be confounded with temperature. However, the weak correlation between fish activity and temperature suggests that in our study these factors influenced the infection intensity in fish almost independently. Nevertheless, an additional experiment with an orthogonal design, i.e., with additional treatment where fish mobility is manipulated, may be needed to confirm our findings.

The presence of mussels in the environment can influence the relationship between temperature and fish activity. Interestingly, in the absence of live mussels, fish post-exposure activity even decreased with temperature increase; However, when mussels were present in the container, a positive relationship between temperature and post-exposure activity was found. It means that in a more risky environment (without mussels filtering cercariae) fish may try to compensate for the increased risk of being infected at higher temperatures by decreasing their activity more radically than in a safer environment with mussels.

It is necessary to stress that our research is likely to reveal only short-term ecological effects of heating within the limits of individual plasticity of studied organisms, while climate change (e.g., global warming) may cause prolonged evolutionary processes. Therefore, extrapolation of our results on a macroecological scale should be done with caution.

In conclusion, in our study, we found that with temperature increase, similar to that predicted for aquatic habitats by the end of this century, fish became more vulnerable to parasitic infection. Though filter-feeders (freshwater mussels) can effectively eliminate cercariae from the water decreasing the parasitic load in fish, this effect remained fairly constant under a relatively broad range of temperatures and is unlikely to compensate for the increased infection risk in fish.

Data accessibility

All data used in the paper are stored in the figshare repository and can be accessed freely (<https://doi.org/10.6084/m9.figshare.8080907>).

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Author contributions – MG and EM contributed equally to this paper. All authors conceived the study. MG and EM conducted the experiments, performed the statistical analysis and wrote the major part of the article. AP, VN, and JT discussed the results of the study, wrote minor passages of the text and revised the manuscript. JT supervised the study.

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Supplementary material (available online as Appendix oik- XXX at <www.oikosjournal.org/appendix/oik- XXX>). Appendix 1.

Figure Legends

Fig. 1. The regression coefficients plot (A) and the partial regression plot (B) showing the influence of the temperature on the infection intensity in rainbow trout for the models fitted on the full (A, B) dataset. The presence of mussels in the container caused a substantial decrease in the infection intensity in fish, while temperature increase led to higher infection intensities. The regression lines for containers with live mussels and control containers are almost parallel confirming the lack of interaction between the temperature and presence of mussels in the environment.

