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Published in:
Freshwater Biology

DOI:
[10.1111/fwb.14239](https://doi.org/10.1111/fwb.14239)

Published: 01/03/2024

Document Version
Final published version

Document License
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[Link to publication](#)

Please cite the original version:

Seppälä, O., Katsianis, G., Stabauer, V., Seppälä, K., & Salo, T. (2024). Resource level modifies heatwave responses in the freshwater snail *Lymnaea stagnalis*. *Freshwater Biology*, 69(5), 692-704. <https://doi.org/10.1111/fwb.14239>


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Resource level modifies heatwave responses in the freshwater snail *Lymnaea stagnalis*

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Funding information

Austrian Science Fund

Abstract

1. Predicting climate change-mediated environmental effects on organisms is difficult because their direction and strength may depend on multiple ecological factors that vary in nature. This is because the effects of environmental factors often interact. One potentially important factor modifying organisms' thermal performance is their resource level.
2. We experimentally examined the dependence of phenotypic responses to heatwaves on resource/food availability in the freshwater snail, *Lymnaea stagnalis*. We maintained snails at different food levels (unlimited, reduced [50% of expected consumption], no food), and exposed them to either a heatwave temperature (27°C) or a benign temperature (17°C) for 1 week, followed by a post-heatwave week (17°C). To estimate snail performance, we quantified their metabolic activity (respiration rate), growth rate, fecundity, and immune activity.
3. All examined traits responded to temperature, and changes in fitness-related traits (growth rate, fecundity) depended on snail resource level. During the heatwave phase, the benefits of the elevated temperature (enhanced growth rate and fecundity) were higher with unlimited than with limited resources. Additionally, during the post-heatwave phase, the performance of previously heat-challenged snails reduced most strongly in resource-limited snails. The high-temperature treatment negatively affected snail immune activity even under the high food supply, but immune traits recovered after the heatwave. Interestingly, the phenotypic effects of the high temperature were mainly direct rather than driven by increased metabolic activity.
4. Our results indicate that heatwave responses of *L. stagnalis* snails are sensitive to their resource level. Such condition dependence may have important ecological and evolutionary implications by: (1) making natural populations in low-quality habitats the most susceptible to heatwaves; and (2) altering climate change-mediated evolution by modifying the expression of phenotypic variation in thermal performance. By demonstrating condition-dependent heatwave responses, our study highlights the importance of examining the interactive effects of

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climate change-mediated environmental alterations and ecological factors that vary in nature.

KEYWORDS

climate change, condition dependence, food availability, global change, heat challenge, Mollusca

1 | INTRODUCTION

Global climate change increases average temperatures on the Earth's surface, as well as the frequency and severity of extreme weather events such as summer heatwaves (Christidis et al., 2015; IPCC, 2021; Perkins-Kirkpatrick & Gibson, 2017). The ecological consequences of such changes can be vast (e.g., Flury & Gessner, 2011; Gehman et al., 2018; Mouthon & Daufresne, 2006; Smith et al., 2023) because temperature is one of the most important drivers of organismal performance (reviewed in Angilletta, 2009). Extreme weather events can dramatically influence population dynamics, species abundance, and species interactions in natural systems (reviewed in Easterling et al., 2000; Smith et al., 2023). This is because heatwaves can, for example, induce mortality and deteriorate organisms' reproductive traits both in aquatic and terrestrial habitats (e.g., Breedveld et al., 2023; Ruthrof et al., 2018). These negative effects are probably partly direct and partly mediated by interactions with other organisms such as natural enemies (e.g., increased parasite-induced mortality, Wegner et al., 2008). Further, owing to differences in the sensitivity of taxa to high temperatures, heatwaves alter the structure of natural communities (e.g., Mouthon & Daufresne, 2006; Sun & Arnott, 2022; Whalen et al., 2023).

The effects of elevated temperatures on organisms may, however, be not only harmful but also beneficial (reviewed in Angilletta, 2009). For example, elevated temperatures often increase the metabolic rate of ectotherms (Brown et al., 2004), which can increase their performance, at least temporarily, by enhancing fitness-related traits such as growth rate and fecundity (e.g., Leicht et al., 2013; Stahlschmidt et al., 2022; Van Dievel et al., 2017). Whether the phenotypic effects of high temperatures are positive or negative, as well as the magnitude of the effects, strongly depend on the level of heat challenge organisms are exposed to, revealed by their thermal performance curves (reviewed in Angilletta, 2009). Additionally, the direction and magnitude of the effects of high temperatures could depend on factors that influence the physiological condition (i.e., metabolic, chemical, hormonal, etc. state) of organisms by altering individuals' ability to take advantage of the beneficial effects and/or tolerate the harmful effects of warming. For example, organisms' thermal performance can vary between sexes and depend on interactive effects with other environmental stressors such as pollution (e.g., Breedveld et al., 2023; Salo et al., 2017).

A naturally varying ecological factor with potentially high importance on organisms' thermal performance is resource/food availability (Giebelhausen & Lampert, 2001), as it strongly influences physiological

condition (reviewed in Reznick et al., 2000). For example, low food availability reduces energy-demanding functions such as fecundity, growth rate and immune activity in a broad range of species (e.g., Kirk, 1997; McCue, 2010; Seppälä & Jokela, 2010). Thus, only individuals with high resource levels could: (1) be able to increase their performance at high temperatures; (2) tolerate/repair their negative effects; and/or (3) efficiently recover after a heat challenge. By contrast, individuals with less resources could potentially only experience the negative impacts of high temperatures. Such condition-dependent responses to elevated temperatures could strongly influence within-population inter-individual variation in thermal performance. Additionally, spatial variation in resource availability/habitat quality could make certain populations more susceptible to the adverse effects of climate change.

