

Gopko, M., Mironova, E., Pasternak, A., Mikheev, V. and Taskinen, J. 2020. Parasite transmission in aquatic ecosystems under temperature change: effects of host activity and elimination of parasite larvae by filter-feeders. – Oikos
doi: 10.1111/oik.07414

Appendix 1

Methods

1. **Mean summer temperatures** in temperate lakes were calculated using data from the ‘laketemps’ package (Sharma et al. 2015). This database contains information concerning summer lake surface temperatures for 291 lakes collected in situ and/or by satellites for the period 1985 – 2009. For our calculations, we used data measured using satellite methods. We considered lakes situated between 40° and 68° latitude as temperate lakes (91 lakes). The mean summer temperatures for each lake over all years of tracking were used for calculations.

Statistical analysis

2. To present the results of the mixed-effect models graphically, **partial regression (=added variable) plots** were drawn. To create them, the dependent variable (log-transformed infection intensity) and the variable of interest (temperature) were regressed against continuous variables (mass and mass + pre-infection activity for full and abridged datasets respectively). Then the residuals from the models were regressed against each other. The influence of the categorical predictor (presence/absence of the mussel in the container) was shown using two types of dots. ‘Filled’ dots represent data points from the treatment with mussels, while ‘empty’ dots are data points from the control treatment. In addition, we plotted models’ estimates with 95% confidence intervals to visualize the magnitude and the range of the regression coefficients.

3. **Influence of environmental conditions and fish phenotype on the infection intensity (when the temperature was considered a factor variable)**. All the details of the analysis were similar to those described in the main text of the paper with only one difference. The biologically sensible model of interest included the temperature predictor turned to a factor as it was initially planned when conceiving the experimental design of the study. The factor has three levels: control temperature, mild heating, and strong heating. In the mild heating treatment, the temperature in containers was

set close to 19.5°C (mean±SD = 19.6±1.6°C), while in the strong heating treatment it was around 22.5°C (22.6±1.5°C). In control containers, the temperature was about 16.0±0.70°C.

The model was the following: log(infection intensity) ~ fish mass (covariate) + temperature (factor) + live mussel presence/absence (factor) + temperature × live mussel presence/absence + pre-exposure activity + experiment identity (random factor). Since we were interested in certain double interaction (temperature × live mussel presence/absence), we included only this interaction in our model of interest. Then, we simplified the model using a backward selection tool from the ‘lmerTest’ package (Kuznetsova et al. 2017). 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). An addition of the interaction of interest ($F_{2,129.0} = 1.14, p = 0.32$; $F_{2,166.0} = 0.89, p = 0.41$) and the fish mass ($F_{1,131.4} = 0.96, p = 0.33$; $F_{1,168.6} = 2.50, p = 0.12$) did not appear to explain substantial amount of variance. However, as it was described in the main text of the article, we decided to leave the mass in the final models, since it seems to be biologically relevant.

Therefore, our final models were the following: log(infection intensity) ~ fish mass (covariate) + temperature (factor) + live mussel presence/absence (factor) + experiment identity (random factor) + pre-exposure activity for the abridged datasets, while for full dataset it was log(infection intensity) ~ fish mass (covariate) + temperature (factor) + live mussel presence/absence (factor) + experiment identity (random factor), i.e. similar but excluding activity, since videos for one of the experiments were accidentally lost.

4. Software used in the analyses

In addition to the R packages mentioned in the main test, we used a 'multcomp' package (Hothorn et al. 2008) to conduct multiple comparisons of means (Tukey contrasts) between the treatments.

Results

Table A1. GLMM on full and abridged datasets summary tables. Log-transformed infection intensity was a response variable.

	Full dataset models					Abridged dataset models				
	df	t	p-value	Est.	SE	df	t	p-value	Est.	SE
Fixed effects										
+ strong heating	170.4	8.23	<0.0001	0.707	0.080	132.9	7.49	<0.0001	0.661	0.088
+ mild heating	170.8	2.45	0.015	0.223	0.092	132.2	0.93	0.36	0.105	0.114
+ live mussel	169.0	-5.02	<0.0001	-0.302	0.060	131.0	-4.46	<0.0001	-0.308	0.069
+ activity (before)							2.21	0.029	0.0015	0.0007