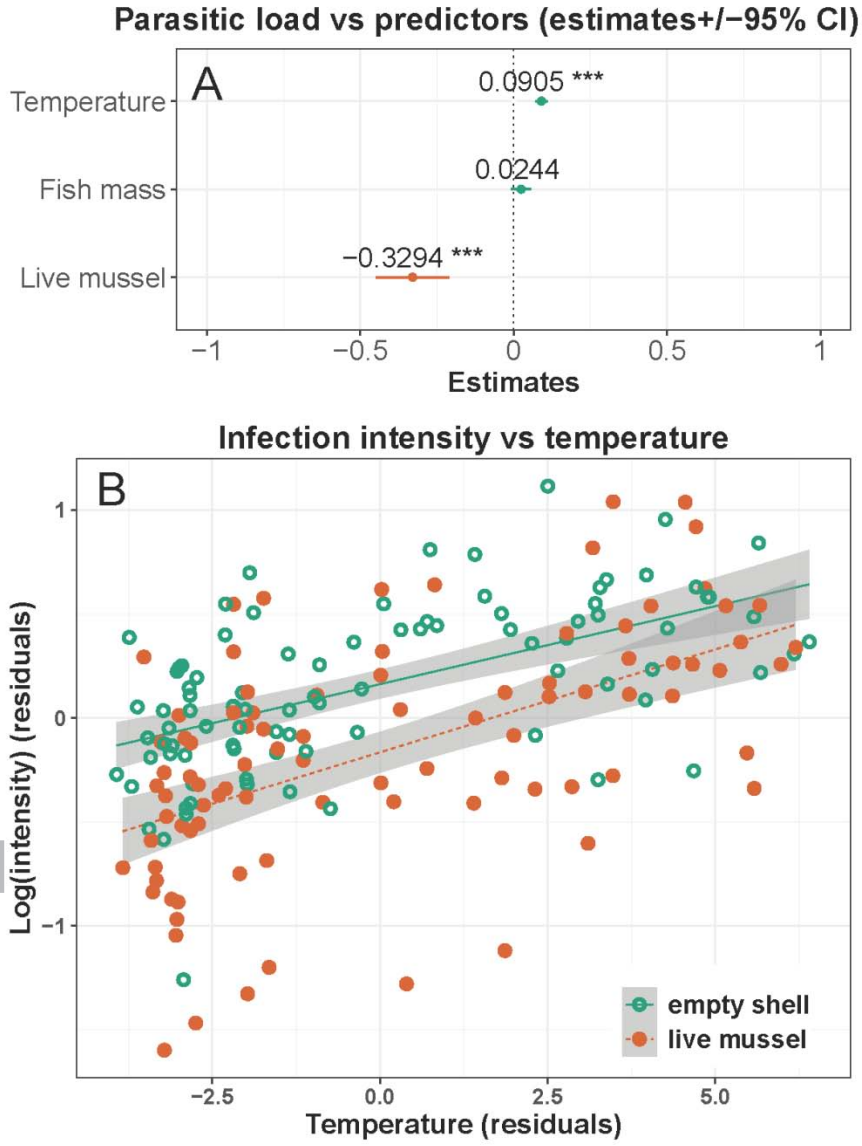

Fig. 2. Rainbow trout pre-exposure (A) and post-exposure (B) activity depending on temperature. (A) Before the exposure to parasites, fish activity increased with temperature both in the presence of the mussel and in the control (empty shell) (Estimate±SE = 4.27±1.47 for the similar slopes model). There was a non-significant trend (p = 0.06), towards a steeper increase in activity in the presence of mussels (dashed line). (B) After exposure, the slopes of regression lines became significantly different. In the presence of mussels, fish increased their activity with increasing temperature, while in the control, fish decreased their activity with temperature rise.

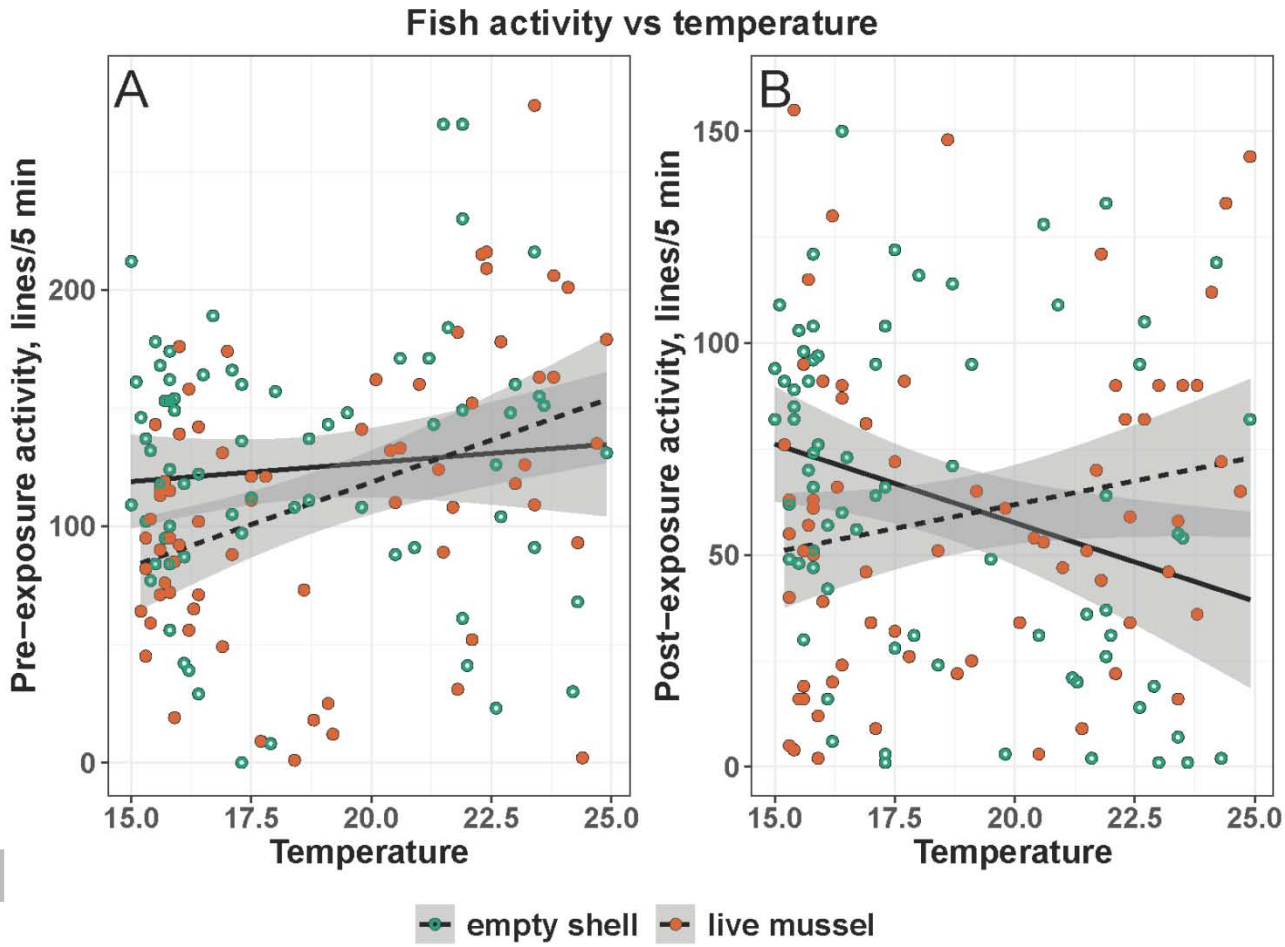

Fig. 3. The decrease in fish activity after the exposure to parasites was temperature-dependent and more prominent at higher than at lower temperatures (extra 4.4 lines crossed per each additional 1ºC). However, this effect was not modified by the presence/absence of mussels (regression lines are almost parallel with overlapping confidence intervals).