In this study, we experimentally examined the condition-dependence of heatwave responses in a freshwater snail, *Lymnaea stagnalis* (Heterobranchia: Lymnaeida). In *L. stagnalis*, exposure to elevated temperatures increases snail metabolic activity (Salo et al., 2017), growth rate, and fecundity (Leicht et al., 2013, 2017, 2019; Salo et al., 2017). However, snails can maintain enhanced performance only for a week (Leicht et al., 2013), after which their growth rate, fecundity, and immune activity are reduced (Leicht et al., 2013, 2017, 2019; Salo et al., 2017; Seppälä & Jokela, 2011). Thus, the net effect of elevated temperatures (i.e., the cost-benefit ratio) could depend on snail physiological condition/resource level. We manipulated snail condition using feeding treatments with different levels of food supply (i.e., unlimited, reduced, no food) and exposed them to distinct thermal regimes (heatwave or no heatwave, followed by a post-heatwave recovery phase). If snail heatwave responses were condition-dependent, we expected the benefits of the high-temperature treatment (e.g., on fecundity and growth rate) to be the strongest under unlimited food supply. Respectively, we expected the costs of the high-temperature treatment (e.g., on immune function) to be the highest in snails with reduced food availability. Additionally, we expected the possible adverse post-heatwave effects of the high-temperature treatment to be strongest in snails with limited resources.

2 | METHODS

2.1 | Experimental animals

Lymnaea stagnalis is a pulmonate gastropod that inhabits stagnant and slowly flowing water bodies in the Northern Hemisphere (Fodor et al., 2020). It is widely used to investigate organisms' responses to

heatwaves (e.g., Leicht et al., 2013, 2019; Leicht & Seppälä, 2019; Salo et al., 2017, 2018; Seppälä & Jokela, 2011). In this study, we used snails from a genetically diverse laboratory stock generated by interbreeding snails from different natural populations in northern Switzerland (see Seppälä & Schlegel, 2023). This stock population represents the study species in the region more generally than individual natural populations that are typically genetically highly differentiated (Kopp et al., 2012). The stock population was maintained at $18 \pm 2^\circ\text{C}$ through mass breeding (ad libitum feeding with fresh lettuce and pulverised *Spirulina* for 6 years; 14–20 generations) before the experiment.

2.2 | Experimental design

We conducted the experiment as a two-by-three fully randomised factorial design consisting of two temperature treatments (a constant benign temperature of 17°C [block 1 {mean \pm SD}: $17.22 \pm 0.54^\circ\text{C}$, block 2 {mean \pm SD}: $17.00 \pm 0.74^\circ\text{C}$], a temporary heatwave temperature of 27°C [block 1 {mean \pm SD}: $26.95 \pm 0.17^\circ\text{C}$, block 2 {mean \pm SD}: $27.10 \pm 0.25^\circ\text{C}$]) and three food treatments (unlimited [i.e., ad libitum] food supply, reduced food supply, no food [i.e., fasting]; see Figure S1). We used a total of 120 haphazardly chosen snails (initial shell length: 23.1–28.0 mm; the initial size of the snails did not differ between treatment groups; Table S1) and exposed each snail to the following experimental phases: (1) a 2-day acclimation period during which we maintained the snails at the benign temperature (17°C) and fed them ad libitum with fresh lettuce (see details of snail maintenance in the following three paragraphs); (2) a 3-day pre-heatwave period (i.e., the benign temperature) when we fed the snails according to their feeding treatments (40 snails per food level, feeding treatments continued until the end of the experiment); (3) a 7-day heatwave period during which we exposed half of the snails in each feeding treatment to the heatwave temperature (27°C ; 20 snails per food level) and kept the rest of the snails at the benign temperature; and, finally, (4) a 7-day post heatwave recovery period when we maintained all the snails at the benign temperature. We conducted the experiment in two blocks (each block included all treatment combinations) that started 2 days apart.

We used 17°C as the benign (i.e., control) temperature in the experiment because it is close to the temperature optimum of adult *L. stagnalis* (Salo et al., 2019) and common in ponds in northern Switzerland (T. Salo, personal observation). Similarly, we chose 27°C as the heatwave temperature because it is close to the near-critically high temperature of *L. stagnalis*, alters snail life history traits and immune activity, and occurs intermittently in habitats of snails during hot summers (Salo et al., 2019). We used a 1-week experimental heatwave because it is close to the length of an average lake heatwave globally (7.7 days; Woolway et al., 2021). We maintained the experimental snails at constant temperatures at all phases of the experiment. This was because the performance of *L. stagnalis* is not influenced by diurnal temperature variation at the temperature treatments tested in this study (Salo et al., 2019).

With the ad libitum and no food treatments, we aimed to maximise the variation in the physiological condition/resource level of experimental snails. We chose fasting as one of the treatments because *L. stagnalis* snails can tolerate it well under comparable laboratory conditions (up to 2.7 times longer than the duration of this study; Zonneveld & Kooijman, 1989). In the reduced feeding treatment, our goal was to limit snail resources and performance to levels that were between ad libitum and no food treatments. Thus, we gave each snail 0.11–0.14 g of fresh lettuce each day, corresponding to approximately 50% of their expected daily consumption based on covariation between snail size and lettuce consumption in a separate test (see Supporting Information). Indicated by the results on several examined traits (see Section 3), the goal of intermediate performance was reached with that feeding level. In the reduced feeding treatment, we did not adjust the amount of lettuce at the individual level based on exact snail sizes but used the same amount for all snails. Thus, food reduction reflected quality of the environment at a general level. Because food consumption varies among individuals (Figure S2), the reduced food supply treatment probably challenged some snails more than others. Additionally, because we did not increase the amount of food given in that treatment during the experiment, food limitation may have gotten more severe towards the end of the experiment as the snails grew larger.

During the experiment, we kept the snails individually in plastic cups filled with 0.3 L of filtered lake water and submerged them in 73-L water baths (30 cups per water bath). At the beginning of the heatwave phase of the experiment, we increased the water temperature in half of the water baths from 17°C to 27°C in 7 hr using aquarium heaters. At the end of the heatwave phase, we let the water temperature decrease back to 17°C (the decrease took 20 hr). Similar temperature changes are detected in small natural ponds during heatwaves (Salo et al., 2019). We fed the snails in all feeding treatments daily. Prior to the feeding, we removed the residues of lettuce from the previous day. We kept all snails in 15/9-hr light/dark cycle (a typical summer light/dark cycle in central Europe) and changed the water in each cup every second day to maintain high water quality.

