# **Recurrent heatwaves slow down the recovery of a phytoplankton community**

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**Data Accessibility Statement:** Data and code to reproduce the analysis and figures is available at <https://github.com/FrancescoPola/rescue_critical_slowing>.

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## **Abstract**

Heatwaves (HWs) are predicted to increase in frequency and severity due to climate change. Yet, there is limited information about how ecological resilience of aquatic communities is going to be impacted by recurrent HWs. Here, we used data from an outdoor freshwater mesocosm experiment where a semi-natural phytoplankton community was exposed to three subsequent HWs. The data was used to test two different hypotheses regarding community and ecosystem responses to recurrent perturbations: critical slowing down and rescue. Slowing down would determine a reduction in resilience and eventually a community or ecosystem collapse, whereas rescue would increase community or ecosystem resilience and maintain stable community and ecosystem properties. The results of our experiment showed evidence for critical slowing down, but not for rescue. The recovery capacity of phytoplankton biomass and dissolved oxygen gradually decreased after the first two HWs, and sharply declined after the third one. The decline in these community and ecosystem properties were linked to a significant compositional turnover in the phytoplankton community. Although we did not find evidence for a transition into an alternative stable state, the results of this study provide insight into how the overall resilience of a phytoplankton community may decline in the presence of recurrent heatwaves. Thus, we highlight the importance of monitoring the slowing down of recovery of aquatic communities experiencing repeated exposure to severe perturbations.

## **Introduction**

Climate change is an urgent global challenge characterized by significant alterations in weather patterns (IPCC 2023). One of the most concerning aspects of climate change is the increasing frequency and intensity of extreme events, such as hurricanes, floods, droughts, and heatwaves (Fischer *et al.* 2021). Particularly, heatwaves (HWs) have been projected to increase in frequency and severity globally (Perkins *et al.* 2012), affecting the freshwater realm (Woolway *et al.* 2021, 2022). Several studies show that HWs can detrimentally impact the diversity and functioning of freshwater ecosystems (Correa-Araneda *et al.* 2020; Mouthon & Daufresne 2006; Polazzo *et al.* 2022; Woodward *et al.* 2016). Yet, the effects of HWs on ecological stability have hardly been assessed (Polazzo *et al.* 2022). The few studies that investigated the effects of HWs on ecological stability have shown that HWs can negatively affect several dimensions of functional and compositional stability of freshwater populations and communities, including resistance, recovery and temporal stability (Polazzo *et al.* 2023; Ross *et al.* 2021).

Additionally, HWs have been reported to decrease the resilience of aquatic ecosystems, causing critical transitions to alternative stable states (Bertani *et al.* 2016; Meunier *et al.* 2024; Turner *et al.* 2020; Wernberg *et al.* 2016). In this context, resilience is defined as the ability of a system to absorb perturbations without transitioning to an alternative equilibrium or stable state (Holling 1973). However, evidence for HW – driven abrupt shifts or collapses is limited in freshwater systems (Bertani *et al.* 2016; Filiz *et al.* 2020; Polazzo *et al.* 2022). Scarce support for HW related collapse may be linked to the fact that most empirical studies analyse the impact of a single HW event (Polazzo *et al.* 2022), and the few available studies considering recurrent HWs have not focused on assessing cumulative effects on ecological resilience (Hermann *et al.* 2023, 2024). Yet, with heatwaves expected to become more common in the future, concerns have raised about whether and how natural communities can sustain multiple recurring HWs.

How repeated perturbations affect the resilience of a system can be understood through two major ecological frameworks: critical slowing down and community or ecosystem rescue. Critical slowing down is the process by which functional and / or structural recovery of communities decreases when they are close to a tipping point because the internal stabilizing forces of the community become weaker (Veraart *et al.* 2012). The exact shifting point is notoriously difficult to predict, as ecosystems exhibit complex, nonlinear interactions among various biotic and abiotic components, where small changes can lead to disproportionate effects (van Nes & Scheffer 2007). Therefore, the focus has shifted to deducing processes from patterns. This involves identifying observable signals in measurable endpoints of a biological system that indicate changes in the system’s behaviour, which may result in a critical transition. In the last two decades, the phenomenon known as critical slowing down has been indicated as a possible early warning signal (EWS) of an approaching abrupt shift, derived from dynamic systems theory (Rietkerk *et al.* 1996; Strogatz 2019). EWSs are based on the idea that recovery rates from repeated perturbations tend to zero as a system approaches a transition point (Rietkerk *et al.* 1996; Strogatz 2019; Veraart *et al.* 2012).

