

Title: Heatwave-induced functional shifts in zooplankton communities result in weaker top-down control on phytoplankton

Running head: Climate change effect on plankton communities

ThuHuong HuynhNgoc^{1,2,3}, Zsófia Horváth^{1,3}, Károly Pálffy^{1,3}, Vivien Kardos¹, Beáta Szabó^{1,3}, Péter Dobosy¹ & Csaba F. Vad^{1,3}

¹Institute of Aquatic Ecology, Centre for Ecological Research, Budapest, Hungary

²Doctoral School of Biology, Institute of Biology, Eötvös Loránd University, Budapest, Hungary

³National Multidisciplinary Laboratory for Climate Change, Centre for Ecological Research, Budapest, Hungary

Corresponding author

ThuHuong HuynhNgoc, Institute of Aquatic Ecology, Centre for Ecological Research, Budapest, Karolina út 29, 1113 Budapest, Hungary

Email: huynh.huong@ecolres.hu

Author Contribution statement

CFV and ZH came up with the original concept, research questions, and experimental design. All authors participated in conducting the experiment, collecting the samples, and taking part in laboratory work. THHN and VK performed zooplankton identification while PD carried out the water chemistry analyses. THHN, ZH, CFV, and KP analyzed the data. The first draft was written by THHN, CFV, and ZH. All authors edited subsequent versions of the manuscript.

Data availability statement: Data will be made available in the Dryad data repository.

27

28 **Abstract**

29 Freshwater ecosystems are increasingly affected by rising annual mean temperatures
30 and extreme heatwaves. While heatwaves are expected to have more immediate effects than
31 mean temperature increases on local communities, comparative experimental studies are
32 largely lacking. We conducted a one-month mesocosm experiment to test the effect of
33 different warming scenarios, constantly raised temperatures (+3°C), and recurring heatwaves
34 (+6°C) on plankton communities. We specifically tested how shifts in zooplankton trait
35 composition and functional groups are reflected in ecosystem functioning (top-down control
36 on primary producers). We found that heatwaves had a stronger and more immediate effect
37 on trait and functional group compositions. Heatwaves were associated with larger body
38 sizes, and the decrease in micrograzers resulted in weaker top-down control, leading to
39 elevated algal biomass. Altogether, our results highlight the importance of the indirect effects
40 of heatwaves via inducing shifts in zooplankton functional groups and trait composition
41 which may foster periodic algal blooms.

42

43 **Keywords:** climate change, warming, heatwave, species interactions, functional traits,
44 microzooplankton, top-down control.

45

46 **Introduction**

47 Heatwaves, periods of acute temperature increase, are expected to occur with
48 increasing intensity, duration, and frequency under climate change (IPCC, 2013). Compared
49 to rising mean temperature, heatwaves might exert a stronger and more immediate effect on
50 communities (Stillman, 2019; Vasseur et al., 2014). However, most experimental studies in
51 freshwater ecology have so far applied static warming treatments in their design (Thompson
52 et al., 2013; Woodward et al., 2016). While the number of studies focusing on the effect of
53 heatwaves on aquatic ecosystems is rising, the majority of these studied single temperature
54 scenarios (but see Striebel et al., 2016; Zhang et al., 2018). This limits our comprehensive
55 understanding of how aquatic ecological communities respond to different warming
56 scenarios, including heatwaves and increasing mean temperatures.

57 Changing temperature regimes can alter population dynamics and species
58 interactions, leading to changes in community composition and ecosystem-level processes
59 (Ohlberger, 2013; Tylianakis et al., 2008). As aquatic species exhibit narrower thermal safety
60 margins compared to terrestrial taxa (Pinsky et al., 2019; Sunday et al., 2012), they may be
61 particularly vulnerable to sudden temperature changes, resulting in high community turnover
62 (Comte & Olden, 2017). Effects of extreme temperatures may be especially strong in shallow
63 lakes, where thermal refugia (e.g., hypolimnetic refuge) are scarce.