2.3 | Measurements

To estimate the effects of the experimental treatments on the snails' growth rate, we measured their shell length at the beginning of the heatwave phase, at the end of the heatwave phase, and at the end of the recovery phase (Figure S1). We quantified shell length (the longest distance between the apex and the edge of the shell's aperture) to the nearest 0.01 mm using a digital calliper (Duratool, 48–500–150). Using these measurements, we calculated the growth rate of each snail as a change in shell length at each 1-week experimental phase (i.e., heatwave phase, recovery phase).

We estimated snail metabolic activity by measuring their respiration rate both at the end of the heatwave phase and the end of the recovery phase of the experiment (Figure S1). To measure

respiration rate, we placed the snails individually in 140-mL glass containers filled with water from their respective temperature treatment at the time of the measurement. We used magnetic stirrers to mix the water in each container. After 10-min acclimation, we measured the oxygen concentration in each container in 3-s intervals for 15 min using a PreSens OXY-4 SMA (G3) oxygen meter and SP-St3-NAU-D5-YOP oxygen sensor spot (PreSens Precision Sensing GmbH, Regensburg, Germany). We calculated the respiration rate (mg O₂/h) of each snail at each measurement event using a linear regression (see Salo et al., 2017).

We assessed the food consumption of each ad libitum-fed snail during the last 24-hr periods of the heatwave phase and the recovery phase of the experiment (Figure S1). At both times, we quantified food consumption separately in two successive 12-hr periods. In the measurements, we offered each snail a blotted and pre-weighed (to the nearest 0.001 g) piece of fresh lettuce (range: 0.45–0.70 g). At the end of the measurements, we blotted the lettuce pieces and measured their weight again. Because lettuce absorbs water, we measured the weight change in three control pieces submerged in water without a snail at each experimental temperature. We then calculated the food consumption of each snail at each 12-hr period as

$$\text{Food consumption} = W_i \times \frac{W_e}{\frac{\overline{W_{ce}}}{\overline{W_{ci}}}}$$

where W_i and W_e are the initial and end weights of the target piece, and $\overline{W_{ci}}$ and $\overline{W_{ce}}$ are the average initial and end weights of the control pieces, respectively. We summed up the 12-hr food consumption measurements for each snail to estimate their daily consumption rates.

To quantify snail reproductive output during the study, we collected all egg clutches laid by the snails and photographed them under Olympus SZX10 stereomicroscope (Olympus Corp., Tokyo, Japan) using Olympus UC90 digital microscope camera with Olympus cellSens Standard 3.1 software. From the pictures, we counted the eggs (with an embryo) in the clutches using Fiji version 1.53f51. We calculated the reproductive output of each snail during each experimental phase (i.e., heatwave phase, recovery phase) by summing up the number of laid eggs within that time period.

For immunological measurements, we took three haemolymph samples (one per examined immune trait) from each snail at the end of the heatwave phase and at the end of the recovery phase (Figure S1). We stimulated the expulsion of haemolymph by gently tapping the undersides of the snails' feet until they retreated into their shells, simultaneously releasing haemolymph through the hemal pore (Sminia, 1981). This behaviour is a normal antipredatory response in *L. stagnalis* (Rigby & Jokela, 2000). We obtained two samples to quantify the activity of phenoloxidase enzymes in snail haemolymph. Phenoloxidases form a component of oxidative defences in molluscs (Le Clec'h et al., 2016; Luna-Acosta et al., 2010; Quinn et al., 2020) and many other taxa (reviewed in Cerenius & Soderhäll, 2021). We took one sample to quantify haemolymph phenoloxidase (PO)-like activity, a composite

measure reflecting the activity of all PO enzymes (Seppälä & Schlegel, 2023). The other sample was for quantifying haemolymph laccase activity, a family of PO enzymes especially important in molluscs, including *L. stagnalis* (Seppälä & Schlegel, 2023). Finally, we took a third sample to quantify haemolymph antibacterial activity, which reflects snails' ability to destroy microbial cells. We collected 60 µL of haemolymph for each sample into a 1.5-mL reaction tube, snap-froze the samples in liquid nitrogen, and stored them at –80°C until processing. During the recovery phase of the experiment, two snails did not release enough haemolymph for all three immunological assays. We did not quantify PO-like activity from those individuals.

We measured the PO-like, laccase, and antibacterial activity of haemolymph samples spectrophotometrically using Epoch 2 microtiter plate reader (BioTek, Winooski, VT, U.S.A., instrument absorbance measurement range: 0–4 with 0 being completely transparent and 4 non-transparent) following the protocols outlined in Seppälä and Schlegel (2023) for PO-like and laccase activity and Seppälä and Leicht (2013) for antibacterial activity. In short, to measure the PO-like and laccase activity, we mixed haemolymph with substrates L-dopa and *p*-phenylenediamine, respectively, and measured the increase in the absorbance of the solution in milliunits. The reactions are due to the enzymatic oxidation of L-dopa and *p*-phenylenediamine. To measure the antibacterial activity, we mixed haemolymph with lyophilised *Escherichia coli* cells and measured the decrease in absorbance of the solution in milliunits. This reaction is caused by the lysis of bacteria cells by antibacterial factors, and probably reflects the activity of multiple antibacterial peptides and proteins (e.g., macins, lipopolysaccharide-binding/bactericidal permeability-increasing proteins; Seppälä et al., 2021). To estimate the repeatability (*R*) of the used immunological assays, we analysed duplicate haemolymph samples from 24 randomly selected snails per trait. Repeatability describes the proportion of variance in a variable arising from differences among individuals rather than from stochastic variation between samples taken from the same individual. It is calculated from variance components derived from an analysis of variance (ANOVA) using individual as a factor (Krebs, 1989). All immunological assays showed significant repeatability (PO-like activity: $R=0.982$, $F_{23,24}=58.020$, $p<0.001$; laccase activity: $R=0.977$, $F_{23,24}=45.087$, $p<0.001$; antibacterial activity: $R=0.680$, $F_{23,24}=2.213$, $p=0.029$). The difference in the repeatability between oxidative defences and antibacterial activity probably arises from differences in the strength of those responses (i.e., magnitude of absorbance changes), which contributes to the proportion of variance in measurements that noise explains.