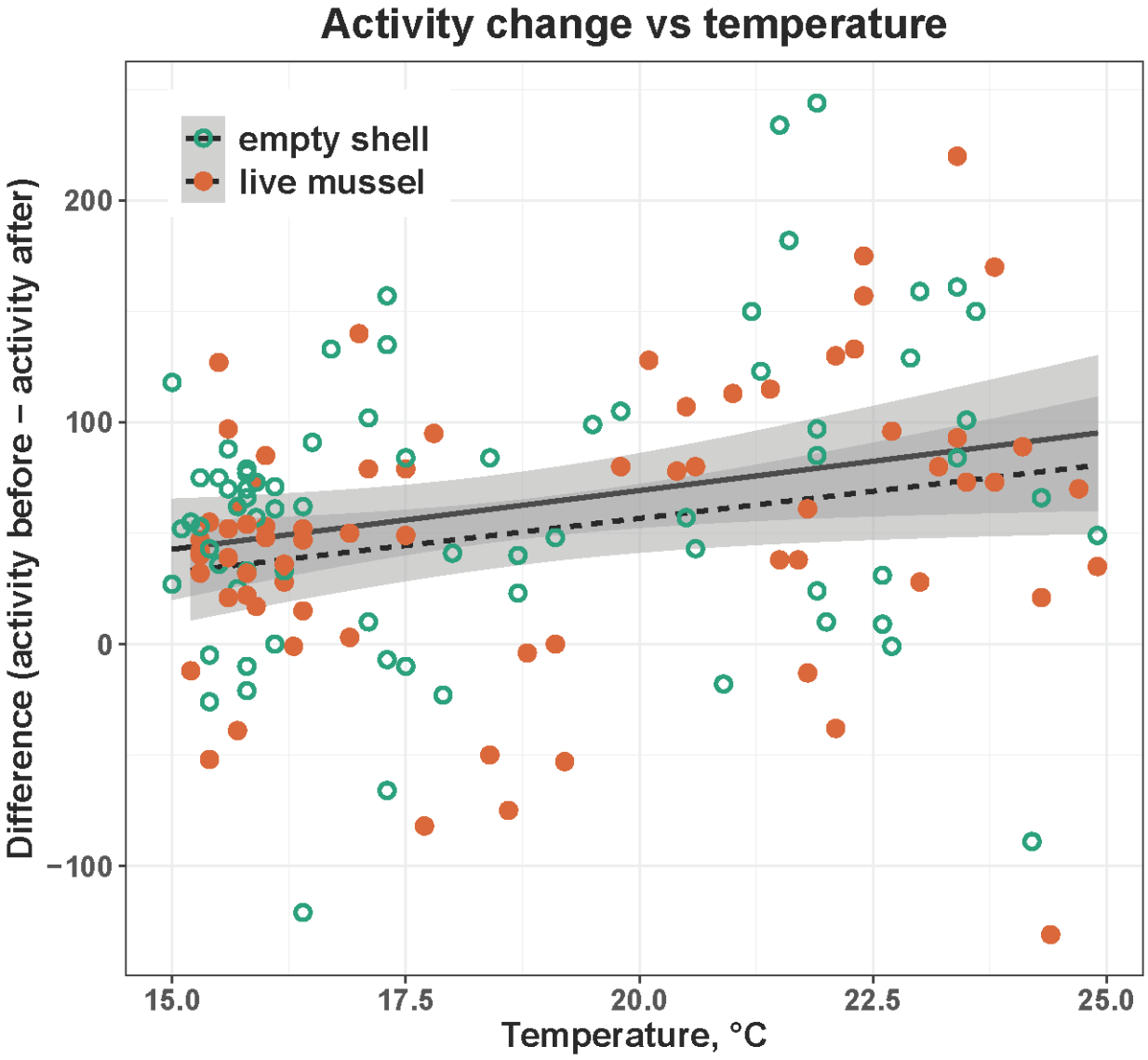

Table Legend

Table 1. GLMM on full and abridged datasets summary tables. Log-transformed infection intensity is a response variable.

	a. Full dataset models					b. Abridged dataset models				
Fixed effects	df	*t*	*p*-value	Est.	SE	df	*t*	*p*-value	Est.	SE
+ temperature	173.8	9.00	<**0.0001**	0.091	0.010	132.1	7.43	<**0.0001**	0.085	0.011
+ live mussel	173.0	-5.46	<**0.0001**	-0.329	0.060	132.0	-4.71	<**0.0001**	-0.327	0.069
+ activity (before)						132.3	2.14	**0.034**	0.0014	0.0007
+ fish mass	173.9	1.50	0.13	0.024	0.016	132.4	0.95	0.35	0.019	0.020