On the other hand, community and ecosystem rescue theory suggests that ecological or evolutionary processes may restore recovery under recurrent stressful conditions, thereby preventing community or ecosystem collapse (Bell & Gonzalez 2011; Carlson *et al.* 2014; Samani & Bell 2010). Although empirical examples of community rescue are scarce (Fugère *et al.* 2020), it is considered a key mechanism that enhances community resistance and helps maintaining aggregate community properties, such as biomass, under stressful conditions. Both frameworks are plausible and have been documented in communities undergoing repeated perturbations (Fugère *et al.* 2020; Veraart *et al.* 2012). However, to the best of our knowledge, they have not been applied to assess the impacts of recurrent heatwaves in freshwater ecosystems. Hence, the extent to which critical slowing down or rescue occur in aquatic communities exposed to recurrent HWs is yet unknown.

The aim of this study was to assess the role of critical slowing down or rescue in explaining the response of phytoplankton biomass and dissolved oxygen when experiencing recurrent HWs. For this, we used data coming from an outdoor pond mesocosm experiment where a semi-natural phytoplankton community was exposed to three subsequent heatwaves separated by one week at ambient temperature. Such experimental design allowed us to assess the impacts of each of the three HWs on phytoplankton biomass, composition, and dissolved oxygen concentration, as well as the change in the short-term recovery of these properties. We hypothesized that if rescue prevails, the first HW will determine a decline in community and ecosystem properties, which will be followed by a compositional change of the phytoplankton community that promotes stress-tolerant species and/or genetic adaptation (Fugère *et al.* 2020). This new community might be more resistant to a following HW, and thus determine higher community stability to future HWs. Conversely, if critical slowing down prevails, the stress accumulation due to recurrent HWs will gradually reduce the recovery rate of the phytoplankton community after each HW, reducing community resilience, and driving the community to a collapse.

## **Materials and Methods**

### **Experimental design**

An outdoor mesocosm experiment was performed at the facilities of the IMDEA Water Institute (Alcalá de Henares, Madrid, Spain) between April and July of 2021. The original experiment comprised 24 mesocosms, different temperature regimes, and an additional chemical stressor (Hermann *et al.* 2024). Here, we used a subset of the original data, only looking at the recurring HW treatment. For this, 8 mesocosms were used. All mesocosm were round, glass fiber ponds with a diameter of 1.2 m, a total depth of 1.2 m, and a volume of approximately 1000 L. Each mesocosm contained a 30 cm to 40 cm layer of silty-sand sediment sourced from the area around the institute and was filled with 850 L of freshwater from an artificial pond at the research facility. To account for water lost due to evaporation, we placed tap water in plastic buckets biweekly and exposed it to outdoor conditions for several days to reduce chlorine levels before refilling the mesocosms to their original volume of 850 L. The biological community of the mesocosms was largely derived from the artificial pond, and was composed of phytoplankton, zooplankton, and macroinvertebrates. Additional macroinvertebrates were collected independently of size and sex from the Henares River near Humanes, Guadalajara (Spain), and evenly distributed in the mesocosms. The biological community was allowed to establish and adapt to the experimental units for 3 months prior to the start of the experiment. Detailed information on the experimental units and the stocking of the biological communities can be found in Hermann *et al.* (2024).