2.4 | Statistical analyses

To estimate the effects of experimental factors (temperature, food availability) and block on snail performance, we analysed the variation in the snails' respiration rate, food consumption (ad libitum-fed snails only), growth rate, fecundity and immune parameters

using mixed-model ANOVAs in IBM SPSS Statistics 27. We conducted these analyses separately for the heatwave phase and recovery phase of the experiment. In these models, we included temperature and food availability as fixed factors, and block as a random factor. Additionally, we included an interaction term between temperature and food availability. We used the following transformations to meet the assumptions of ANOVA: respiration rate (both heatwave and recovery phase): $\ln(x+0.1)$; growth rate, heatwave phase: $\ln(x+0.5)$, recovery phase: $\sqrt{x+0.8}$; fecundity, heatwave phase: $\sqrt{x+1}$, recovery phase: $\ln(x+1)$; haemolymph PO-like activity, recovery phase: $\ln(x)$; laccase activity, heatwave phase: $\ln(x)$, recovery phase: x^{-2} ; antibacterial activity, recovery phase: \sqrt{x} . Food consumption and haemolymph PO-like and antibacterial activity during the heatwave phase did not require transformations.

To estimate the causality of responses of different phenotypic traits on experimental treatments (temperature, food availability), we analysed the data with structural equation modelling (SEM) using the package lavaan (Rosseel, 2012) in R. We conducted separate analyses comparing unlimited and reduced food treatments (i.e., unlimited food supply vs. reduced food supply, unlimited food supply vs. no food) at each experimental phase (heatwave phase, recovery phase), resulting in four SEMs. This approach allowed us to test if the effect of food limitation depends on its strength. Note that separate analyses for different food treatment pairs were needed because lavaan allows only two levels for an exogenous categorical variable (Rosseel, 2012). We included all response variables that were measured from all individuals in the starting models. Thus, food consumption that was only assessed in the unlimited food supply was excluded from the analyses. The starting models included all relevant links between variables based on the existing knowledge of their phenotypic responses to environmental variation and relationships among them. The model fit estimations were based on Chi-square, comparative fit index, root mean square error approximation and standardised root mean square residual, and model selection on Akaike information criterion and parsimony (Grace, 2006). We calculated standardised regression weights and squared multiple correlations (R^2) for each path and endogenous variable. The R^2 summarises the direct and indirect effects of treatments on different traits. Because several variables had non-normal distributions, we used the transformations described above.

3 | RESULTS

During the heatwave phase of the experiment, snails exposed to the high-temperature (27°C) treatment showed an increased respiration

rate compared to snails maintained at the benign temperature (17°C; Figure 1a, Table 1). This indicates higher metabolic activity at the elevated temperature. The respiration rate of snails also increased with the increasing food availability, but this effect was partly temperature-dependent (Figure 1a, Table 1). Specifically, the respiration rate of fasted snails increased more at the high-temperature treatment compared to snails in other feeding treatments (Figure 1a). After the heatwave (i.e., the recovery phase of the experiment), the respiration rate of snails depended only on the amount of provided food (Figure 1a, Table 2). In the group of snails with unlimited food supply, food consumption did not differ between temperature treatments in either phase of the experiment (i.e., heatwave phase, recovery phase; Figure 1b, Tables 1 and 2).

During the heatwave phase of the study, exposure to the high-temperature treatment increased snail growth rate and fecundity (Figure 1c,d, Table 1). However, this effect depended on food availability, the positive effects of the high temperature being the strongest when resources were abundant (Figure 1c,d, Table 1). This effect was especially clear for snail growth rate, which increased only a little in individuals maintained under no food or reduced food supply but threefold in snails fed ad libitum (Figure 1c). The interactive effect of temperature and food availability on snail fecundity was similar, although weaker (Figure 1d). During the recovery phase of the experiment, snails with unlimited food supply that had earlier been exposed to the high temperature grew equally fast to snails maintained at the benign temperature throughout the study (Figure 1c, Table 2). However, heat-challenged snails in the other food treatments (reduced food supply, no food) did not grow during the recovery phase (Figure 1c, Table 2). Additionally, snail fecundity during the recovery phase was negatively affected by the earlier exposure to the high temperature, independently of the feeding treatment (Figure 1d, Table 2).

During the heatwave phase of the experiment, the high-temperature treatment reduced snail immune activity (Figure 1e–g, Table 1). Also, food limitation negatively affected immune traits, especially at the benign temperature (Figure 1e–g, Table 1). At the high-temperature treatment, snails' haemolymph antibacterial and laccase activity were relatively low at all feeding treatments compared with the highest levels observed in fed snails at the benign temperature. However, the negative effects of the high temperature on snail immune activity were no longer visible during the post-heatwave recovery period, food availability being the only factor influencing immune traits (Figure 1e–g, Table 2).

All final SEMs had a good fit (χ^2 : $p=0.13$ – 0.26 , root mean square error approximation = 0.06 – 0.09 , standardised root mean square residual = 0.04 – 0.07 , comparative fit index = 0.98 – 0.99 for all). During the heatwave phase of the study, temperature and food availability influenced snail phenotype both directly and indirectly (Figure 2a,b).

FIGURE 1 (a) Respiration rate (mg O₂/h), (b) food consumption (g/day), (c) growth rate (mm/week), (d) fecundity (number of produced eggs), (e) haemolymph antibacterial activity, (f) haemolymph phenoloxidase (PO)-like activity, and (g) haemolymph laccase activity (back-transformed least squares means \pm SE for all) of *Lymnaea stagnalis* snails exposed to different temperatures (17°C, 27°C) at different levels of food availability (unlimited food supply, reduced food supply [50% of expected consumption], no food [exception: food consumption was measured only under unlimited food supply]) at the end of different experimental phases (heatwave phase, post-heatwave phase) of the study.