64 Zooplankton plays a crucial role in aquatic food webs by transferring energy from
65 primary producers to higher trophic levels. Shifts in dominant species or trait composition
66 can alter energy flow in these ecosystems (Hébert et al., 2016; Ye et al., 2013), e.g., changes
67 in abundance and community size structure affect their overall grazing impact on
68 phytoplankton (Carpenter et al., 1985). Physiology, behaviour, and life history traits
69 (Litchman et al., 2013) can be similarly important when predicting the responses of
70 zooplankton communities to environmental change. Hence zooplankton, with a high
71 diversity of traits and ecological strategies (Barnett et al., 2007) and their key position in
72 aquatic food webs, provide an excellent model for understanding the mechanisms of
73 community reorganization in response to different warming scenarios.

74 Trait-based approaches are powerful tools for addressing the mechanisms of
75 ecosystem change beyond community shifts (Litchman & Klausmeier, 2008). By focusing
76 on key traits, we can forecast functional community reorganization under warming scenarios
77 and link this to changes in ecosystem functioning (Litchman et al., 2006; Schleuning et al.,
78 2020). For example, decreasing body size of ectothermic organisms is a universal response
79 to warming in freshwater systems at both population and community levels (Daufresne et
80 al., 2009), which can weaken the strength of trophic cascades by reducing top-down control
81 (DeLong et al., 2015). These changes can alter the magnitude and direction of energy flow,
82 eventually reflected in the provision of ecosystem services.

83 The aim of this study was to record and contrast the responses of zooplankton
84 communities to warming scenarios of constantly elevated mean temperatures versus
85 heatwaves, and to link community-level shifts to changes in the strength of top-down control
86 on phytoplankton. Warming treatment was designed with a constantly elevated average
87 temperature compared to the ambient (control) treatment, whereas heatwave treatment was
88 designed as pulses of higher temperature for pre-determined time periods. We expected that,

from these two temperature treatments, heatwaves would have a stronger and more immediate effect on community composition and ecosystem functioning. In line with this, we predicted that heatwaves would be associated with small body size, as populations of smaller-sized species would respond faster to sudden temperature changes due to their short generation times. The dominance of smaller zooplankton species may imply weaker top-down control on primary producers, hence we expected increased phytoplankton biomass, especially in response to heatwaves.

Material and Methods

Experimental design and environmental parameters

We conducted a one-month outdoor mesocosm experiment between June and July 2020 at the Balaton Limnological Institute of the Centre for Ecological Research. Twelve mesocosms were filled with water directly from Lake Balaton two days before the start of the experiment. The water level was set to 1.20 m, resulting in an experimental volume of 3,000 L (inner diameter of mesocosms: 2 m; maximum depth: 1.5 m). The water column of each mesocosm was constantly mixed with an airlift system (with 0.6 m³ h⁻¹ carrying capacity) to prevent vertical stratification (Striebel et al., 2013) hence representing shallow lake ecosystems that are regularly mixed by the wind. Besides, it also ensured that dissolved oxygen levels were saturated in all mesocosms during the experiment, which was followed with daily DO measurement with a sensor. The airlift consisted of a PVC pipe hanging in the center of each mesocosm, in which compressed air released from a tube produced a gentle upward current. As the primary focus of the experiment was to study the responses of pelagic plankton communities, we did not include fish, macrophytes, or sediment in the mesocosms. Mesocosms were covered with a mosquito mesh to prevent larger debris from falling into the tanks and the colonization of macroinvertebrates that could influence the nutrient level and species interactions in the water.

Three treatments, each replicated four times, were randomly assigned to the 12 mesocosms: (1) ambient environmental conditions (C - control); (2) warming set to a constant elevation of 3°C above the control conditions (W - warming); and (3) recurring 1-week 6°C heatwaves (H - heatwaves) during weeks one and three (**Figure 1a**). Both warming scenarios (W and H) received an identical total energy input but in different pulses, and both started on the first day of the experiment. Water temperature throughout the experiment was

recorded automatically in 10-minute intervals. Regular nutrient measurements (detailed in the **Supplemental Information**) were carried out to monitor and, if necessary, ensure relatively constant levels of basal resources by nutrient addition, which was eventually not necessary as they were comparable across treatments (**Figure S1**).

