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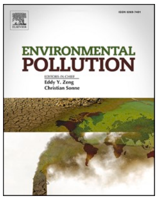

Heatwaves, elevated temperatures, and a pesticide cause interactive effects on multi-trophic levels of a freshwater ecosystem☆

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ABSTRACT

Climate impacts of elevated temperatures and more severe and frequent weather extremes like heatwaves are globally becoming discernible on nature. While a mechanistic understanding is pivotal for ecosystem management, stressors like pesticides may interact with warming, leading to unpredictable effects on freshwater ecosystems. These multiple stressor studies are scarce and experimental designs often lack environmental realism. To investigate the multiple stressor effects, we conducted a microcosm experiment for 48 days comprising benthic macroinvertebrates, zooplankton, phytoplankton, macrophytes, and microbes. The fungicide carbendazim (100 μg/L) was investigated combined with temperature scenarios representing elevated temperatures (+4 °C) or heatwaves (+0 to +8 °C), both applied with similar energy input on a daily fluctuating ambient temperature (18 °C ± 1.5 °C), which served as control. Measurements showed the highest carbendazim dissipation in water under heatwaves followed by elevated and ambient temperatures. Average carbendazim concentrations were about 50% in water and 16% in sediment of the nominal concentration. In both heated cosms, zooplankton community dynamics revealed an unexpected shift from Rotifera to Cladocera and Copepoda nauplii, indicating variations in their thermal sensitivity, tolerance and resilience. Notably, warming and heatwaves shaped community responses similarly, suggesting heat intensity rather than distribution patterns determined the community structure. Heatwaves led to significant early and longer-lasting adverse effects that were exacerbated over time with Cladocera and Copepoda being most sensitive likely due to significant carbendazim interactions. Finally, a structural equation model demonstrated significant relationships between zooplankton and macrophytes and significantly negative carbendazim effects on zooplankton, whereas positive on macroinvertebrate abundances. The relationship between macroinvertebrate feeding and abundance was masked by significantly temperature-affected microbial leaf litter decomposition. Despite the thermal tolerance of zooplankton communities, our study highlights an increased pesticide threat under temperature extremes. More intense heatwaves are thus likely to cause significant alterations in community assemblages which will adversely affect ecosystem's processes and functions.

1. Introduction

Global climate change (GCC) and chemical pollution are considered to be among the current and future emerging threats to freshwater ecosystems, raising the risk of species and population extinction *ergo* biodiversity loss (Reid et al., 2019). One example of how this trend is manifested is the continuously declining Living Planet Index with an average loss of 84% for monitored freshwater populations (Almond et al., 2020). Research to date has put effort into understanding the effects of GCC-driven temperature changes and their underlying mechanisms on ecosystems (Petchey et al., 1999; Arenas-Sánchez et al., 2018; Van de Perre et al., 2018; Cremona et al., 2020). Temperature effects can be versatile since they strongly impact life-history traits of ectotherms (O'Gorman et al., 2017). Additionally, dissolved oxygen concentrations, nutrient cycling, and litter decomposition are only few of the ecosystem's properties, processes, and functions that are directly or indirectly influenced by temperature (Pinsky et al., 2019). Temperature may also affect toxicity of chemicals by mitigating or meliorating the effects on

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organisms via increased uptake, metabolization or excretion rates (Mangold-Döring et al., 2022; Huang et al., 2023), and affect the fate among others through modified degradation rates and adsorption kinetics (Noyes et al., 2009).

Multiple stressor studies which translated temperature-related GCC-scenarios into experimental designs have almost exclusively applied an increased mean temperature within a constant temperature regime, rather than using environmentally realistic temperature variations (Willming and Maul, 2016; Barbosa et al., 2017; Li et al., 2021). Yet, recent studies demonstrate the importance of incorporating daily and seasonal temperature fluctuations in ecotoxicological experiments, as under chemical stress more severe effects on species were noted under daily fluctuating temperatures than using constant temperatures (Vasseur et al., 2014; Verheyen and Stoks, 2019). Conducting climate-chemical multiple stressor experiments with constant temperature regimes may thus not be representative for realistic habitats with thermal variation. Future climate predictions and global change scenarios (IPCC, 2021) expect higher mean temperatures and more severe, more frequent, and lasting weather extremes, such as heatwaves and warm spells, until the end of this century (Meehl and Tebaldi, 2004; Perkins et al., 2012; Woolway et al., 2021). Although research on the effects of these climate phenomena is still in its infancy, the reviewed literature point out that they can harm freshwater ecosystem biota at different trophic levels both as single and combined stressor (Polazzo et al., 2022).

Multiple stressor studies using micro/meso-cosms are still relatively scarce because of, for instance, logistical issues with a sufficient number of replicates and the preparation efforts required to set up these complex test systems. Yet, there is lately a growing interest in these studies because they allow for ecological and environmental complexity by species interactions across different trophic levels (Paiva et al., 2021).

The goal of this 48 days indoor microcosm study was to investigate how the single and combined stressors of temperature and the benzimidazole-carbamate pesticide carbendazim affect structure and dynamics of a simplified freshwater ecosystem. The temperature scenarios comprised daily temperature patterns of a fluctuating ambient and elevated regime and repeated heatwaves. Recent studies examining the single and combined stress posed by different climate change scenarios and pesticides demonstrate the complexity to unravel the direct and indirect effects on community- or ecosystem-level (Allen et al., 2021; Roth et al., 2022; Vijayaraj et al., 2022). Furthermore, these studies point out the unpredictable environmental risk of such unprecedented multiple stressor interaction effects. We chose the fungicide carbendazim as model pesticide because it is among the most frequently found compounds in pesticide mixtures of Dutch and other European aquatic ecosystems (Schreiner et al., 2016). Despite the wide distribution of fungicides and their potential toxicity-related risk to aquatic organisms, their effects have received less attention (Zubrod et al., 2019). Previous studies examining carbendazim effects showed significantly negative effects on macroinvertebrates at concentrations of 33 μg/L and on a zooplankton community at concentrations of 100 μg/L, whereas positive effects were noted on phytoplankton, mollusks, and macrophytes (Cuppen et al., 2000; Van den Brink et al., 2000). Here, we studied the effects of elevated temperature and heatwaves, both representing climate change scenarios, on different trophic levels of a carbendazim-stressed freshwater ecosystem. Three research questions with hypotheses were investigated: How do elevated temperatures and heatwaves:

1. affect the chemical fate of carbendazim? Hypothesis: The elevated temperature and heatwave treatments will increase carbendazim degradation rates compared to the ambient temperature treatment (Cuppen et al., 2000; Daam et al., 2009).
2. influence directly and indirectly organisms' sensitivity to carbendazim? Hypothesis: Direct and indirect effects are more pronounced in the elevated temperature and heatwave treatments compared to the ambient temperature treatment because of temperature-induced higher uptake rates with regards to organisms' increased metabolism and food consumption (Noyes et al., 2009; Huang et al., 2023).
3. affect the structure and functioning of a freshwater ecosystem? Hypothesis: There is a shift from large organisms to smaller ones under temperature increase because the thermal tolerance of smaller organisms is higher than for larger ones (Daufresne et al., 2009; Saucedo-Ríos et al., 2017; Li et al., 2019).

We disentangled the effects of the combined stressors to distinguish between temperature- and chemical-induced effects and finally evaluated ecological relationships and cause-effect chains.