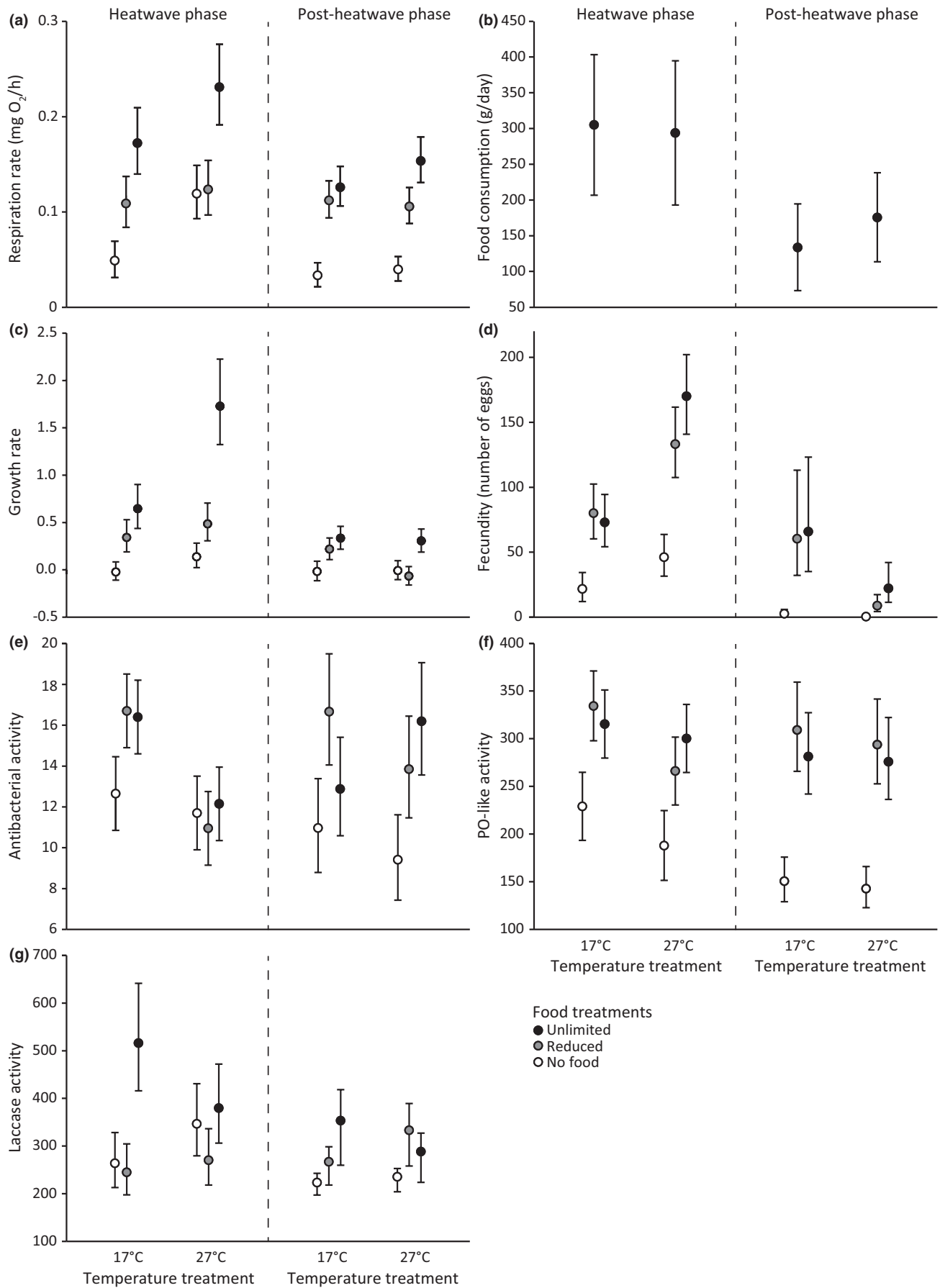


TABLE 1 Mixed-model analyses of variance for the respiration rate, food consumption, growth rate, fecundity, and immune activity (antibacterial activity, phenoloxidase [PO]-like activity and laccase activity of haemolymph) of *Lymnaea stagnalis* snails by water temperature (17°C, 27°C), food availability (unlimited food supply, reduced food supply, no food), block (two blocks), and their relevant interactions during the heatwave phase of the experiment.

| Trait | Effect | df | MS | F | p |
|--|-----------------------|-----|-------------|--------|--------|
| Respiration rate | Temperature (T) | 1 | 1.403 | 16.958 | <0.001 |
| | Food availability (F) | 2 | 2.651 | 32.043 | <0.001 |
| | T×F | 2 | 0.255 | 3.086 | 0.050 |
| | Block | 1 | 0.013 | 0.157 | 0.693 |
| | Error | 113 | 0.083 | | |
| Food consumption (unlimited food supply) | Temperature (T) | 1 | 1224.870 | 0.026 | 0.873 |
| | Block | 1 | 169.642 | 0.004 | 0.952 |
| | Error | 36 | 46,941.451 | | |
| Growth rate | Temperature (T) | 1 | 4.131 | 20.114 | <0.001 |
| | Food availability (F) | 2 | 11.291 | 54.979 | <0.001 |
| | T×F | 2 | 0.692 | 3.371 | 0.038 |
| | Block | 1 | 5.072 | 24.697 | <0.001 |
| | Error | 113 | 0.205 | | |
| Fecundity | Temperature (T) | 1 | 279.246 | 40.060 | <0.001 |
| | Food availability (F) | 2 | 302.702 | 43.425 | <0.001 |
| | T×F | 2 | 15.777 | 2.263 | 0.109 |
| | Block | 1 | 29.663 | 4.255 | 0.041 |
| | Error | 113 | 6.971 | | |
| Antibacterial activity | Temperature (T) | 1 | 399.675 | 24.128 | <0.001 |
| | Food availability (F) | 2 | 48.900 | 2.952 | 0.056 |
| | T×F | 2 | 60.300 | 3.640 | 0.029 |
| | Block | 1 | 49.408 | 2.983 | 0.087 |
| | Error | 113 | 16.565 | | |
| PO-like activity | Temperature (T) | 1 | 50,879.768 | 7.834 | 0.006 |
| | Food availability (F) | 2 | 119,699.802 | 18.431 | <0.001 |
| | T×F | 2 | 7,013.879 | 1.080 | 0.343 |
| | Block | 1 | 4,336.922 | 0.668 | 0.416 |
| | Error | 111 | 6,494.582 | | |
| Laccase activity | Temperature (T) | 1 | 0.014 | 0.058 | 0.810 |
| | Food availability (F) | 2 | 3.099 | 12.971 | <0.001 |
| | T×F | 2 | 0.882 | 3.691 | 0.028 |
| | Block | 1 | 0.230 | 0.964 | 0.328 |
| | Error | 113 | 0.239 | | |