Four mesocosms were used to simulate the HW scenario (n = 4), which was formed by three repeated HWs (Figure 1); while the remaining four mesocosms were kept at ambient temperature for the whole experimental duration and were used as temperature controls (n = 4). The HWs treatment consisted of three HWs lasting 7 days each and separated one another by 7 days of ambient temperature. In the HWs treatment, the temperature was +8 °C above the control temperature, meaning that the absolute temperature of the HWs increased progressively from the first to the third HW as the water temperature in the control mesocosm warmed because of seasonality. The duration of the HW treatment was chosen as it is comparable to the average duration of recorded lake heatwaves with 7.7 ± 0.4 days (Woolway *et al.* 2021), while the intensity (+8 °C) was selected based on projections of future HW intensity for the end of the century (Woolway *et al.* 2021).

All temperature manipulations and recordings were carried out using a transportable temperature and heatwave control device (TENTACLE) applicable for aquatic micro-and mesocosm experiments (Hermann *et al.* 2022). Additionally, we placed a Hobo logger (Onset Computer Corporation, Bourne, MA, USA) in one control mesocosm and in one mesocosm undergoing the HWs treatment to have an independent water temperature measurement.

### **Phytoplankton sampling, biomass quantification, and photosynthetic activity**

The phytoplankton community was sampled on days -4, 3, 10, 15, 24, 30, and 38, relative to the start of the first HW. Depth-integrated samples were collected from the mesocosm until a total volume of 5 L was obtained. Next, 250 mL of this water sample were introduced into glass amber bottles and 10% Lugol’s iodine was added for preservation. Phytoplankton taxa identification and counts were performed on a 1 mL subsample by means of an inverted microscope and a Sedgewick-Rafter counting cell (Graticules Optics).

Every phytoplankton taxon was digitally photographed with scale reference using a camera Samsung 12 mp (4032 X 3024, JPG format), and measured using the Image J software (Schneider *et al.* 2012). The biovolume (μm3/org) of the phytoplankton individuals was calculated using geometric models according to Hillebrand *et al.* (1999) and Sun & Liu (2003). Biovolume was transformed to fresh weight using the following ratio 1 μg = 106 μm3, assuming that the specific density of water was 1 kg/L (CEN, 2006).

We also quantified phytoplankton photosynthetic activity Chl *a* was measured *in situ* by using a portable multi-meter (YSI Pro DSS 626,973–01) calibrated using a rhodamine standard.

### **Ecosystem property**

Dissolved oxygen (DO; mg/L) was measured as ecosystem property during the experimental period. Oxygen is essential to all aerobic organisms, and its dynamics in freshwater involves interconnected physical and biological processes that form the basis of the functioning of freshwater ecosystems. DO was measured on days -4, 3, 7, 10, 15, 24, 30, and 38. DO was measured *in situ* by using a portable multi-meter (YSI Pro DSS 626,973–01).

**Zooplankton sampling and biomass quantification**

Since phytoplankton biomass dynamics can be heavily influenced by zooplankton grazing activity (Huỳnh *et al.* 2024), we also analysed zooplankton biomass and compositional dynamics.

Zooplankton was sampled from each mesocosm on days -4, 10, 24, and 38 using a PVC tube. Depth-integrated samples were collected from the mesocosm until a total volume of 5 L was obtained. The entire sample was then concentrated into a 100 mL polyethylene bottle using a 55 µm zooplankton net, preserved with Lugol's solution, and stored in the dark in the laboratory for later species identification. A binocular microscope (Olympus SZX7) was used to examine and count all individuals from the Cladocera, Copepoda, and Ostracoda taxa (macro-zooplankton). To analyse micro-zooplankton, a 1 mL subsample was taken from the concentrated sample, and counts were adjusted to individuals per litre. Micro-zooplankton (primarily Rotifera and nauplii) were identified and counted using a microscope (Olympus CX41).

Every phytoplankton taxon was digitally photographed with scale reference using a camera Samsung 12 mp (4032 X 3024, JPG format), and measured using the Image J software (Schneider *et al.* 2012). The biovolume (μm3/org) of the phytoplankton individuals was calculated using geometric models according to (Alcaraz *et al.* 2003). Biovolume was transformed to fresh weight using the following ratio 1 μg = 106 μm3, assuming that the specific density of water was 1 kg/L (CEN, 2006).