2. Materials and methods

2.1. Experimental design

The microcosm-experiment was conducted at Wageningen University (WU) in the Netherlands from 3rd of September to November 21, 2019. The 80 days of experimentation included 32 days of acclimatization followed by 48 treatment days. We used a fully randomized, 2*3 factorial design with a chemical treatment of 0 (i.e., control) and 100 μg/L of the fungicide carbendazim (PESTANAL®, Sigma-Aldrich 45368) and ambient temperatures, elevated temperatures, and repeated heatwaves as temperature treatments. All six treatments were replicated five times, resulting in 30 microcosms. The TENTACLE device coupled with 150 W glass heaters (Eheim) was used to achieve indoor temperature manipulation and recording, taking into account daily temperature variation in accordance to a 24 h day-night rhythm (Hermann et al., 2022). All microcosms were placed in two water baths which were additionally temperature controlled by two aquarium chillers (TECO) to gain sinusoidal daily temperature patterns. Furthermore, day-night temperature patterns were synchronized with the photoperiod (14:10 h day:night) provided by programmable Hg-fluorescent tubes (80 W BlueWhite, Red Sea). We set the in- and decrease of the light intensity to an hourly time interval, starting at 20% starting at 7 a.m. in the morning with the highest light intensity from 11 a.m. until 5 p.m. and switched off lights at 9 p.m. At the end of the acclimatization period and with the start of the experiment (day 0), both temperature and chemical stressors were applied simultaneously.

2.2. Microcosm set-up

All microcosms, hereafter referred to as cosms, were cylindrical glass tanks and due to stock availability with either a 0.25 m diameter and a 0.38 m height (18 cosms) or with the same diameter but a 0.34 m height (12 cosms) and a total volume of about 18 and 15 L, respectively. Each cosm contained a 3 cm layer of dried natural river sediment (i.e. 1.76 kg dry weight) from a clean flood plain of the river Waal in the Netherlands, and 12 L of aerated groundwater from the same location. We collected natural phytoplankton and zooplankton communities (55 μm net) from the WU campus pond; added 20 individuals of the macrocrustaceans *Asellus aquaticus* and *Gammarus pulex* from a stream; and six individuals of the freshwater snail *Lymnaea stagnalis* of similar size (27 ± 2.56 mm in length) as a representative grazer on periphyton from a ditch in the surroundings of the WU campus to each cosm. Lastly, we added from the same location one shoot (10 cm in length) of the macrophyte species *Elodea nuttallii* and *Myriophyllum spicatum* planted in separate small glass beakers (4 cm diameter, 8 cm height) to the cosms, which we filled with the same sediment as the bottom of all cosms. All organisms were randomly collected independent of size and sex. For chemical fate analyses, we additionally placed five of the same small glass beakers filled with a 5 cm sediment layer in each cosm. Apart from that, we filled 180 nylon bags (420 μm mesh size, 14 × 11 cm) with 2 g of dried *Populus* leaves without branches, and hanged on day 0 sets of six of them (without previous leaching or conditioning) in each cosm with a nylon string on a bamboo stick to assess leaf litter degradation. Finally, we weighted in 2 g of the same dried leaf material into 30 glass petri dishes (11 cm diameter), placed one on the sediment of each cosm, and covered it with a metal mesh. Two holes in the metal mesh provided access for the benthic macroinvertebrates to assess their feeding behavior on the leaf material. Gentle aeration provided in all cosms through an air stone diffuser prevented temperature stratifications and hypoxia. Water losses by evaporation were compensated weekly with Milli-Q water. From the start of the acclimatization phase, nutrients in the form of 90 μg/L ammonium nitrate (NH_4NO_3) and 15 μg/L potassium phosphate (KH_2PO_4), corresponding to a molar N:P ratio of 20:1, were added (Van den Brink et al., 2016) two times a week.

2.3. *Temperature scenario simulations by TENTACLE*

The ambient temperature treatment simulated natural, daily temperature variations following a day-night pattern with a set maximum temperature of 19.5 °C (Hermann, personal observations; Salo et al. (2017) for temperature references) and a starting time for water temperature increase by heating at 9:00 a.m. and stopping time at 16:00 p.m. (Fig. 1). By doing so, 16.5 °C was reached over the course of the night, resulting in daily temperature fluctuations of 3 °C and ±1.5 °C around an average temperature of 18 °C. The elevated temperature treatment followed the simulated temperature fluctuations of the first treatment with a +4 °C offset by calculating the mean temperature from all ambient cosms at a measuring interval of 2 min. The temperature increase and decrease was set to 0.18 °C/h which is based on personal observations and within the range of approximately 1 °C/h observed in small ponds in Central Europe (Salo et al., 2017). The duration interval of the heatwaves treatment was set at 8 days, which is close to the current average duration (i.e., 8.4 days) of such extreme events in Western Europe (Meehl and Tebaldi, 2004). Additionally, our applied heatwave duration covers the average duration of recorded lake heatwaves of 7.7 ± 0.4 days (Woolway et al., 2021). During the heatwave, the target temperature was set at 26 °C while returning after 8 days back to ambient mode. The cosm experiment included three series of reoccurring heatwaves, each with an 8-day recovery time at ambient temperature. The amounts of added energy was equal for both temperature treatments over the experimental time.

2.4. *Carbendazim spike and analysis in water and sediment*

For the single spike of the model compound carbendazim, a stock solution with a nominal target concentration of 2 g/L was prepared with the solvent dimethyl formamide (DMF). Prior to the spiking event, analytical analysis resulted in 1.96 g/L for the stock solution. Subsequently, 612 μL of the analyzed stock was applied to each cosm on day 0 using a micropipette to gain the nominal concentration of 100 μg/L. By doing so, we took per cosm a 0.5 L filtered (0.45 μm, Acrodisc syringe filter, Merck) water sample to which the fungicide was added. The same volume of DMF without carbendazim was added to the negative controls. Finally, we returned the subsamples to the cosms and stirred the water column gently with a glass stick.

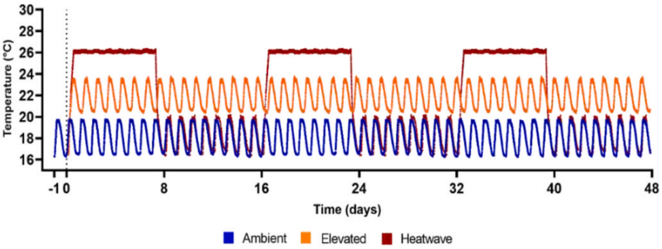

Fig. 1. Temperature treatments of the microcosms subject to three different scenarios (i.e. ambient (blue), elevated (orange), and heatwave (red) in the course of the experiment. The dotted line indicates the experiment start (day 0). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Carbendazim concentrations in water were measured on day −1 prior to the chemical application to check for cross contamination and 4 h after the spiking event followed by day 1, 4, 8, 16, 24, 32, and 48. On these days 1 L of depth-integrated water samples were taken using the Perspex tube. Before putting back to the cosm, a subsample of 3 mL was collected with a 5 mL syringe, filtered (0.45 μm, Whatman, Spartan 13/0.45 RC), and transferred to a 4 mL amber glass vial (BGB, 130401-W) containing a share of 1:3 mL acetonitrile.

To measure carbendazim concentrations in sediment, we collected one glass beaker with a sediment sample on day −1, 4, 16, 32, and 48. We followed the advices by Hogendoorn et al. (2006) for the chemical extraction of carbendazim from sediment. Details of the chemical analytical analyses are provided in *Description S1*. For each temperature scenario, dissipation and accumulation rates of carbendazim in water and sediment were determined by means of exponential models assuming first-order decay and association kinetics, respectively. Dissipation half-lives (DT_{50}) were calculated by ln(2)/k, with k as the dissipation rate constant of the least squares fit. Time weighted average concentrations (TWAs) were calculated by the sum of the carbendazim concentrations of each measured time point, multiplied by the time period and finally divided by the total time of observation.