Chlorophyll a

After thorough mixing of water column, a vertically integrated water sample was collected with a PVC tube sampler for chlorophyll *a* (Chl*a*) and nutrient measurements pooled from three random locations in each mesocosm and filtered through a 100- μ m plankton mesh to obtain water free of large zooplankton. To measure Chl*a* concentration, a proxy for phytoplankton biomass, 500-850 mL of water (depending on algal densities) was filtered through glass microfiber filters (Whatman GF/F) twice a week. Subsequently, the spectrophotometric determination was carried out after hot methanol extraction, using the absorption coefficients determined by Iwamura et al., (1970). Data was converted to phytoplankton dry weight (μ g dry weight L⁻¹) by a multiplication factor of 100 for subsequent analyses (Reynolds, 2006).

Additionally, to obtain higher-frequency data on Chl*a*, and hence phytoplankton dynamics, we measured Chl*a* fluorescence daily (maximum fluorescence yield; **Figure 1b**) with a handheld fluorometer (AquaPen AP 110-C, Photon System Instruments). We took samples from each mesocosm every morning during the experiment from the central surface water of the mesocosms (next to the airlift, where water was well-mixed). Measurements were carried out after a 30-minute dark adaptation period to avoid potential bias resulting from short-term physiological changes. Chl*a* fluorescence was measured using the OJIP protocol (Stirbet & Govindjee, 2011). We assessed the overall precision of Chl*a* fluorescence measurements by testing the relationship between Chl*a* concentration and fluorescence via linear regression (**Figure S2, S3**).

Zooplankton

To determine initial densities and community composition, zooplankton were sampled at the start of the experiment (day 0, before the start of the warming treatments). Subsequently, samples were collected twice a week with a Schindler-Patalas plankton trap

(volume: 10 L, with a net mesh size: 45 μm) from three randomly chosen locations of the mesocosms, which represented different vertical layers, hence resulting in a depth-integrated sample, and preserved in 70% ethanol. We counted and identified zooplankton with an inverted microscope at 20 \times magnification. For microcrustaceans, we used the average body lengths obtained from the regular monitoring data of Lake Balaton (provided in **Table S1**), and then we converted body length to biomass (dry weight), following published length-weight relationships (McCauley, 1984). As similar data were not available for Rotifera, here literature data (Cieplinski et al., 2018; Gosse, 1851; Roche, 1993; Skorikov, 1986) was used as average body length and biomass (dry weight) was calculated similarly based on length-weight relationships (Finlay & Uhlig, 1981). Traits were assigned to each species, including body length, body mass, reproduction mode, feeding mode, generation time, and clutch size, according to **Table S1**.

Biomass ratios as indices for changes in top-down control

Biomass ratio of organisms at higher and lower trophic positions can be used as a measure of the strength of top-down control (Shurin et al., 2012). We, therefore, calculated the ratio between zooplankton (ZP, $\mu\text{g dry weight L}^{-1}$) and phytoplankton (PP, $\mu\text{g dry weight L}^{-1}$) biomass, referred to as ZP:PP, and tested its potential response to the different warming treatments. As a further measure of changes in the trophic structure and functioning, we also analyzed the temporal trend of the biomass ratio of the two dominant functional groups, the small-bodied micrograzer Rotifera and the larger omnivorous Copepoda across treatments (R:C).

Data analysis

To visualize the temporal dynamics of Chl a , zooplankton biomass (ZP), and ratios of zooplankton:phytoplankton (ZP:PP) and Rotifera:Copepoda (R:C) in the different treatments, we fitted generalized additive models (GAMs) on the respective data for each treatment over the experimental duration. For this, we used the “geom_smooth” function of the package “ggplot2” (Wickham, 2016), with $k=7$ for Chl a and $k=4$ for the other datasets. To test for treatment-specific differences for ZP, ZP:PP, and R:C, we performed non-parametric Kruskal–Wallis (KW) tests for each sampling date after the double square root

transformation of data. Subsequently, we applied Dunn's *post hoc* test to reveal pairwise differences (p-values were adjusted with the Holm method) using the package “FSA”.