### **Statistical analyses**

To investigate the effect of the HWs on DO, chl *a,* phytoplankton biomass in the mesocosms, we employed a linear mixed-effects model (LMM) using the *lmer* function from the “*lme4*” package (Bates *et al.* 2015). The model included HW (factor with two level: HW or Control), time, and their interaction as fixed effects, with mesocosm identifier as a random effect to account for the repeated measures within each mesocosm. Since water temperature directly affect oxygen solubility in water, we included in the model only values measured in days when HWs were not occurring (i.e. all mesocosms were at ambient temperature). The model diagnostics were performed using the *“check\_model”* function from the *“performance”* package (Lüdecke *et al.* 2020) to visually inspect that the assumptions of the LMM were met. We log- transformed the raw data of DO and chlorophyll-a concentration to meet the model’s assumptions. When an interaction effect between HW and time was found we performed a post-hoc comparison across different days using the *“emmeans”* package (Lenth *et al.* 2024) to perform estimated marginal means (EMMs) analysis.

To quantify whether there was a change in the resilience after each HW, we quantified resilience following the method proposed by Baert *et al.* (2016). Resilience was calculated as the proportional change in deviation in the variable between the HW treatment and the control between the sampling before each HW (days -4, 10, 24) and the sampling after each HW (days 10, 24, 38).

Where represents the value of the variable in the control the sampling before the beginning of a HW, and represents the value of the variable in the control the sampling after the beginning of a HW. Whereas represents the value of the variable in the mesocosms experiencing the HW treatment the sampling before the beginning of a HW, and represents the value of the variable in the mesocosms experiencing the HW treatment the sampling after the end of a HW.

This resulted in 3 values of resilience of DO, chl *a,* and phytoplankton biomass, calculated respectively between days -4 – 10, 10 – 24, and 24 – 38.

Resilience is >1 when differences between the control and HW treatment decrease between before and after an HW, and <1 otherwise. Please note that in the figures below resilience was log10 transformed, so the benchmark becomes 0.

In case of critical slowing down, we would expect an erosion of resilience with each consecutive HW, and thus negative resilience values. In case of rescue, we expect resilience to go progressively from negative to positive after each HW, which would suggest an increase resilience due to previously experienced stress.

Both critical slowing down and rescue are mechanistically underpinned by compositional changes that can reduce resilience in the case of critical slowing down or improve it in the case of community rescue. To quantify changes in phytoplankton community composition, we performed a non-parametric permutational multivariate analysis of variance (PERMANOVA), function “*adonis2”* of the R package “*vegan*” (Oksanen *et al.* 2019) with 999 permutations and based on Bray-Curtis distances calculated on the biomass of phytoplankton taxa.

To further assess the dissimilarity in community composition between the control and the HW treatment, the Similarity Percentage (SIMPER) analysis was conducted using the function *“simper”* of the package *“vegan”* (Oksanen *et al.* 2019). This method identifies the contributions of individual taxa to the dissimilarity between groups to the overall Bray-Curtis dissimilarity.

All statistical analysis and figures were done in R (R version 4.2.2; R Core Team 2022). Data and code to reproduce the analysis and figures is available at <https://github.com/FrancescoPola/rescue_critical_slowing>.

## **Results**

### **Temperature manipulations**

Throughout the whole experiment the average ambient water temperature ranged between 13 °C and 30 °C, with a mean temperature of 20°C (Fig. 1). Except for a few temporary declining temperature periods, the ambient water temperature gradually increased during the experiment. The average HW water temperature was 24°C, and ranged between 13°C and 36 °C.

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**Figure 1**. Water temperature dynamics over time in ambient mesocosms (blue line) and HW mesocosms (orange line). The drop in temperature in the HW treatment during the first HW was caused by a technical failure of the TENTACLE machinery during few hours.

### **Dissolved oxygen**

DO declined sharply during the first HW but recovered quickly to values higher than the control between the first and the second HW (Fig. 2a). The second HW determined a similar decrease in DO, but with a less steep recovery trajectory. However, the third HW drastically decreased DO during the HW event. Following the last HW (day 38), DO further declined, showing no signs of recovery (Fig. 2a). The trend was also confirmed by the calculated resilience, which became negative after the third HW (Fig. 2b).