2.5. *Phytoplankton and physico-chemical water variables*

Phytoplankton was sampled in all cosms on day −1, 4, 12, 20, 28, 36, 44, and 47 by collecting depth-integrated-samples by means of a Perspex tube until a final sample volume of 1 L. Before returning the sampled volume back to the corresponding cosm, subsamples of 2 mL were removed with plastic falcon cups and measured for chlorophyll-a concentrations of green-, brown-, and blue-algae (Phyto-Pam-II, Walz). Water quality was measured *in situ* by using a portable multi-meter (Multi 3630 IDS, WTW) on day −1, 0, 6, 8, 14, 16, 22, 24, 30, 32, 38, 41, 46 and 47. Single measurements in the morning comprised pH, electrical conductivity (EC), and dissolved oxygen (DO) while the latter was also measured in the evening to determine ecosystem productivity rates as the difference between both measurements (Kersting and van den Brink, 1997). Light intensity was measured on the water surface of the cosms in the beginning of the experiment to ensure equal lightning (LI-1500, LI-COR). At a weekly interval and always a day before the next addition of nutrients, 1 L of depth integrated water samples were collected from all cosms with a Perspex tube for nutrient analyses. From this sampled volume, two 50 mL aliquots were collected, one filtered with a 0.45 μm filter (Acrodisc syringe filter, Merck) to analyze nitrate (NO_3^-) and nitrite (NO_2^-), ammonia (NH_3), total nitrogen (N_{tot}), and ortho-phosphate (PO_4^-). The other one was unfiltered to analyze total phosphorus (P_{tot}). Finally, aliquots were transferred to 100 mL polyethylene bottles and analyzed with a Skalar San++ Continuous Flow Analyzer (Skalar, Netherlands) by taking 1 mL subsamples from each bottle.

2.6. *Benthic species' feeding behavior and abundance*

To assess macroinvertebrates feeding behavior before and during treatment phase, petri dishes had been submerged for the first time 8 days prior treatment and were collected on day 0. By doing so, the remaining wet leaf material has been gently collected, dried for at least 24 h at 60 °C to determine the difference in dry weight between the cosms before the carbendazim exposure. Subsequently, all emptied petri dishes were refilled with 2 g of new dried leaf material and placed back to their assigned cosm. After an exposure time of always 16 days during treatment phase, petri dishes were collected and returned on day 16, 32, and 48.

The abundances of the benthic macrocrustaceans (*G. pulex, A.*

aquaticus), and the freshwater snail *L. stagnalis* (small: ≤16 mm, big: ≤37 mm) was assessed at the end of the experiment by emptying and multiple sieving (0.5, 1, and 2 mm) of all cosms.

2.7. Leaf litter decomposition and macrophyte biomass

For each of the three sampling events on day 16, 32, and 48, duplicates of hanging nylon bags were retrieved from each cosm. Thereafter, leaf material was transferred to aluminum bowls and dried at 60 °C for at least 24 h to determine leaf dry weight. Macrophyte biomass was assessed by sampling the whole cosms at the end of the experiment and total plant weight (shoots and roots) per species was determined in the same way as the leaf material.

2.8. Zooplankton

To yield equal zooplankton and phytoplankton community compositions in all cosms at the start of the treatments, water exchanges and mixing were conducted in week −3 and −1. Cosms were emptied to at least 75% of their original water volume and all of the water was mixed in large buckets before refilling. Zooplankton was sampled on day −1 followed by day 6, 14, 22, 27, 33, 38, and 47 from each cosm using a Perspex tube with a length of 40 cm and a diameter of 4.6 cm. Depth-integrated-samples were collected from the cosms until a final sample volume of 1 L was obtained. The total sample was concentrated by means of a 55 μm zooplankton net into a 100 mL polyethylene bottle, preserved with lugol solution, and stored at 7 °C in the fridge for later species identification, while the filtrate was added back to the respective cosm. All macro-zooplankton individuals belonging to the taxa Cladocera, Copepoda, and Ostracoda were identified and counted with a binocular. For the micro-zooplankton individuals of Rotifera and nauplii, a subsample of 1 mL was collected from the concentrated sample and counts were made with an inverted microscope and recalculated to numbers per liter.

2.9. Data analysis

Generalized linear mixed models (GLMMs) were applied to examine the significance of the fixed effects of the different temperature scenarios, the chemical, the sampling days, and their interactions on the different endpoints. The test systems (i.e. microcosms) were considered as random effect to account for background variation. Best model fit and the contribution of the random effect to explained variances was tested by comparing residual errors (sigma), marginal and conditional R^2-values, AIC- and BIC-values. Data distribution skewness was analyzed to see if a log-transformation of the dependent variable in the model is required (Webster and Oliver, 2007). Additionally, visual inspections of normally-distributed residuals were conducted by using histograms and Q-Q-plots. The results of the best fit model with estimates of the fixed, random, and interaction effects were assessed in an ANOVA table with F-tests and p-values using Satterthwaite's correction method. Subsequently, estimated marginal means (EMMs) and their contrasts of the best model were calculated to determine significant effects with Tukey p-value and confidence level adjustment method. Standardized effect sizes (Cohen's d) were calculated (Nakagawa and Cuthill, 2007) for further comparison of noted series of significant effects. The applied GLMM structure was:

$$O_{p,q,r,s,n} = \mu + C_p + T_q + D_r + C_p \times T_q + C_p \times D_r + T_q \times D_r + C_p \times T_q \times D_r + MC_s + \varepsilon$$

where $O_{p,q,r,s,n}$ represents the *n*th observation of a response variable with μ as the mean (i.e. intercept) and ε as the error component, under *p*th chemical treatment (*C*, two levels), *q*th temperature treatment (*T*, three levels), *r*th sampling day (*D*, levels were response variable dependent), and *s*th microcosm test system (*MC*, thirty levels).

The structure of the zooplankton community was analyzed with the principle response curves (PRCs) method using CANOCO version 5 (Van den Brink and Ter Braak, 1998, 1999; Ter Braak and Smilauer, 2012) for the analysis of significant effects caused by the different temperature treatments, the chemical and their interactions. Prior to the community analyses, zooplankton abundance data was $\ln(2x + 1)$ transformed (see Van den Brink et al. (2000) for rationale). The PRC technique is based on redundancy analysis (RDA) and extracts responses of the community composition to the stressors in time from the total variation in community composition. In the PRC, all treatments and the interaction with time are included as explanatory variables, while sampling day is included as covariate. Thus, treatment effects versus control are displayed as a regression coefficient (c_{dt}) on the y-axis against time on the x-axis. Calculated species weights (b_k) next to the main analysis present the affinity of each individual taxon within the community response. The overall significance of the treatment effects on the variation in community composition displayed in the PRC diagram was tested by performing Monte Carlo permutations. Significance of effects on each individual sampling day were assessed by conducting Monte Carlo permutation tests under the RDA option (Van den Brink and Ter Braak, 1999).

Finally, ecological relationships and effects of temperature and carbendazim on macroinvertebrates, leaf litter decomposition, macrophytes, and zooplankton were assessed with a structural equation model (SEM). Additionally to the direct effects of the stressors, relationships for macroinvertebrate feeding and between macrophytes and zooplankton were assessed. By doing so, ecological relationships were tested. Since sampling intervals differed among the observed nodes, mean scores were calculated for the macroinvertebrates feeding behavior, leaf litter decomposition, macrophyte dry weight and ash-free dry weight combined, and zooplankton data to obtain a uniform data matrix. SEM fit was ensured by a maximum likelihood estimation method. Model quality was evaluated by the results of Chi-squared tests, goodness of fit index (GFI), comparative fit index (CFI), standardized root mean square residual (SRMR), and root mean square error of approximation (RMSEA). Statistical tests were performed with a significance level $\alpha = 0.05$ indicated by *0.01, **0.001, and ***0. The univariate statistics and SEM analyses (lavaan-package) were performed with R (R Core Team, 2020). Temperature effects were analyzed by contrasting elevated temperatures and heatwave scenarios against ambient conditions and by comparing both with each other across non-chemical and chemical treatments. Chemical effects were analyzed by contrasting carbendazim against chemical control across all temperature scenarios.