To test the effect of treatments on daily Chla fluorescence, we excluded the first days (days 0-3) when Chla still showed a decreasing trend in all treatments. Then, we split the dataset into two periods: days 4-14 including the first heatwave (until day 7) and its direct aftermath (days 8-14) and days 16-28 including the second heatwave (days 15-21) and its direct aftermath (days 22-28). For each dataset, we used a generalized additive mixed model (GAMM) with treatment as the main linear predictor, adding time with varying shapes of smooth according to individual mesocosms and a temporal autocorrelation within individual mesocosms to account for random effects. In case of a significant treatment effect, we performed a pairwise test for multiple comparisons based on the GAMM with single-step p-value adjustment with the package “multcomp” (Bretz et al., 2010).

To test how environmental predictors, or in our case, treatments, can filter species traits based on species abundances, we performed RLQ analyses (Dolédéc et al., 1996) with the package “ade4” (Dray & Dufour, 2007) separately for each date. The RLQ method performs a double inertia analysis including three data matrices (environmental variables by samples - R; species by samples - L table; and traits by species - Q table) and produces their simultaneous ordination. Fourth-corner statistics were computed based on 9999 permutations by permuting both sites and species (with “modeltype=6”), to avoid Type I error (Dray et al., 2014). We used the log(x+1) transformed biomass of zooplankton species and the traits presented in **Table S1**.

To quantify the direct and indirect trophic relationships between the main organism groups across treatments, we applied a structural equation model (SEM) on the copepod (double-square-root transformed) and rotifer (cubic-root transformed) biomasses and Chla fluorescence (untransformed). We started with an initial SEM that included all the plausible pathways between plankton groups and the treatments using the R package “piecewiseSEM” (Lefcheck et al., 2015). Each relationship within the SEM was estimated with linear mixed-effects models with a temporal autocorrelation structure of order 1 (AR-1) and mesocosm number as a random factor. We expected that any top-down effect on phytoplankton would manifest with a time lag. Therefore, separate SEMs were fitted using the Chla values on the zooplankton sampling days and the values 0 to 6 days after the samplings. The SEM yielding

the highest explanatory power for Chl a (based on R^2) was selected to describe the causal network between the three organism groups and the treatments.

All statistical analyses were performed using R version 4.0.2 (R Core Team, 2020).

Results

Temperature varied between 18.5–26.0 °C in C, 18.9–28.9 °C in W, and 19.4–31.1 °C in H during the four weeks of the experiment (**Figure 1a, Figure S4**). We found significantly higher Chl a fluorescence in treatment H than in W and C during and after the first heatwave (day 4-14) according to the GAM model and the pairwise test (H-C estimate: 0.27, $p=0.001$; H-W estimate: 0.24, $p=0.005$). Differences were not significant in the second experimental period (day 15-28). A peak in Chl a fluorescence was observed in treatment H after the first heatwave (**Figure 1b**). This difference disappeared by the second part of the experiment (no significant treatment effect).

In our experiment, the copepods *Mesocyclops leuckarti* and *Eudiaptomus gracilis*, and the rotifer *Polyathra remata* dominated zooplankton, by accounting for 19.0 ± 24.6 , 13 ± 9.8 and 4 ± 6.6 % (mean \pm SD) of total biomass over the experiment. We found an overall increase in zooplankton biomass over time, largely comparable across treatments (**Figure 1c**). We found statistically significant differences between W and H (KW test: $\chi^2=7.65$, $df=2$, $p=0.02$, W-H: $p<0.01$) only at the end of the second heatwave (day 21, **Table S2**). We found overall higher zooplankton biomass in W than in H and C, while the temporal trends in H and W were similar (**Figure 1c**). Overall, the R:C biomass ratio decreased significantly as a response to H, which became evident after the first heatwave (KW test: $\chi^2=7.04$, $df=2$, $p=0.02$, H-C: $p<0.01$) and lasted until the first part of the second heatwave (significant differences between H and C on days 14 and 17; **Figure 1d, Table S2**). We found an increasing trend in the ZP:PP biomass ratio with significant differences (KW test: $\chi^2=4.77$, $df=2$, $p=0.03$, H-W: $p<0.03$) between W and H at the end of the second heatwave (day 21; **Figure 1e, Table S2**).