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**Figure 2**. Dissolved oxygen dynamics over time. (a) shows the difference in DO between mesocosm undergoing the HWs treatment and the control mesocosms (dashed line at zero). The red areas show the timing of the three heatwaves. (b) boxplot of resilience of DO after each HW. Coloured dots represent the different mesocosms. Values of resilience > 0 mean increased resilience (HW and control mesocosms became more similar after a HW), whereas values of resilience <0 mean decreased resilience (HW and control mesocosms became more dissimilar after a HW).

The LMM showed a significant effects of the HW treatment (estimate: 0.12, 95% confidence interval of 0.013 to 0.23, p-value: 0.046), and a significant interaction between HW and time (estimate: -0.008, 95% confidence interval of -0.013 to -0.003; p-value: 0.003) suggesting a time-dependent effect of the HWs on the DO concentration, that became more negative with time (Appendix, Table 1).

The post-hoc analysis showed that there was a significant difference (p < 0.05) in the DO concentration between the control mesocosms and the mesocosms undergoing HWs on day -4, and 38 (Appendix Table 2).

Resilience of DO was negative after the first HW (Fig 2b). It became positive after the second HW, but eventually turned to negative after the third HW (Fig 2b).

### **Chlorophyll - *a***

Chl *a* showed a slight decline after the first two HWs. Though, after the third HW, the chl *a* concentration sharply declined in the mesocosms experiencing the HWs compared to control levels (Fig. 3). This trend was highlighted also by analysis of resilience. After the first and second HWs, resilience gradually declined. Yet, after the third HW, resilience showed the largest decline, with all HW mesocosms having large negative resilience values .

The LMM analysis showed that the HW treatment had a significant effect on the chlorophyll- a concentration (estimate: -0.65, 95% confidence interval of -1.22 to -0.078; p-value: 0.041; Appendix, Table 3).

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**Figure 3**. Chlorophyll *- a* concentration dynamic over time. (a) shows the difference in Chlorophyll *- a* between mesocosms undergoing the HWs treatment and the control mesocosms (dashed line at zero). The red areas show the three heatwaves. (b) boxplot of resilience of Chlorophyll *- a* after each HW. Coloured dots represent the different mesocosms. Values of resilience > 0 mean increased resilience (HW and control mesocosms became more similar after a HW), whereas values of resilience <0 mean decreased resilience (HW and control mesocosms became more dissimilar after a HW).

### **Phytoplankton community biomass and composition**

Phytoplankton biomass declined during and after the first HW, but it recovered during and after the second HW. Eventually, it declined during and after the third HW. Yet, neither HW (estimate: -0.42, 95% confidence interval of -1.21 to 0.29; p-value: 0.26; Appendix, Table x) nor time (estimate: 0.004, 95% confidence interval of -0.019 to 0.027; p-value: 0.75; Appendix, Table 4) had a significant effect on phytoplankton biomass. There was, however, a marginally significant (p-value = 0.085) interaction between HW and time, indicating a time-dependent effect of the HWs on phytoplankton biomass (Appendix, Table 4).

The post-hoc analysis showed that there was a significant difference (p < 0.05) in the phytoplankton biomass between the control mesocosms and the mesocosms undergoing HWs from day 10 onwards, with mesocosms experiencing HW having a significantly lower phytoplankton biomass (Appendix Table 5).

Resilience of phytoplankton biomass was reduced after the first HW (Fig. 4b). However, it recovered after the second HW but became negative again after the third HW (Fig. 4b).

A graph of heatwave and heatwave

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**Figure 4**. Phytoplankton biomass dynamic over time. (a) shows the difference in phytoplankton biomass between mesocosms undergoing the HWs treatment and the control mesocosms (dashed line at zero). The red areas show the three heatwaves. (b) boxplot of resilience of phytoplankton biomass after each HW. Coloured dots represent the different mesocosms. Values of resilience > 0 mean increased resilience (HW and control mesocosms became more similar after a HW), whereas values of resilience <0 mean decreased resilience (HW and control mesocosms became more dissimilar after a HW).