3. Results

3.1. Carbendazim concentrations in water and sediment at elevated and heatwave temperatures

One day after carbendazim application, measured carbendazim concentrations in water were on average 93 μg/L (i.e. 93% of the nominal concentration) with moderate dissipation ($R^2 = 0.93$–0.97, Fig. 2A). Significantly lower carbendazim concentrations were noted for the heatwave treatment on day 32 ($p = 0.001$) and day 48 ($p \leq 0.001$) compared to ambient treatment. Additionally, carbendazim concentrations were significantly lower for heatwave on day 32 ($p = 0.02$) and day 48 ($p = 0.002$) compared to the elevated treatment. Lowest DT_{50}-values and time weighted averages (TWAs) in water were noted under heatwaves followed by elevated and ambient temperatures (Table 1). TWAs for carbendazim in sediment (Table 1) were highest under elevated temperatures, followed by almost similar concentrations under ambient temperatures and heatwaves ($R^2 = 0.62$–0.89, Fig. 2B).

Chlorophyll-a concentrations ranged from about 6 to 300 μg/L for green algae (Fig. S1A) and 3–28 μg/L for brown algae (Fig. S1B), while blue algae were not detectable. Except for a temperature*chemical

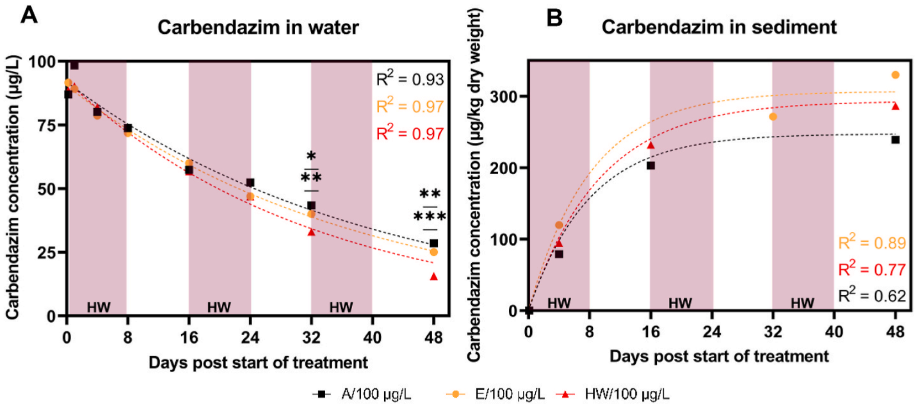

Fig. 2. Mean carbendazim concentrations (µg/L) in (A) water and (B) sediment (µg/kg dry weight) under ambient (A), elevated (E) and heatwave (HW) temperatures over the course of the experiment (48 days). Red blocks indicate the presence of a HW. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
DT_{50}-values with 95% confidence intervals (C.I.) and time weighted averages (TWA) of carbendazim (µg/L) in water and sediment under ambient (A), elevated (E), and heatwave (HW) conditions.

	Water			Sediment
	DT_{50} (days)	95% C.I.	$TWA_{48\ days}$ (µg/L)	$TWA_{48\ days}$ (µg/L)
A	27.97	25.08–31.40	47.9	240.3
E	26.26	24.39–28.37	45.9	251.6
HW	22.37	20.87–24.03	41.8	237.4

interaction effect on day 36 for green algae, no significant differences were found. Among the different physico-chemical water variables (Table S2), dissolved oxygen (DO) in the morning and evening (Fig. 3A and B), ecosystem productivity rates (Fig. 3C), and electrical conductivity (Fig. 3F) show lasting and significant temperature- and chemical-induced differences while pH (Fig. 3D and E) seemed less affected. Similar effect sizes for DO and ecosystem productivity rates were noted under elevated temperatures and for EC under heatwaves, however, effects were stronger with time for DO while exposed to heatwaves (Table S2). Significant temperature*chemical interaction effects appeared for all variables when a heatwave was present. The

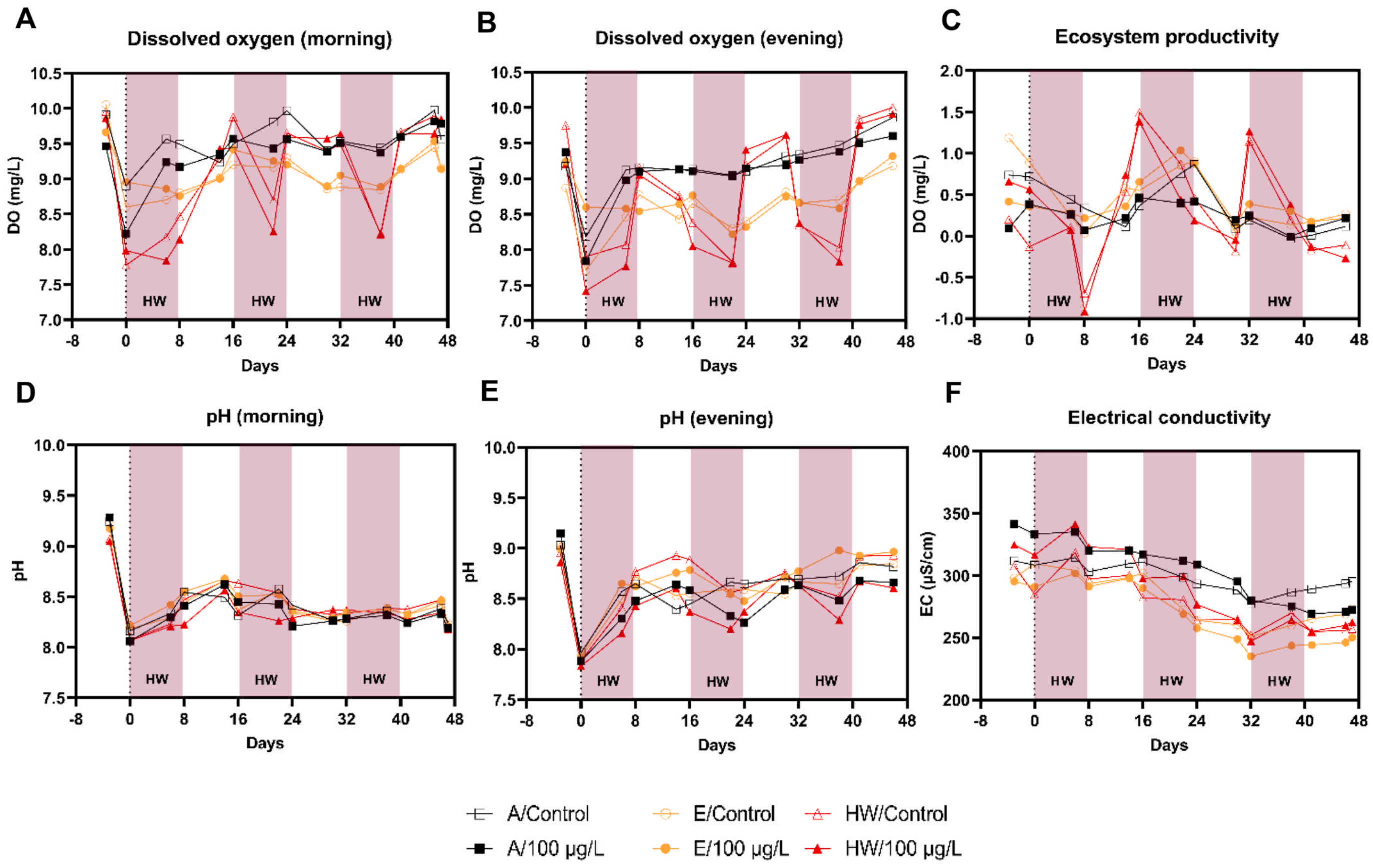

Fig. 3. Physico-chemical variables (geometric mean, n = 5) with (A,B) dissolved oxygen (DO), (C) ecosystem productivity, (D,E) pH, (F) electrical conductivity. DO and pH were measured in the morning and evening. Red blocks indicate the presence of HWs and the dotted line the experiment start. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

photoperiod ranged from 0 to 170 μmol/m^2/s for a day-night cycle equally across all cosms. Nutrient concentrations were similar across all treatments and followed the same patterns over time with only significant differences at isolated timepoints (Fig. S2). Sudden changes in physico-chemical dynamics and nutrients during day 0 (Fig. 3, Fig. S2) are likely the result of resuspended sediment, adding litter bags and DMF to the cosms and were not stressor-associated.