The negative effects of the high-temperature treatment were always direct, while the positive effects were partly mediated by metabolic activity (i.e., respiration rate). Unlimited food supply enhanced phenotypic traits both directly and through increased metabolic activity (Figure 2a,b). These general patterns were consistent, independent of the strength of the food limitation (i.e., reduced food supply, no food). During the recovery phase of the experiment, the negative effects of the previously experienced heatwave on snails were again direct (Figure 2c,d). Similarly to the heatwave phase of the study, the effects of food availability were partly direct and partly mediated by snail metabolism. However, compared to the heatwave phase of

the study, the comparison between unlimited food supply and fasting showed a stronger direct effect on fecundity (Figure 2d). This suggests resource limitation in the no-food treatment getting more severe towards the end of the study.

4 | DISCUSSION

Climate change-induced extreme weather events such as summer heatwaves often negatively influence population dynamics, species abundance, and species interactions in nature (e.g., Easterling

TABLE 2 Mixed-model analyses of variance for the respiration rate, food consumption, growth rate, fecundity, and immune activity (antibacterial activity, phenoloxidase [PO]-like activity and laccase activity of haemolymph) of *Lymnaea stagnalis* snails by earlier water temperature (17°C, 27°C), food availability (unlimited food supply, reduced food supply, no food), block (two blocks) and their relevant interactions during the recovery phase of the experiment (i.e., post heatwave).

| Trait | Effect | df | MS | F | p |
|--|-----------------------|-----|------------|--------|--------|
| Respiration rate | Temperature (T) | 1 | 0.055 | 1.295 | 0.258 |
| | Food availability (F) | 2 | 3.348 | 78.301 | <0.001 |
| | T×F | 2 | 0.053 | 1.232 | 0.296 |
| | Block | 1 | 0.334 | 7.810 | 0.006 |
| | Error | 111 | 0.043 | | |
| Food consumption (unlimited food supply) | Temperature (T) | 1 | 17,154.575 | 0.960 | 0.334 |
| | Block | 1 | 9257.194 | 0.518 | 0.476 |
| | Error | 36 | 17,871.186 | | |
| Growth rate | Temperature (T) | 1 | 0.086 | 5.248 | 0.024 |
| | Food availability (F) | 2 | 0.303 | 18.602 | <0.001 |
| | T×F | 2 | 0.074 | 4.545 | 0.013 |
| | Block | 1 | 8.584E-10 | <0.001 | 1.000 |
| | Error | 111 | 0.016 | | |
| Fecundity | Temperature (T) | 1 | 48.958 | 25.141 | <0.001 |
| | Food availability (F) | 2 | 92.157 | 47.324 | <0.001 |
| | T×F | 2 | 2.271 | 1.166 | 0.315 |
| | Block | 1 | 0.567 | 0.291 | 0.591 |
| | Error | 113 | 1.947 | | |
| Antibacterial activity | Temperature (T) | 1 | 0.086 | 0.161 | 0.689 |
| | Food availability (F) | 2 | 5.415 | 10.158 | <0.001 |
| | T×F | 2 | 1.691 | 3.171 | 0.046 |
| | Block | 1 | 9.071 | 17.016 | <0.001 |
| | Error | 111 | 0.533 | | |
| PO-like activity | Temperature (T) | 1 | 0.050 | 0.431 | 0.513 |
| | Food availability (F) | 2 | 6.127 | 52.747 | <0.001 |
| | T×F | 2 | 0.003 | 0.030 | 0.971 |
| | Block | 1 | 0.141 | 1.217 | 0.272 |
| | Error | 111 | 0.116 | | |
| Laccase activity | Temperature (T) | 1 | 3.974E-11 | 0.538 | 0.465 |
| | Food availability (F) | 2 | 8.591E-10 | 11.641 | <0.001 |
| | T×F | 2 | 1.681E-10 | 2.277 | 0.107 |
| | Block | 1 | 6.350E-11 | 0.860 | 0.356 |
| | Error | 111 | 7.380E-11 | | |

et al., 2000; Mouthon & Daufresne, 2006; Ruthrof et al., 2018; Smith et al., 2023). However, the effects of elevated temperatures on organisms' performance (e.g., growth rate, fecundity; Leicht et al., 2013; Stahlschmidt et al., 2022; Van Dievel et al., 2017) could vary in their direction and magnitude depending on other factors that influence the physiological condition of organisms. In this study, we found both positive (growth rate, fecundity) and negative (immune activity) effects of the elevated temperature on the fitness-related traits of the freshwater snail *L. stagnalis*. However, the observed positive effects were condition-dependent, with the increase in growth rate and fecundity being strongest in snails with unlimited food supply.

Additionally, the growth rate and fecundity of heat-challenged snails were reduced post-heatwave (snail immune activity recovered to the level of unchallenged snails within 1 week). Regarding growth rate, the post-heatwave effect was condition-dependent, with only ad libitum-fed snails showing positive growth. These findings suggest that both the immediate and post-heatwave effects of exposure to high ambient temperature can be context-dependent, and especially the positive effects of the high temperature are likely to occur only when individuals are in high physiological condition.