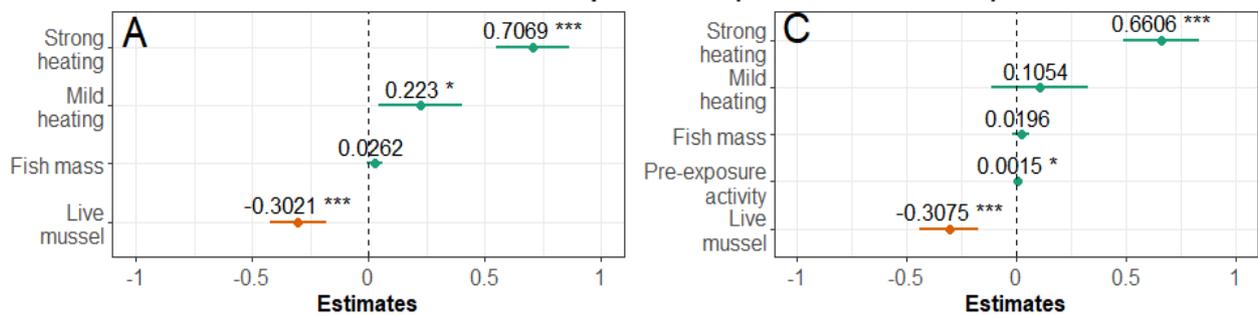
Table A1. GLMM on full and abridged datasets summary tables. Log-transformed infection intensity was a response variable.

+ fish mass 169.6 1.57 0.12 0.026 0.016 131.4 0.98 0.33 0.0196 0.020

Multiple comparisons of means: Tukey contrasts

Linear hypotheses	z-value	p-value	Estimate	SE	z-value	p-value	Estimate	SE
Strong vs control	8.82	<0.001	0.707	0.080	7.51	<0.001	0.661	0.088
Mild vs control	2.45	0.036	0.226	0.092	0.93	0.61	0.105	0.113
Strong vs mild	-3.96	<0.001	-0.482	0.122	-3.91	<0.001	-0.555	0.142

Parasitic load vs predictors (estimates+/-95% CI)



Infection intensity vs heating treatments

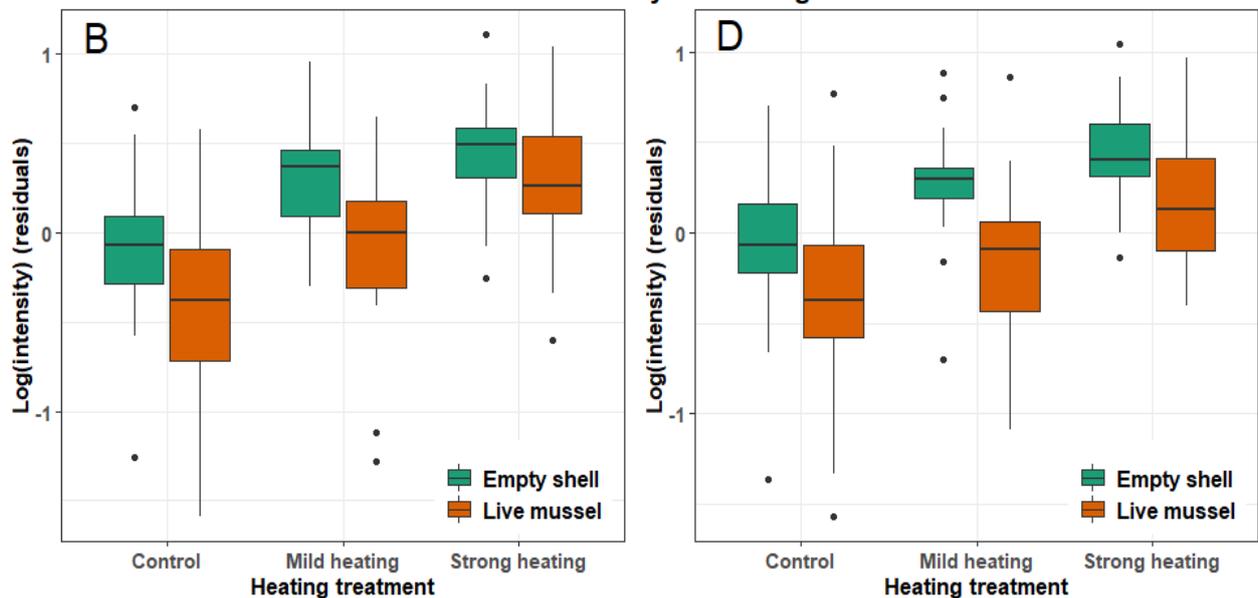


Figure A1. Regression coefficients plots (A, C) and boxplots (B, D) showing the influence of heating treatments on the infection intensity in rainbow trout for the models fitted on the full (A, B) and abridged dataset (C, D). In both cases, the presence of live mussels in the container substantially (26% and 27% respectively) decreased the infection intensity in fish, while temperature increase led to higher infection intensities. In the full dataset both strong and mild heating lead to increased infection intensity in fish (25% and 102% respectively), while in the abridged dataset only the influence of strong heating was substantial (94%), most likely because of

the smaller sample size in mild treatment in the abridged dataset. There was no significant interaction between the temperature and presence of live mussels in the environment.