3.2. Benthic species' feeding and abundance

An overview of all the significant results of section *3.3* and *3.4* is given in Table S1. A heatwave effect with significantly increased feeding ($p = 0.003$) was noted on day 32 compared to ambient temperatures (Fig. S3A). On the same day, a significant temperature*chemical interaction effect ($p = 0.0003$) was noted. Furthermore, temperature increased feeding on day 48 significantly for elevated temperatures ($p = 0.01$) and heatwave ($p = 0.02$) compared to ambient temperatures. Considering benthic species' abundance on day 48, a significantly higher abundance ($p = 0.04$) of small *L. stagnalis* individuals was found for elevated temperatures (Fig. S3B). Carbendazim effects ($p = 0.01$) on these individuals resulted in significantly higher abundances for the chemical treatment. For the big individuals of *L. stagnalis*, heatwave effects resulted in significantly ($p = 0.002$) higher abundances (Fig. S3B). Similarly as for the small individuals of *L. stagnalis*, significantly higher abundances ($p = 0.001$) of big individuals were found under carbendazim stress. By grouping all snail sizes together, a significantly higher abundance ($p = 0.03$) was found for elevated temperatures with significantly higher abundances ($p = 0.002$) when exposed to carbendazim (Fig. S3B).

3.3. Leaf litter decomposition and macrophyte biomass

Considering leaf litter decomposition in the course of the experiment (Figs. S4A,B,C), a significant temperature*chemical interaction effect ($p = 0.02$) was found on day 16 (Fig. S4A). Additionally, carbendazim caused a significantly lower leaf litter decomposition ($p = 0.04$) on day 48 (Fig. S4C). Since almost all planted shoots of *E. nuttallii* became stunted, only the results of *M. spicatum* were considered and showed a significantly lower macrophyte biomass ($p = 0.03$) for the heatwave treatment compared to elevated temperatures (Fig. S4D).

3.4. Zooplankton community

We identified in total 29 different zooplankton taxa, with 12 classified as macro- and 17 classified as micro-zooplankton. Three cladocerans (*Chydorus sphaericus*, *Alona costata,* and *Daphnia magna*), one Copepoda (nauplius), and five rotifers (*Notholca labis*, *Lecane lunaris*, *Anuraeopsis fissa*, Ascomorpha, *Monommata longiseta*) dominated the community. The zooplankton taxa which had the highest positive species weights in the overall PRC were *C. sphaericus*, *A. costata*, *D. magna,* and nauplius (b_k-values in Fig. 4), indicating a decrease in abundance, while *N. labis* and Ascomorpha had lower positive values, and *L. lunaris* presented a negative weight (Fig. 4), indicating an increase in abundance. The first PRC of the community revealed that it displays a significant 36% of the variation in species composition ($p = 0.0001$) due to the carbendazim and the different temperature treatments (Fig. 4). Of the total variance present, 43% was explained by factor time and 16% by factor treatments, while the remaining variance is due to differences between replicates. The PRC diagram showed a clear chemical-dependent separation of the treatments and not a temperature separation (Fig. 4). The second PRC of the zooplankton community significantly displayed 15% of the variation in species composition ($p = 0.0473$) describing temperature effects (Fig. S5A). Finally, non-significant individual community effects were only visible by disentangling the combined stressor effects of the temperature treatments and the chemical by making separate PRC diagrams (Figs. S5B and C). Monte Carlo permutation tests confirmed the differences shown in the PRCs and showed that heatwaves have longer lasting effects on community structure than elevated temperatures indicated by the consistent pattern of significant effects, especially in the presence of carbendazim (Table 2). No significant effects were found when comparing heatwaves versus elevated temperatures across non-chemical and chemical treatments. By contrasting the carbendazim treatments against chemical control under the different temperature treatments, an early and longer-lasting series of significant effects were found for chemically stressed communities under heatwaves compared to elevated and/or ambient temperatures with delayed significant effects. Significant and lasting temperature*chemical stressor interactions were noted after the second heatwave on day 27 through 38 (Table 2).

3.5. Zooplankton populations

Individual taxa dynamics of the nine most affected species are shown in Fig. 5A–I, including significant effects on abundance of the individual and interacting stressors per individual day. While nauplius revealed significantly adverse carbendazim effects early from day 6 continuously until the end of the experiment, *L. lunaris* showed significantly positive effects on its abundance due to the chemical treatment on day 14, 22 and on day 47. In comparison to these, chemical effects on Copepoda (nauplius) and the rotifer, the cladocerans *C. sphaericus* and *A. costata*

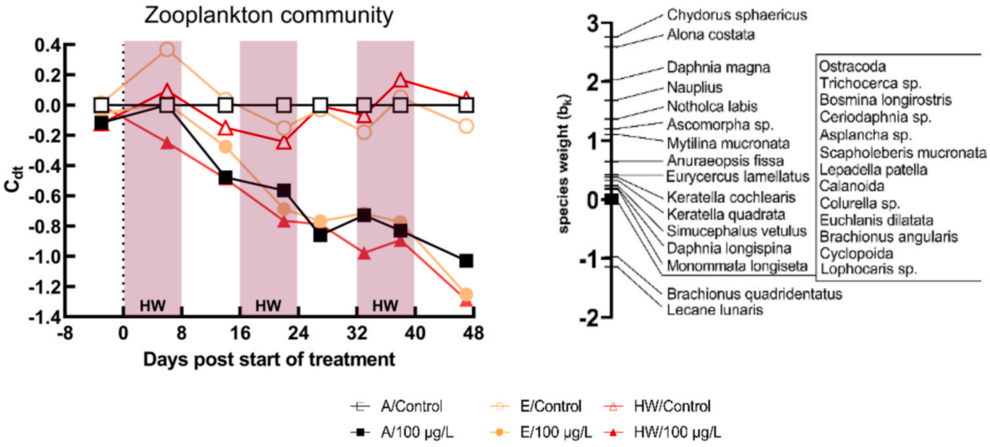

Fig. 4. Principle response curve (PRC) depicting combined temperature and carbendazim effects on the zooplankton community over time. Samples weights (c_{dt}) present differences between control and treatments on a sampling day. Taxon specific affinity of the community related to the response indicated by the PRC is presented by the species weight (b_k). The dotted line indicates the experiment start.

Table 2

Significant results (p-values) from Monte Carlo permutation tests performed under the redundancy analyses (RDA) option testing individual temperature effects of elevated (E) and heatwave (HW) versus ambient (A), between E and HW, the chemical effects of carbendazim (C1) versus control (C0), and the interaction of temperature (T) and chemical (C) on the zooplankton community on each sampling day.

Day	Temperature									Chemical				Combination
	E vs. A			HW vs. A			HW vs. E			C1 vs. C0				Interaction
	C0	C1	C0C1	C0	C1	C0C1	C0	C1	C0C1	A	E	HW	AEHW	T*C
−1					**0.02**									
6												**0.04**	**0.01**	
14													**0.0072**	
22				**0.0072**	**0.02**	**0.0004**						**0.0072**	**0.0003**	
27					**0.0072**	**0.0092**				**0.0072**		**0.0072**	**0.0001**	**0.04**
33					**0.0072**	**0.02**				**0.0072**	**0.03**	**0.0072**	**0.0001**	**0.03**
38	**0.05**	**0.03**			**0.0072**					**0.0072**	**0.0072**	**0.0072**	**0.0001**	**0.02**
47											**0.0072**	**0.0072**	**0.0001**	

demonstrated significantly adverse carbendazim effects from day 22 until the end of the experiment, while *D. magna* showed, with the exception of day 33, significant effects from day 14 until the end of the experiment. *N. labis* and Ascomorpha showed significantly adverse carbendazim effects only on day 33. Significant temperature effects were discernible for all taxa except for *A. costata* and *D. magna*. In particular, elevated temperatures resulted on day 6 in a significantly increased abundance for Ascomorpha. Further significant effects of elevated temperatures were either adverse (*N. labis*) or positive (nauplius) at isolated timepoints. Heatwaves caused significantly positive and adverse effects during the first and second occurrence, respectively, for *L. lunaris* (day 6 and 22). Adverse effects of the second heatwave were significant for *C. sphaericus* (day 22), *M. longiseta* (day 22), and for *N. labis* (day 22 and 27), while the third heatwave adversely affected Ascomorpha (day 33 and 47) and *A. fissa* (day 47). Significant temperature*chemical interaction effects were noted for all taxa in the course of the experiment except for *A. costata, D. magna*, *L. lunaris*, and *A. fissa*.