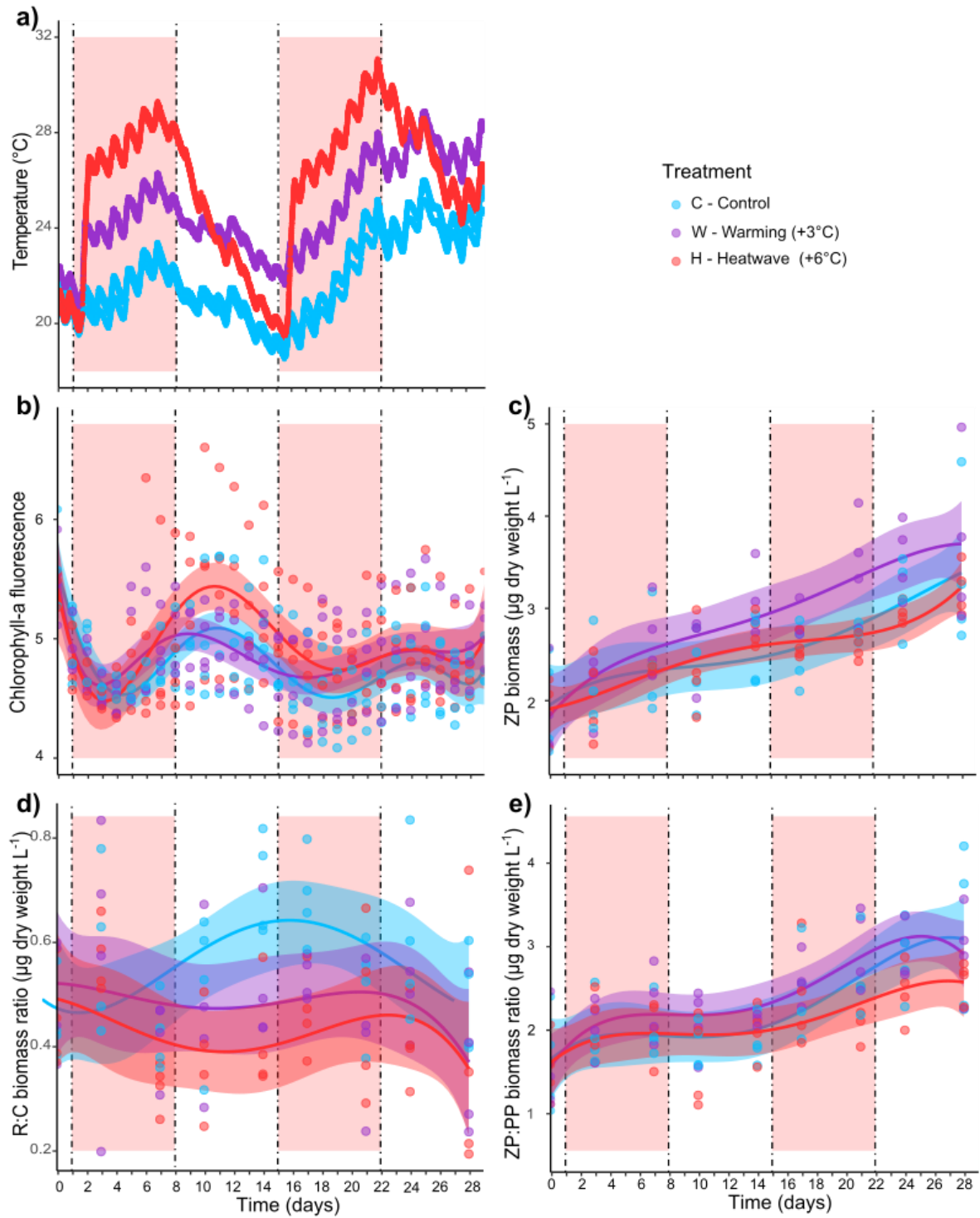


Figure 1. Temporal patterns in (a) observed water temperature ($^{\circ}\text{C}$), (b) log-transformed chlorophyll *a* (Chla) fluorescence (proxy of phytoplankton biomass), (c) zooplankton (ZP) biomass (dry weight, $\mu\text{g L}^{-1}$) (c), (d) biomass ratio of Rotifera (R) to Copepoda (C), and (e) biomass ratio (dry weight, $\mu\text{g L}^{-1}$) of total zooplankton (ZP) to phytoplankton (PP) in the three (colour-coded) treatments. Biomass data were double-square-root transformed for the

analyses. Solid trend lines and error bands represent fitted GAMs \pm SE. Red backgrounds indicate the lengths of the two experimental heatwaves (H, +6°C).