The relative biomass of the different phytoplankton groups in the mesocosms undergoing the HW treatment showed a gradual turnover over the course of the experiment and gradually became more and more dissimilar to the control mesocosms (Fig. 5). The increased compositional dissimilarity was confirmed by the PERMANOVA analysis, which highlighted a significantly different community composition between the control and the mesocosms exposed to the HWs on day 10 (F = 4.21, p = 0.027), 30 (F = 3.10, p = 0.032), and 38 (F = 2.55, p = 0.026; Table 1).

**Table 1**. Results of the PERMANOVA analysing the effects of the HWs on phytoplankton community composition in different days of the experiment.

| **Day** | **df\_model** | **df\_residual** | **F** | **R2** | **p\_value** |
| --- | --- | --- | --- | --- | --- |
| -4 | 1 | 6 | 0.895 | 0.130 | 0.367 |
| 3 | 1 | 6 | 3.091 | 0.340 | 0.067 |
| **10** | **1** | **6** | **4.376** | **0.422** | **0.027** |
| 15 | 1 | 6 | 1.766 | 0.227 | 0.15 |
| 24 | 1 | 6 | 0.783 | 0.115 | 0.569 |
| **30** | **1** | **6** | **3.109** | **0.341** | **0.032** |
| **38** | **1** | **6** | **2.553** | **0.298** | **0.026** |

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**Figure 5**. Non-metric multidimensional scaling (NMDS) plots of phytoplankton community composition over time. Facets show different experimental days. Treatments are defined by colour. The dots present the replicated mesocosms.

Significant differences on day 10 were related to a significant decline in Charophyta, Cryptophyta, and Bacillariophyta biomass in the HW treatment compared to the control (Fig. 6; SIMPER, Table 5 of the Appendix). On day 30, the compositional change was driven by a significant decline in Cryptophyta (Fig. 6; SIMPER, Table 5 of the Appendix), whereas on day 38 there was a significant reduction in Cryptophyta and Chlorophyta biomass in HW mesocosms (Fig. 6; SIMPER, Table 5 of the Appendix).

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**Figure 6**. Mean relative biomass of different phytoplankton taxa in the control and the mesocosms exposed to HWs in different time points of the experiment. Day facets in white represent non-HW days, whereas light red facets represent HW days.

### **Zooplankton community biomass and composition**

Zooplankton biomass was less affected by the HWs than phytoplankton biomass, and it showed only an initial decline after the first HW (Fig. 7). Later the variability among replicates was massive, but the general trend showed an increase after the second HW, and a subsequent decline after the third. LMM analysis found a significant effect of time on zooplankton biomass (estimate: 0.006, 95% confidence interval of 0.004 to 0.009, p-value < 0.001; Table 7 of the Appendix).

Community composition was also not significantly affected by the HWs, as showed by the PERMANOVA analysis (Table 8 of the Appendix). However, a general increase in Cladocera could be noted after the third HW (Fig. 7).

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Description automatically generated**Figure 7**. Zooplankton biomass dynamic over time. (a) shows the difference in zoolankton biomass between mesocosms undergoing the HWs treatment and the control mesocosms (dashed line at zero). The red areas show the three heatwaves. (b) mean relative biomass of different zooplankton taxa in the control and the mesocosms exposed to HWs in different time points of the experiment

## **Discussion**

Our experiment highlights the profound impact of recurring HWs on the resilience of aquatic communities. Our findings align with the growing body of literature reporting the detrimental effects of HWs on aquatic ecosystems (Hermann *et al.* 2023, 2024; Polazzo *et al.* 2022; Ross *et al.* 2021). Critically, we show that a series of three, repeated HWs can erode the resilience of phytoplankton communities in freshwater ecosystems.