3.6. Structural equation modelling of ecological relationships and cause-effect chains

The SEM analysis showed that temperature and carbendazim both significantly had a positive relationship with the abundance of the benthic macroinvertebrate species, having a stronger chemical relation indicated by the standardized path coefficients (SPCs) of 0.33 and 0.51, respectively (Fig. 6). Temperature and carbendazim stressors were negatively related to macrophytes but this was non-significant (SPCs of -0.13, −0.29, respectively). Macrophytes had a significantly positive relation to zooplankton (0.29). Temperature presented only a minor non-significant negative relation (−0.04) with the zooplankton community, while carbendazim had a significantly adverse relationship (−0.56). Temperature had a significant negative relationship with leaf litter decomposition (−0.39), whereas carbendazim related positively, but non-significant (0.29). Leaf litter decomposition plays a significant, positive role in the feeding of macroinvertebrates (0.63). However, macroinvertebrates abundance showed a non-significant, negative relationship with feeding (−0.20). Temperature and carbendazim together explained 37% of the variation in macroinvertebrates abundance, 23% of the variation in leaf litter decomposition, 11% of the variation in macrophytes, and 50% of the variation in zooplankton. Leaf litter decomposition and macroinvertebrates abundance together explained 43% of the variation in feeding.

4. Discussion

4.1. Carbendazim fate and effects under elevated temperatures and heatwaves

Our study found carbendazim to be moderately persistent in the water layer with DT_{50}-values of about 27 days under ambient temperatures, 26 days under elevated temperatures and 22 days under heatwaves (Table 1). In comparison, carbendazim degradation (DT_{50}) ranged from 15 to 17 days in tropical outdoor microcosms under 30 ± 3 °C (Daam et al., 2009) and 6–25 weeks under constant temperate temperatures (19 ± 2 °C) in a climate chamber microcosm study (Cuppen et al., 2000). The reported temperature effects on carbendazim fate in these studies are consistent with our findings and imply that carbendazim degrades faster at higher temperatures. Both referring studies applied a pesticide formulation instead of our analytical standard which might explain the discrepancies in the DT_{50s} which may also be caused by variations in compound stability *per se*. Indeed, previous experiments demonstrated high persistence of the formulated carbendazim (Van Wijngaarden et al., 1998; Daam and van den Brink, 2007; Del Arco et al., 2015). Additionally, the microcosms and jars used in those studies were simplified test systems containing no sediment and macrophytes which may absorb carbendazim from water and thus influence dissipation. Apart from the direct temperature induced effects on degradation, alterations in physico-chemical properties in the microcosms, such as the significant differences in DO and pH, may have also have affected the fate of carbendazim since previous studies demonstrated a positive correlation between degradation rate, DO and pH for similar experimental conditions (Panadés et al., 2000). Carbendazim is thermally stable at temperatures up to 50 °C with degradation in water and soils being primarily attributed to microbial communities (Tomlin, 2009). Microbes can be affected by biotic (e.g., macrophytes, soil) and abiotic (e.g., chemical, temperature) factors which can indirectly alter chemical fate as well (Singh et al., 2016). In fact, leaf litter decomposition, as an indicator for microbial activity, was significantly lower under carbendazim at the end of the experiment (Fig. S4C).

Significant chemical effects under elevated temperatures adversely affected the zooplankton community and appeared late in the course of the experiment when compared to early and longer-lasting heatwave effects (Table 2). Previous cosm experiments demonstrated a slow response of various ectotherms towards carbendazim, however, more pronounced and faster responses were observed at increasing doses (Cuppen et al., 2000; Van den Brink et al., 2000). Moreover, assuming all adverse effects were direct, our results suggest that carbendazim acts temperature-dependent with a slower mode of action on the community under elevated temperatures compared to heatwaves. Longer-lasting significant chemical effects on the community were found under heatwaves compared to elevated temperatures (Table 2) with no prompt recovery (Fig. S5B). Such a temperature-induced increase in carbendazim ecotoxicity is comparable to the temperature-chemical toxicity relationship of other fungicides, such as pyrimethanil and azoxystrobin (Seeland et al., 2012; Billet et al., 2022). Some of the most reported rationales behind this are increased biotransformation of pesticides to more toxic and bioactive metabolites (Harwood et al., 2009), and elevated uptake rates due to an increased food consumption and