We found significant relationships between traits and treatments revealed by fourth-corner analysis (related RLQ plots presented in **Figure 2**), with body-size related traits (body mass, body length) responding positively to H. Specifically, body mass (day 10, $p=0.048$) and body length (day 14, $p=0.046$) were positively associated with H in the period after the first heatwave (**Figure 2**). At the same time, both traits were negatively associated with the control treatment on day 14 (body length: $p=0.031$; body mass: $p=0.046$). During and after the second heatwave, we did not find any significant treatment effects.

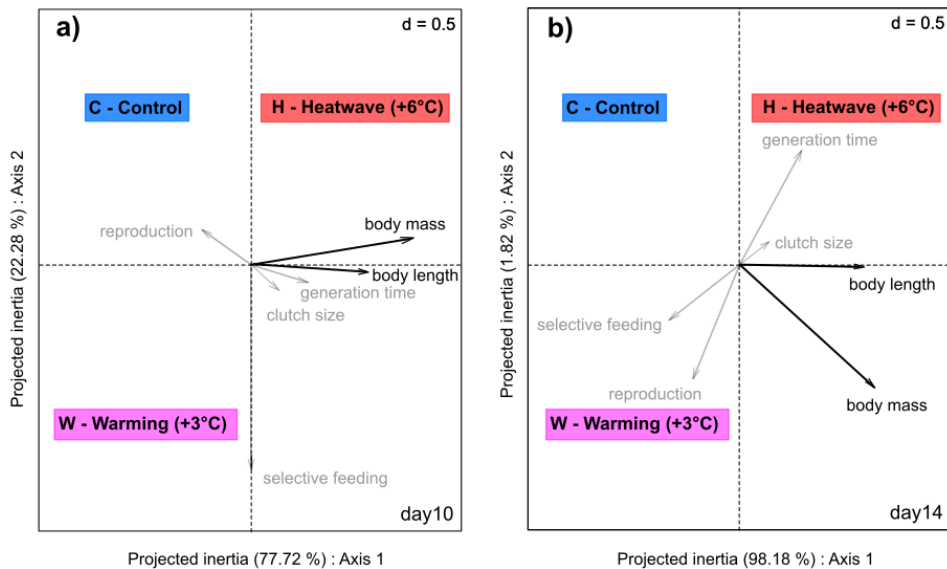
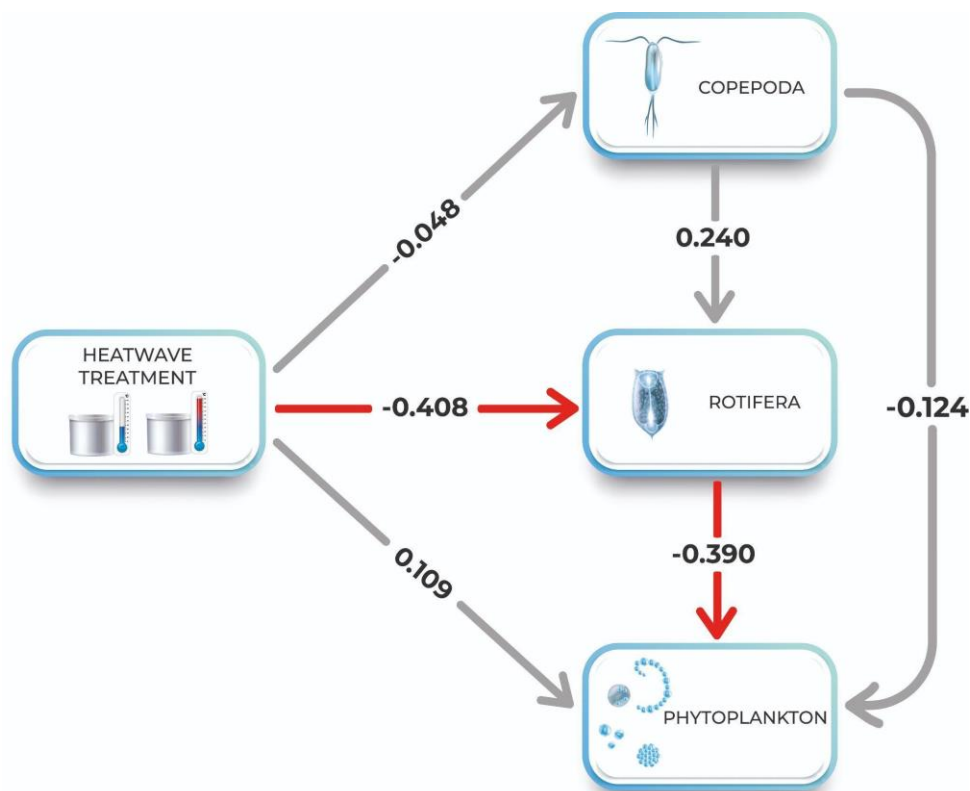