The observed trends in dissolved oxygen (DO) levels suggest a pattern of critical slowing down, indicated by the gradual reduction in recovery trajectory with repeated disturbances that eventually determined an erosion of resilience (Veraart et al. 2012). It should be noted that the positive value of resilience after the second HW, was only determined by the high DO concertation on day 10, which resulted in a positive resilience value as DO concentration on day 24 at the same level as in the control mesocosm, but we overall observed a gradual decline in DO after each HW (Fig. 2a).

HWs can cause abrupt increases in O2 consumption (Yvon-Durocher *et al.* 2012), ultimately modifying the hourly amplitude of the balance between CO2 and O2 without altering the diurnal frequency of the lake metabolism cycle. The increased O2 consumption determined by the HWs, together with the heat-driven reduced photosynthetic activity determined an overall decline in DO after each HW, and decreased its resilience.

Chlorophyll – *a*, on the other hand, showed a decline in concentration, as well as in resilience after each HW. The steady decline in resilience after every HW event, suggests an impaired recovery potential for chlorophyll – *a,* and thus aligns with the critical slowing down hypothesis. A recent experiment found that chlorophyll – *a* was actually increased by a first HW, but then returned to control level after a second HW (Huỳnh *et al.* 2024). The different responses of chlorophyll – a in Huỳnh *et al.* (2024) and our study may be related to the intensity of the HW treatment. Indeed, we applied a temperature difference of +8°C in the HW treatment, whereas a difference of +6°C was applied in Huỳnh *et al.* (2024). Additionally, the maximum temperature in the HW mesocosms in our study was 36 °C, whereas it never reached 32 °C in Huỳnh *et al.* (2024). The difference in absolute temperature may have determined the larger decline and loss of resilience in chlorophyll – a in our study, as the higher stress could have led to a larger reduction in the photosynthetic activity of phytoplankton. Yet, our results align with those of Veraart *et al.* (2012), where a gradual decline in photosynthetic activity was found in a phytoplankton species exposed to an increasing level of stress.

Phytoplankton biomass exhibited a less clear response. During and after the first HW, phytoplankton biomass declined. The biomass decline was associated to a significant compositional change after the first HW (day 10), and a loss of resilience. Yet, after the second HW, phytoplankton biomass recovered to control level, and no compositional difference was note between control and HW on day 24. The compositional similarity between communities in the control and HW mesocosms on day 24 suggests that after the first HW, which caused a significant change in composition, phytoplankton community composition recovered and was undistinguishable from the control. This recovery in biomass and composition determined an increased in resilience, suggesting a possible community rescue. However, the third HW determined a decline in biomass which was associated to a significant compositional turnover, and to reduced resilience. Particularly towards the end of the experiment, the compositional dissimilarity was driven by a significant reduction in the biomass of Cryptophyta, Dinophyta, and Chlorophyta in the HW treatment. The new community composition did not promote stress-tolerant species able to maintain phytoplankton biomass and increase resilience, as testified by the decline of both biomass and resilience. Although community rescue is usually linked to a strong compositional change, this compositional change should determine an increased resistance to stress and consequent ability to maintain community biomass and restore resilience (Fugère *et al.* 2020). Since we found the opposite (i.e. compositional change determined a decline in resistance to following HW and decline in biomass), we exclude that any rescue process happened in our experiment.

On the contrary, the increased compositional dissimilarity, linked to the reduced resilience, rather supports the critical slowing down hypothesis. Indeed, critical transitions to alternative stable states are often related to dramatic shifts in composition (Bertani *et al.* 2016; Meunier *et al.* 2024; Wernberg *et al.* 2016). The classic example is the shift from clear water state dominated by macrophyte to a turbid water state dominated by phytoplankton in shallow lakes (Scheffer 2009). The compositional shift is a common feature of critical transition across ecosystems and has been reported in marine (Meunier *et al.* 2024) and terrestrial systems (Eby *et al.* 2017). Hence, our study aligns with the body of literature describing a strong compositional shift which relates to dramatic changes in community biomass that may result in critical transitions (Eby *et al.* 2017; Meunier *et al.* 2024).