Our findings on *L. stagnalis* heatwave responses (i.e., increased growth rate and fecundity, reduced immune activity) largely align

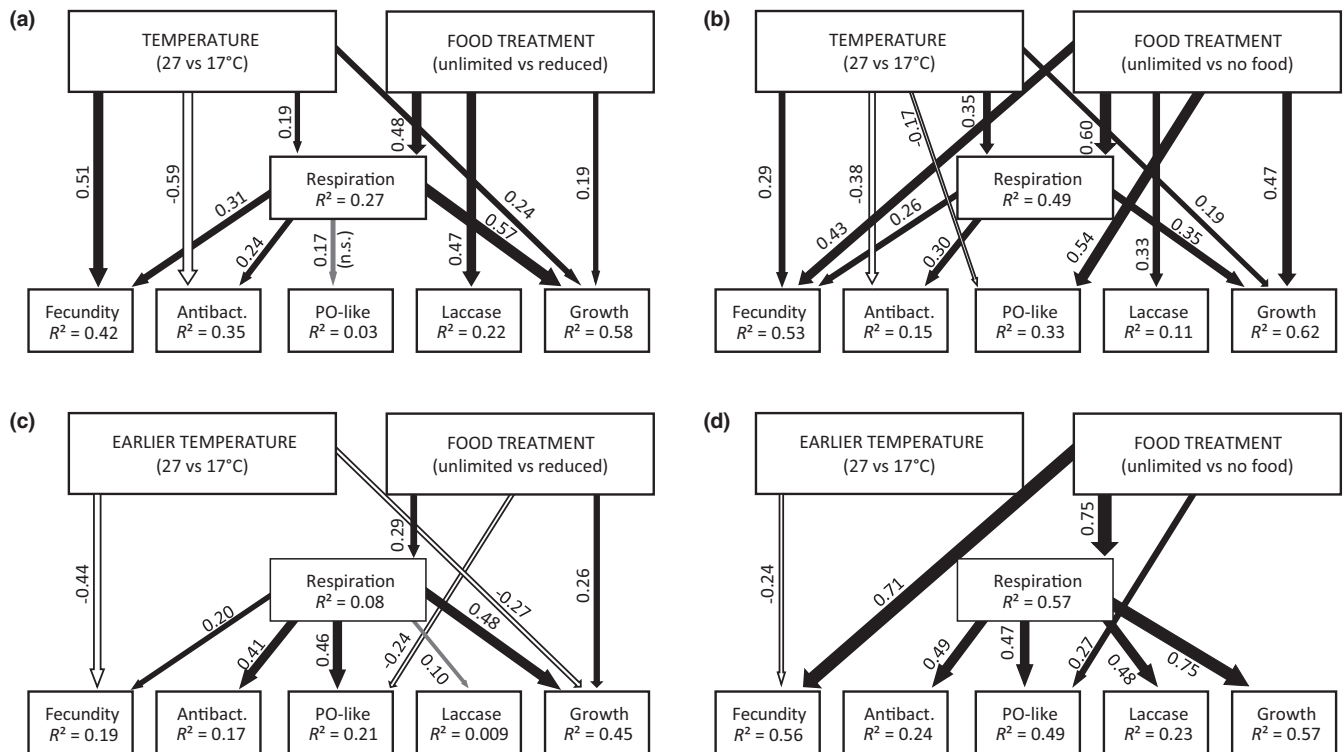


FIGURE 2 Structural equation models for snail respiration rate, growth rate, fecundity, phenoloxidase (PO)-like activity, laccase activity and antibacterial activity during (a, b) the heatwave and (c, d) recovery phases of the experiment when (a, c) unlimited and reduced food supplies and (b, d) unlimited food supply and fasting are compared. Black and white arrows indicate positive and negative relationships, respectively. The arrow's thickness indicates the strength of the relationship, and the number next to the arrow shows the standardised correlation coefficient. R² summarises the treatment effects on each trait.

with earlier studies on this species (Leicht et al., 2013, 2017, 2019; Salo et al., 2017, 2018, 2019; Seppälä & Jokela, 2011), but also show some interesting differences. For example, in an earlier study in which heat-challenged snails were fed ad libitum with lettuce (Salo et al., 2017), snail growth rate and fecundity recovered quickly after the heatwave. The stronger post-heatwave effect in the present study could be due to the higher heatwave temperature (27°C) that was closer to the critically high temperature of snails (30–33°C, Salo et al., 2019) compared to 24°C used in Salo et al. (2017). Additionally, in another study in which snails were fed with food types snails have access to in nature (i.e., aquatic macrophytes, leaf litter), snails showed lower performance in general, and the fecundity of heat-challenged (24°C) snails was reduced post-heatwave (Salo et al., 2018). Together, the present and the earlier studies by Salo et al. (2017, 2018) suggest that the ability of snails to recover from heatwaves probably depends on their resource level and the level of heat challenge the snails experience. It is, however, important to note that the present study investigated the condition dependence of snail heatwave responses by manipulating only the quantity of resources for snails. As suggested by the studies by Salo et al. (2017, 2018), variation in resource quality could also be important. Because wild snails consume a broad range of different food types ranging from microalgae and plant detritus to animal corpses (Doi et al., 2010; Elger et al., 2004; Zhang et al., 2018), and snail performance depends on, for example, the

protein content of consumed food (Li et al., 2018), examining the role of variation in resource quality on heatwave responses would be relevant in future studies. However, such experiments might be technically challenging because snails tend to consume different amounts of different food types (Salo et al., 2018), which may confound the observed results.

The above effects of the high temperature on the fitness-related traits of snails could arise from alterations in organismal metabolism and energetic balance. Similarly to many other ectotherms (Brown et al., 2004), snail metabolic activity (i.e., respiration rate) increases with the ambient temperature (see also Salo et al., 2017), which could explain their increased growth rate and fecundity during the heatwave. However, the foraging rate of snails under the unlimited food supply did not differ between the temperature treatments (see also Salo et al., 2017). This suggests that snails could not increase their resource intake despite the elevated metabolic activity during the heatwave, leading to a metabolic imbalance. Such an imbalance also probably occurs in other organisms, although some species increase resource consumption at high temperatures (Lemoine et al., 2013; Van Dievel et al., 2017). This is because the increase in food intake is typically lower than the increase in organismal metabolic rate (e.g., Lindmark et al., 2022; Vucic-Pestic et al., 2011). When exposure to high temperatures leads to metabolic imbalance, trade-offs among phenotypic traits (Reznick et al., 2000; Van Noordwijk & Dejong, 1986) should always lead to some negative phenotypic

effects, even if certain traits are positively affected. In *L. stagnalis*, reduction in immune activity during the heatwave and lower growth rate and fecundity post-heatwave compared to controls suggest such costs (see also Lemoine & Burkepile, 2012; Rall et al., 2010). However, while the structural equation modelling indicated both direct and metabolism-mediated effects of the high temperature on snails, the effect of temperature on respiration rate was modest compared to its direct effects on snail phenotypes. This finding also suggests that mechanisms other than the metabolic imbalance are important in determining heatwave responses in *L. stagnalis*. Such mechanisms are currently unknown but could include, for example, biochemical and transcriptional changes at the molecular level (e.g., Bernal et al., 2020; Seppälä et al., 2021; Zinta et al., 2014).