Figure 2. RLQ plots for the relationships between zooplankton traits and experimental treatments after the first heatwave, on day 10 (a) and day 14 (b). Traits having at least one significant ($p < 0.05$) relationship with at least one treatment based on fourth-corner analyses are shown with black arrows, while others are in grey. (Grid size: $d=0.5$)

Treatment H exerted a direct and significant negative effect on Rotifera biomass based on the SEM (standardized beta estimate: -0.408 , $p < 0.05$, **Figure 3, Table S3**). Besides, the SEM revealed a direct negative effect of Rotifera on Chl a fluorescence, which appeared with a time lag of 2-5 days (**Figure 3, Table S3**). This relationship was strongest directly after the first heatwave and diminished over time. The results obtained for W were not significantly different from those obtained for treatment C (**Table S3**).



268

269 **Figure 3.** A structural equation model (SEM) of the linkages between the dominant
270 zooplankton groups, Rotifera and Copepoda (biomass), and phytoplankton (Chl*a*
271 fluorescence) in the heatwave treatment during the first two weeks of the experiment. The
272 model presented here is based on a time lag of 5 days between Chl*a* and zooplankton. Solid
273 red arrows represent direct, significantly negative pathways ($p < 0.05$), while grey arrows
274 stand for non-significant direct pathways. Numbers represent standardized parameter
275 estimates. The amount of variation explained by the model (along with others with different
276 time lags) is presented in **Table S3**.

277

278 **Discussion**

279 The results of this study supported our expectation that heatwaves exert stronger and
280 more immediate effects on community composition than a constantly warmer temperature
281 of the same energy input. However, contrary to our expectation, heatwaves had a negative
282 impact on the biomass of small-bodied zooplankton (Rotifera) in our study. This result has
283 important implications for ecosystem functioning, as the decline in small-bodied grazers in

turn resulted in reduced top-down control of phytoplankton and contributed to elevated algal biomass in treatment H during the first heatwave.

The positive association between body size and heatwaves was counterintuitive as most existing studies reported zooplankton body size to decline with warming, spanning from population to community levels (Brans et al., 2017; Brucet et al., 2010). However, these studies largely derived their conclusions from natural temperature gradients (where species can adapt over time) or experiments with constant warming setups. Heatwave effects, in contrast, may be less predictable due to stronger pressure on individual physiology, potentially compromising physiological or genetic adaptations (Dam & Baumann, 2017), resulting in increased mortality rates (Stillman, 2019), or sudden changes in species interactions and phenology (Zhang et al., 2018). In our study, the positive association with larger body size resulted from the relative decrease in microzooplankton (Rotifera) to mesozooplankton (Copepoda) biomass in the H vs the other treatments.

The decline in Rotifera biomass may be a direct effect of the heatwave through the change in metabolic activity (Lansing, 1942) or decrease in lifespan (Kauler & Enesco, 2011). At the same time, our results rather point to the possible relevance of other indirect effects for several reasons. First, the negative effect was only observed after the first heatwave, which peaked at lower temperatures compared to the second one. Second, the most dominant Rotifera was *Polyarthra remata*, a eurythermic summer species (Bērzins & Pejler, 1989). These altogether point at the possible relevance of indirect trophic effects, such as increased predation by copepods. Both cyclopoid and calanoid copepods are known to be efficient predators of rotifers (Brandl, 2005; Lapesa et al., 2002). Although we did not observe a negative correlation between the biomass of Copepoda and Rotifera, Copepoda could have exerted higher predation rates due to increased metabolic demands in treatment H.