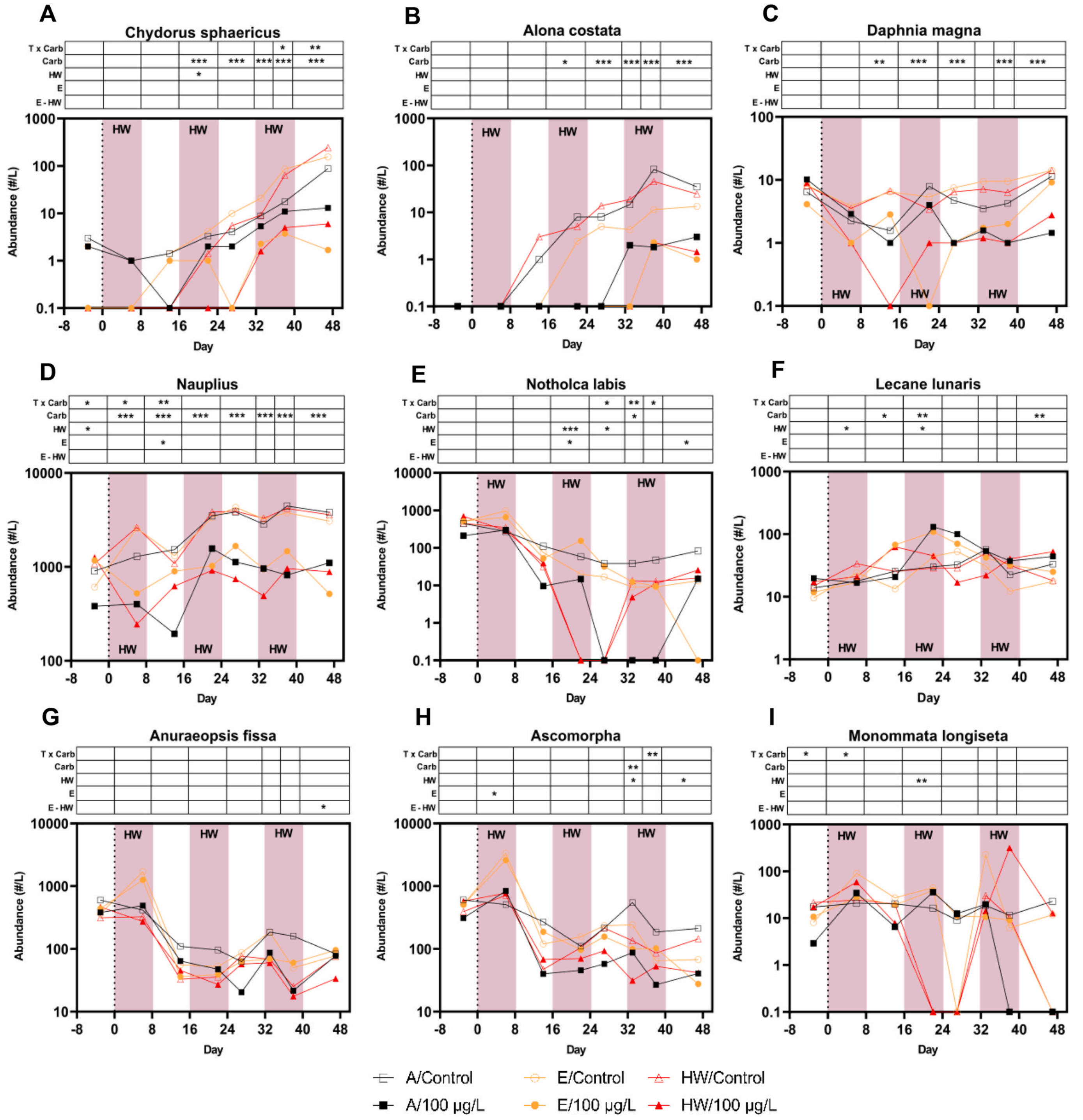

Fig. 5. Zooplankton taxa dynamics of the nine most important species in the PRC community analyses. Figure A to I show the geometric means of the counted organisms per individual treatment of (A) C. sphaericus, (B) A. costata, (C) D. magna, (D) Nauplius, (E) N. labis, (F) *L. lunaris*, (G) A. fissa, (H) Ascomorpha, and (I) M. longiseta. The absence of individuals is denoted by 0.01. Red blocks indicate the presence of HWs and the dotted line the experiment start. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

metabolism (Noyes et al., 2009; Huang et al., 2023). Albeit carbendazim caused adverse effects under both elevated temperature and heatwave regimes, the community demonstrated resilience towards thermal extremes, with recovery back to the initial state after the second heatwave (Fig. S5B). Similarly, zooplankton community recovery dynamics towards insecticides were more apparent and faster under higher temperatures (Arenas-Sánchez et al., 2018; Vilas-Boas et al., 2021). Given the observed rise in community tolerance, it is likely that sensitive species have been diminished by heatwaves and carbendazim while the non-sensitive ones survived over time, implying the pollution induced community tolerance (PICT) concept (Tlili et al., 2016). Conversely to these community dynamics, persistent adverse effects of carbendazim with no recovery were noted under elevated temperatures (Fig. S5B). Besides direct temperature and chemical effects, our observations could be the result of disadvantageous changes in the competitiveness of some taxa because strength of interspecific competition was revealed as one of the mechanisms determining recovery (Knillmann et al., 2013). Consistent carbendazim effects under the different temperature

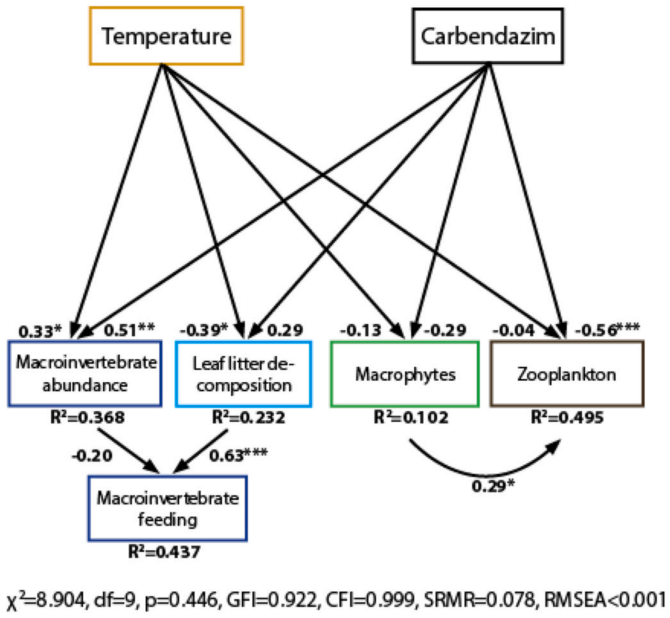

Fig. 6. Structural equation model (SEM) presenting the effects of temperature and carbendazim on macroinvertebrate abundance, leaf litter decomposition, macrophytes, and zooplankton. Further relationships for macroinvertebrate feeding and between macrophytes and zooplankton are displayed. Arrows denote significantly and non-significantly positive or negative relationships with standardized path coefficients. Goodness-of-fit statistics for the SEM are below.

scenarios manifested over time at the species level for *C. sphaericus* (Fig. 5A), *A. costata* (Fig. 5B), *D. magna* (Fig. 5C), and nauplius (Fig. 5D), suggesting increased chemical sensitivity for these cladocerans and the copepod when compared to the studied rotifers (Fig. 5E–I). Furthermore, the individual dynamics showed, especially for nauplius (Fig. 5D), *L. lunaris* (Fig. 5F), *A. fissa* (Fig. 5G), and Ascomorpha (Fig. 5H), greater adverse effects of carbendazim under heatwaves than elevated temperatures, indicating lower tolerance of these taxa towards thermal extremes. This goes in line with previous toxicity studies with copepods and rotifers with an increased chemical sensitivity under elevated, constant temperatures (Cairns et al., 1978; Li et al., 2014).

4.2. Temperature effects

There was not a consistent pattern in adverse effects on the zooplankton community structure for elevated temperature fluctuations and repeated heatwaves under chemical control conditions (Table 2). Similar temperature effects on community characteristics have been observed in cosm experiments from Spain (Arenas-Sánchez et al., 2018), Denmark (Šorf et al., 2015), and the UK (McKee et al., 2002). These studies indicated that subtle community effects caused by experimental warming can be inconsistent. One of their common findings is a temperature-induced shift from larger cladocerans to smaller rotifers. Conversely to these findings, our study revealed relatively low rotifer densities (e.g., *N. labis, L. lunaris,* Ascomorpha*, A. fissa,* and *M. longiseta*), compared to cladocerans (e.g., *E. lamellatus, D. magna, A. costata,* and *C. sphaericus*) and Copepoda nauplii in the elevated temperature and heatwave treated cosms compared to the ambient ones (Fig. S5C). Temperature effects on cladocerans are more studied than on rotifers. One of the major drivers causing variations in zooplankton responses are strong interlinks between food availability and quality, thermal stress and tolerance, and species metabolism (Starke et al., 2021). Considering phytoplankton as a potential food source of zooplankton, our study showed that food availability was likely not a limiting factor due to non-significant effects of warming on chlorophyll-a concentrations. Although this is contradictory to an expected increase in algal growth rates towards warming (Padilla-Gamiño and Carpenter, 2007), increased zooplankters metabolism may have resulted in higher grazing rates and thus suppressing the increase in chlorophyll-a (Sommer and Lewandowska, 2011). Similarly to our observations, Strecker et al. (2004) found no temperature effects on total phytoplankton biomass, however, reported a significant alteration in community composition by favoring phytoflagellates over larger filamentous green algae. Although we miss information on phytoplankton species composition for this experiment, freshwater phytoplankton collectively showed temperature-dependent growth responses favoring smaller-sized individuals (Strecker et al., 2004; Zohary et al., 2021). Thus, alterations in the food quality for zooplankters can be assumed. One possible explanation for the significant increase in rotifer abundances of Ascomorpha followed by collapsing populations under elevated temperatures (Fig. 5H) is the observed warming response of a model rotifer which demonstrated first significantly increased offspring numbers but reduced survival times of the next generations under heating conditions of +10 °C and +20 °C (Zhang and Geng, 2012). Similar but non-significant population dynamics have been also observed for *N. labis* and *A. fissa* (Fig. 5E,G). The rotifer species *N. labis*, *L. lunaris*, and *M. longiseta* (Fig. 5E,F,I), and the cladoceran *C. sphaericus* (Fig. 5A) showed a direct temperature effect with significantly lower abundances during the second heatwave, whereas lagged adverse heatwave effects occurred for *A. fissa* and Ascomorpha (Fig. 5G and H). Interestingly, *C. sphaericus* increased in abundances in the course of the experiment, suggesting differences in species' thermal resilience and adaptability (Saucedo-Ríos et al., 2017; Li et al., 2019). Similarly for both temperature scenarios and as observed likewise by Vilas-Boas et al. (2021), the population dynamics of Ascomorpha indicated declining abundances compared to increases of copepod nauplii and cladocerans in the course of the experiment. Besides differences in organisms' thermal traits to deal with temperature-altered environments, the observed population responses may be explained by variations in life-history strategies. Copepods, for instance, showed under heating (+5 °C) higher energy allocation in reproduction by significantly higher egg production (Devreker et al., 2009), possibly explaining the increasing abundances in our study. The rotifer species *N. labis, A. fissa,* Ascomorpha, and *M. longiseta* (Fig. 5E,G,H,I) demonstrated a decline in species abundances towards heatwaves with no recovery back to initial population sizes in the course of the experiment. Heating characteristics across space and time, such as intensity, duration, and frequency, may play a crucial role in explaining the mechanisms which modulate recovery (Walberg and Green, 2021). While rotifers significantly declined under heatwaves in our experiment, cladocerans indicated a positive response likely because thermal conditions were still within their acceptable range of up to 28 °C (Starke et al., 2021). Furthermore, our observed increasing abundances of the cladoceran species *C. sphaericus, A. costata,* and *D. magna* under heatwave and elevated temperatures (Fig. 5A,B,C) during the experiment are in line with increases in cladoceran population dynamics under heatwave-similar conditions as observed by Miracle et al. (2011). Higher abundances of these large-bodies zooplankton taxa may have further increased predation and competitive pressures for food on small-bodied rotifers (MacIsaac and Gilbert, 1989), which are thus likely reasons for the observed suppression. Interestingly, the community responses to both thermal stressors followed a similar pattern which was initially positive, then negative (Fig. S5C). Since the effects of heatwaves compared to elevated temperatures on the community did not differ significantly, warming may shape community responses similarly and independently of the heating patterns when the amount of induced energy is equal over time.