The condition-dependence of organisms' heatwave responses could have important ecological consequences for their natural populations. In general, resource/food availability varies both spatially and temporally in diverse aquatic and terrestrial environments (e.g., Jankowski et al., 2014; Wright et al., 2015). This is also the case for lakes and ponds inhabited by *L. stagnalis* (e.g., Sayer et al., 2010; Wondie et al., 2007), although, to our knowledge, how often snails experience food limitation in nature has not been examined. Owing to variation in resource level, populations in habitats with abundant resources (e.g., primary production due to high nutrient levels) could benefit the most from (short-term) heatwaves. For example, in *L. stagnalis*, increased fitness in terms of fecundity and growth rate (larger snails lay more eggs; Koene et al., 2007) when subjected to elevated temperatures could increase population sizes. Additionally, populations with high resource levels should be the best at avoiding the negative effects of high temperatures. Instead, populations in resource-poor environments could be the most susceptible to heatwave-induced population bottlenecks or even extinctions (Binzer et al., 2016). Furthermore, the time of the season when a heatwave occurs could be important in determining its effects on natural populations (Huber et al., 2010). Heatwaves in Europe are most likely to occur in the mid and late summer when resources for, for example, aquatic grazers are the most abundant (Sayer et al., 2010; Wondie et al., 2007). Thus, our results suggest that heatwaves that occur early or late in the season could be most harmful to them. In our study system, this is because of smaller positive effects during the heatwave and larger negative effects post-heatwave. However, the negative effects of high temperatures on snail immune activity could have the strongest epidemiological effects in populations with abundant resources. This is because snails could not maintain two of the examined immune traits at high levels when exposed to 27°C at any feeding treatments, the levels being close to those of fasted individuals at the benign temperature.

In addition to its potential ecological effects, the condition-dependence of organisms' heatwave responses could influence evolutionary processes relevant to the ability of natural populations to adapt to climate change. This is because both the form and strength of selection (Monro et al., 2010; Svanfeldt et al., 2018) and the expression of genetic variation (i.e., evolutionary potential) in phenotypic traits subject to selection (Saltz et al., 2017; Valdar et al., 2006) can be condition dependent. Among-individual variation

in organismal resource levels could increase variation in phenotypic responses to high temperatures and their fitness consequences, thus strengthening heatwave-mediated selection in organisms. However, population-wide food limitation could reduce individual-level variation in thermal performance, thus hindering phenotypic selection on heatwave responses. Similarly, the condition dependence of thermal responses could alter the evolutionary potential of organisms subject to heatwave-mediated selection (i.e., genetic variation in thermal performance). *Lymnaea stagnalis* has been found to show within-population family-level variation (among full-sib families) in the negative effects of an experimental heatwave on immune activity (Leicht et al., 2017, 2019). That finding suggests a genetic potential to adapt to the adverse effects of heatwaves. Because snail immune activity is strongly condition-dependent (Seppälä & Jokela, 2010; Seppälä et al., 2022; this study), the expression of (additive) genetic variance and, thus, evolutionary potential in their heatwave responses could depend on snail resource levels. For example, resource limitation could reduce additive genetic variance in immune activity, a typical pattern for quantitative phenotypic traits in qualitatively different environments in other organisms (Hoffmann & Merilä, 1999).

Taken together, we found the heatwave responses of *L. stagnalis* snails to be dependent on their resource level. During an experimental heatwave, the fitness-related traits (growth rate, fecundity) of snails under unlimited food supply benefitted the most from the high-temperature treatment. Additionally, the post-heatwave performance of heat-challenged snails was less reduced if offered unlimited resources. The high temperature negatively affected snail immune activity, with heat-challenged snails unable to maintain some of the examined immune parameters at high levels even under ad libitum food supply. However, immune traits recovered quickly after the heatwave. The condition dependence of heatwave responses potentially has important ecological and evolutionary implications by influencing: (1) the performance of natural populations during heatwaves depending on habitat quality; and (2) the expression of phenotypic and quantitative genetic variation in thermal performance critical for its evolution. Previous research on the ecological effects of climate change has increasingly considered their interactive effects with other anthropogenic environmental changes (e.g., Hermann et al., 2023; Salo et al., 2017; Taniwaki et al., 2017). Our results highlight the role of ecological variation in determining organisms' responses to climate change and a need to integrate such variation into predictions of climate change-mediated effects in nature.

AUTHOR CONTRIBUTIONS

O.S., T.S.: conceptualisation. O.S., G.K., V.S., K.S.: conducting the research. O.S., G.K., T.S.: data analysis. O.S., T.S.: preparation of figures and tables. O.S., G.K., V.S., K.S., T.S.: developing methods, data interpretation, writing.

ACKNOWLEDGMENTS

We thank S. Mayer for his help with installations for the experiment and anonymous reviewers for their helpful comments on the manuscript.

FUNDING INFORMATION

This work was funded by the Austrian Science Fund (grant no. P 34687) to OS.

CONFLICT OF INTEREST STATEMENT

We have no conflict of interest.

DATA AVAILABILITY STATEMENT

Data are available online: <https://zenodo.org/records/10718388>.

ETHICS STATEMENT

The study was conducted following the laws and regulations of Austria and the University of Innsbruck.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Seppälä, O., Katsianis, G., Stabauer, V., Seppälä, K., & Salo, T. (2024). Resource level modifies heatwave responses in the freshwater snail *Lymnaea stagnalis*. *Freshwater Biology*, 69, 692–704. <https://doi.org/10.1111/fwb.14239>