Increased algal biomass as a response to warming and heatwaves is a commonly observed phenomenon, which may result from direct and indirect (i.e., trophic) effects (Ger et al., 2016; Viitasalo & Bonsdorff, 2022). A moderate increase in temperature increases both the phytoplankton growth rate and the metabolic demands of consumers, shifting to higher feeding rates and, consequently, higher consumer-to-producer biomass (Kratina et al., 2012; O'Connor et al., 2009). Heatwaves, at the same time, may disrupt the predator-prey relationships, thus increasing algal growth (Ross et al., 2022; Vad et al., 2023). Our results highlight the importance of copepods and microzooplankton in trophic cascades. Most freshwater studies on trophic cascades focus on Cladocera-dominated communities

(Carpenter & Kitchell, 1993; Sommer, 2008), while in our experiment, zooplankton was largely dominated by Copepoda and Rotifera. In contrast to cladocerans, copepods exert a stronger top-down control on the larger fraction of phytoplankton (Sommer et al., 2001; Sommer & Sommer, 2006) and microzooplankton grazers (Adrian & Schneider-Olt, 1999; Williamson, 1987). This can release small-sized phytoplankton from grazing pressure (Sommer & Sommer, 2006), which was most likely the key mechanism in our experiment as well, where the algal biomass peaks of the H treatment were dominated by small-celled *Chlorella* and *Monoraphidium* species (K. Pálffy, pers. obs.). It is important that elevated phytoplankton biomass appeared without external nutrient input (TP concentration was similar among treatments). Therefore, we argue that it resulted from direct and indirect effects of temperature.

An intriguing observation of our study is that the heatwave effect on phytoplankton biomass occurred only after the first heatwave. This effect diminished later on, even though the second heatwave peaked at higher temperatures. It is unlikely that the lack of phytoplankton peaks in the second half of the experiment was related to grazing by Rotifera, as they did not recover in the heatwave treatment (**Figure S5**). A possible explanation is that copepods, with steadily increasing biomass over time (**Figure S5**), performed a diet shift to the more abundant food sources, i.e., phytoplankton over Rotifera (Kiørboe et al., 1996). Omnivorous copepods can become more herbivorous with increasing temperature (Boersma et al., 2016), representing another possible explanation for the disappearance of higher phytoplankton biomass in the heatwave treatment.

Mesocosm experiments are useful tools to identify community- and ecosystem-level shifts to climate change by representing a compromise between experimental control and realism (Fordham, 2015; Stewart et al., 2013). In our study, the lack of top-down control due to the absence of higher trophic levels (e.g. fish, macroinvertebrates) likely explains the temporal increase in zooplankton biomass in all treatments. However, by this reduction of trophic complexity, planktonic community shifts and food web interactions could be revealed without confounding factors. Though the one-month duration of our study limits long-term forecasts, it delivers relevant information on the short-term effects of intermittent heatwaves on community dynamics. The relatively short experimental duration may also be the reason for the absence of strong effects in treatment W, which can be considered a press disturbance, typically resulting in permanent restructuring of communities on longer time

scales, while the pulse disturbance in treatment H can cause sudden changes followed by a certain extent of recovery (Glasby & Underwood, 1996; Vad et al., 2023).

Here we showed that heatwaves could exert a stronger short-term pressure on planktonic communities than a more moderate warming scenario. Most importantly, we showed how heatwave-driven planktonic interactions indirectly lead to increased algal biomasses. At the same time, the lack of lasting effects at this temporal scale implies that communities in shallow lakes may be to some extent resilient to such short-term heat perturbations. As natural systems are facing heatwaves of increasing magnitude and duration, stronger effects on communities and ecosystems are expected in the future. Longer-term studies are required to be able to create more accurate predictive models and thereby improve our forecasting ability, while experimental studies should incorporate temperature fluctuations besides focusing on the predicted increase in mean temperature.

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