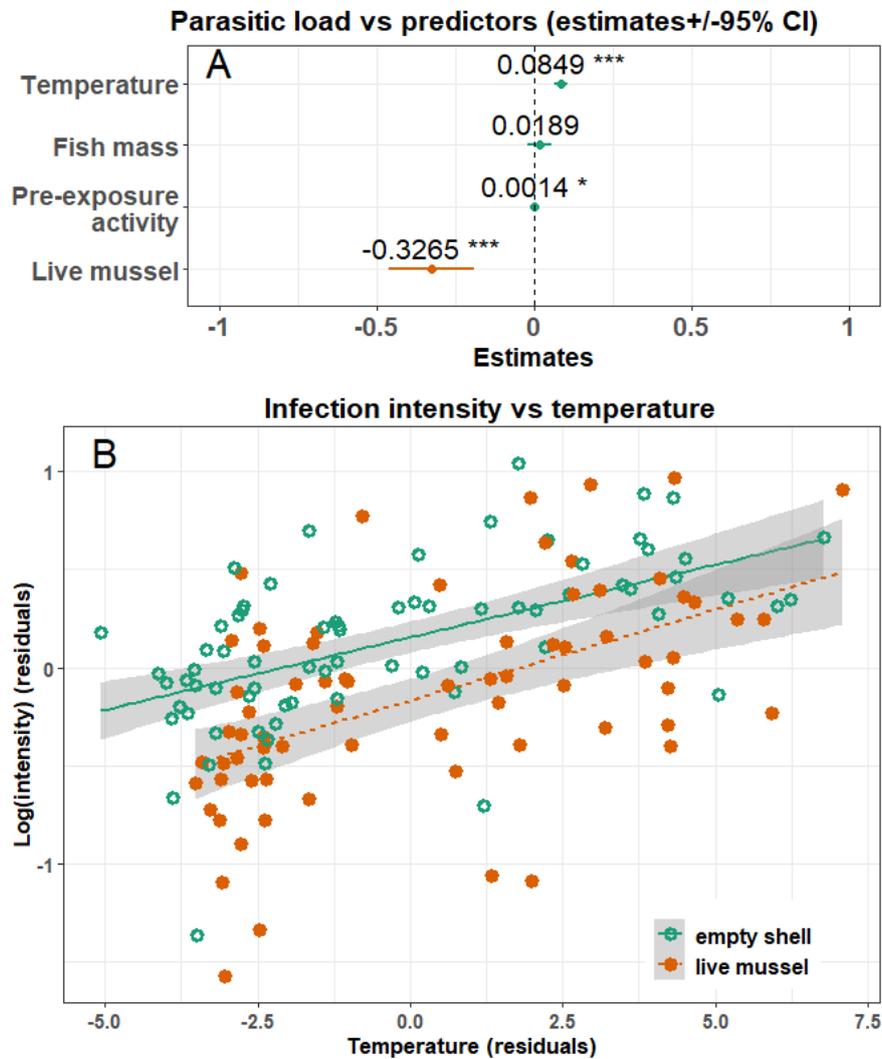


Figure A2. The regression coefficients plot (A) and the partial regression plot (B) showing the influence of the temperature on the infection intensity in rainbow trout for the model fitted on the abridged dataset. The presence of mussels in the container caused a substantial decrease in the infection 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. Fish, which were more active before the exposure, were more infected compared with less active fish (A) (about 15% increase in the infection intensity per 100 additional lines crossed by fish in five min).

References

Hothorn, T. et al. 2008. Simultaneous inference in general parametric models. – *Biometrical J.* 50: 346–363.

Kuznetsova, A. et al. 2017. lmerTest package: tests in linear mixed effects models. – *J. Stat. Softw.* 82: 1–26.

Sharma, S. et al. 2015. A global database of lake surface temperatures collected by in situ and satellite methods from 1985–2009. – *Sci. Data* 2: 150008.