4.3. Interaction effects of elevated temperatures, heatwaves and carbendazim

Our analyses showed that carbendazim adversely affected the zooplankton community over time, with effects being more severe for heatwaves than at elevated or ambient temperatures (Table 2, Fig. 4). While heatwaves and carbendazim had significant interactive effects on the zooplankton community immediately after the first heatwave, ambient or elevated temperatures and carbendazim had rather lagged community effects. These combined stressor responses are in line with studies showing rapid and adverse zooplankton community responses towards pesticide stress under heatwave-like conditions (Arenas-Sánchez et al., 2018; Vilas-Boas et al., 2021). The lack of recovery dynamics of the zooplankton community across all combined treatments in our experiment (Fig. 4) is possibly the result of exacerbating effects through temperature*chemical interactions similar to those observed on cladocerans by nullifying or converting former positive heatwave effects through carbendazim (Miracle et al., 2011; Vilas-Boas et al., 2021). In fact, the most sensitive populations in our study, Cladocera and Copepoda (Fig. 5A,B,C,D), showed a clear compensating temperature effect by increasing species abundances over time. However, significant temperature*chemical interaction effects demonstrate an attenuating chemical-dependent effect, resulting in diminished population sizes. Given that temperature is the primary stress factor in ectotherms to chemical sensitivity (Heugens et al., 2003), observed differences in multiple stressor responses of rotifers, including more tolerant taxa like *L. lunaris,* may be explained by species-specific temperature preference ranges (Mikschi, 1989).

4.4. Ecological relationships and cause-effect chains

The SEM confirmed our previous findings on profound carbendazim effects on the zooplankton community by showing a significantly negative relationship compared to a modest negative impact of temperature. Interestingly, the zooplankton community positively related to macrophytes, highlighting the significantly positive impact of macrophytes on zooplankton microhabitats by determining community structure and diversity (Choi et al., 2014). Temperature and carbendazim significantly explained the positive effects observed on the abundances of macroinvertebrates with carbendazim playing a more important role. This was especially true for *L. stagnalis*, which showed higher abundances for combined treatments in contrast to *A. aquaticus* and *G. pulex,* which showed lower and no abundances, respectively (Fig. S3B). Our findings are consistent with previous reported carbendazim effects on macroinvertebrate communities at the same carbendazim dose (Cuppen et al., 2000) and single species tests (Van Wijngaarden et al., 1998). Additionally, increased *L. stagnalis* populations are conclusive with these studies, indicating higher chemical tolerance (Kwakye et al., 2021). Leaf litter decomposition was significantly, negatively related to temperature while non-significantly, positively to carbendazim. Our results go in line with recent findings on significantly increased dry biomass loss under similar warming and heatwave treatments (Li et al., 2021). While higher temperatures can increase microbial metabolic rates which in turn lead to higher leaf decomposition (Brown et al., 2004), they may also affect the microbial community composition by favoring species of warmer climate (Fernandes et al., 2012). Furthermore, the addition of untreated leaf material in the litter bags presumably leached high amounts of dissolved organic carbon (Li et al., 2021), which could have facilitated microbial growth and activity on the leaf litter. The reported carbendazim effects by Cuppen et al. (2000) contradict our findings. However, the differences in their study were noted at ten times higher doses than in the present study, indicating concentration dependent leaf degradation effects. Additionally, the fungicide may have caused community shifts by replacing decomposing aquatic hyphomycetes with bacteria (Feckler et al., 2018), which would have indirectly lowered the palatability of the leaf material for leaf-shredding organisms (Bundschuh et al., 2011). In agreement with previous studies that showed no significant carbendazim effects on dry biomass of the macrophyte *Myriophyllum* (Van den Brink et al., 2000), carbendazim was more important than warming in explaining the negative relationship for *M. spicatum*. Other indirect effects, such as the feeding pressure of *G. pulex* or *A. aquaticus*, as observed by Van den Brink et al. (2000), cannot be primarily the reason for our observed differences in macrophyte biomass because of the low abundances of these taxa. However, the high prevalence of *L. stagnalis*, particularly in the combined stressor treatments, and their potential impact through macrophyte grazing (Wong et al., 2010) is not neglectable and may indicate a higher vulnerability of the macrophyte to grazing than feeding effects. Macroinvertebrate feeding was significantly positively related to microbial leaf litter decomposition whereas the macroinvertebrate abundances *per se* seemed less important. Considering the low numbers of leaf shredders like *G. pulex* and *A. aquaticus* at the end of the experiment, macroinvertebrate feeding was significantly masked and shaped by microbial leaf litter decomposition. In this context, low functional diversity of decomposers is associated with reduced litter decomposition (Handa et al., 2014). It remains questionable though to what extent functional redundancy serves as a mitigation strategy and how the stressors affected the microbial community structure.

5. Conclusion

Our research demonstrated that temperature-induced stress of environmentally realistic elevated temperatures and heatwaves may affect freshwater ecosystems under carbendazim stress, causing significant alterations and adverse effects on the chemical fate, ecosystem properties, and freshwater biota, respectively. Temperature-induced ecosystem effects are complex because, depending on the endpoint and trophic level under investigation, responses may be significantly positive, negative, or interactive with other stressors like carbendazim, resulting in significant ecological cause-and-effect chains across trophic levels. Considering an increased threat of carbendazim under temperature extremes, our findings motivate further investigations on freshwater ecosystem responses towards various temperature patterns in combination with pesticide stress to elucidate how these ecosystems are at risk from climate change.

Author statement of the study

Markus Hermann: Conceptualization, Methodology, Software, Formal analysis, Investigation, Data Curation, Writing – original draft, Writing – Review & Editing, Visualization. **Edwin T.H.M. Peeters:** Conceptualization, Methodology, Formal analysis, Writing – review & editing, Supervision. **Paul J. Van den Brink:** Conceptualization, Methodology, Formal analysis, Resources, Writing – review & editing, Supervision, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The supporting data of this study can be found online at https://doi.org/10.17632/dygk3rcgy6.1.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2023.121498.

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