Ultimately, the response of phytoplankton to an HW depends on the thermal sensitivity of the species composing the community (Polazzo *et al.* 2022), and on the interactions between species that are established during and after the HW (Huỳnh *et al.* 2024; Polazzo *et al.* 2023; Seifert *et al.* 2015). In our experiment, although the intensity defined as the temperature difference between the HW treatment and the control was +8 °C for all HW events, the temperature in the control increased as we progressed from April to July. This resulted in the HWs having different absolute temperature. The strong decline in biomass and chlorophyll – *a* after the third heatwave may have been determined by the higher portion of species unable to cope with the stress posed by another, stronger HW. Despite the cumulative stress caused by recurring HWs, the intensity of an HW has been shown to impact differently planktonic communities not only during, but especially after the HW event (Seifert *et al.* 2015).

Yet, also a change in zooplankton activity could have contributed to the decline in phytoplankton biomass and chlorophyll - *a*. Although, the recurring HWs did not significantly affect zooplankton biomass or composition, at the end of the experiment mesocosms exposed to the recurring HWs had a larger proportion of Cladocera compared to control mesocosms. Cladocera are much more efficient grazers than Copepods and Rotifers (Sommer *et al.* 2002), and an increase in Cladocera may have resulted in an increased grazing, and thus stronger top-down control on primary producers, contributing to the overall phytoplankton biomass decline. A decline in Copepods and an increase in Cladocera is opposite to what was recently found in another freshwater mesocosm experiment, where Copepods increased after two consecutive HWs, leading to an overall weaker top-down control (Huỳnh *et al.* 2024). However, an increase in small Cladocera was reposted in another experiment where a zooplankton community was exposed to a HW of similar duration and intensity (Roth *et al.* 2022).

Overall, we highlight that it is not necessary for a community to tip in order to show a slowing down in recovery. van Nes & Scheffer (2007) suggested that critical slowing down may not only be related to a critical transition or tipping points but could also generically indicate a reduced tolerance of the system to repeated perturbations. Critical slowing down may thus provide important information in cases where the threshold leading to a critical transition has not yet been reached, thus working as an EWS, and may be informative for systems that do not have multiple stable states at all.

In conclusion, we show here that the repeated stress caused by increasingly stronger HWs led to a drastic change in the composition of the phytoplankton community. The new compositional configuration was unable to maintain and / or recover photosynthetic activity or biomass levels like unperturbed systems, suggesting an overall increase in vulnerability to subsequent perturbations. These results force us to evaluate the consequences of climate change-induced extreme weather events on the functioning of communities and ecosystems, particularly as they become more recurrent and severe in the near future.

## **Competing Interests Statement**

The authors declare no competing interests.

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## **Appendix**

**Table 1**. Results of the LMM analysing the effects of HW and time on the DO concentration.

| **effect** | **term** | **estimate** | **2.5%** | **97.5%** | **p.value** |
| --- | --- | --- | --- | --- | --- |
| fixed | (Intercept) | 2.418 | 2.342 | 2.494 | 0.000 |
| **fixed** | **TreatmentHW** | **0.121** | **0.013** | **0.228** | **0.046** |
| fixed | Day | 0.001 | -0.002 | 0.005 | 0.483 |
| **fixed** | **TreatmentHW:Day** | **-0.008** | **-0.013** | **-0.003** | **0.003** |

**Table 2**. Results of the post-hoc test (estimated marginal means (EMMs) analysis) of the LMM assessing the effects of HWs and time on the DO concentration.

| **contrast** | | **Day** | | **estimate** | | **SE** | | **df** | | **t.ratio** | | **p.value** | |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **Control - HW** | | **-4** | | **-0.153** | | **0.064** | | **24.889** | | **-2.376** | | **0.026** | |  |
| Control - HW | | 10 | | -0.040 | | 0.042 | | 8.521 | | -0.950 | | 0.368 | |  |
| Control - HW | | 24 | | 0.073 | | 0.042 | | 8.521 | | 1.721 | | 0.121 | |  |
| **Control - HW** | | **38** | | **0.185** | | **0.064** | | **24.889** | | **2.882** | | **0.008** | |  |

**Table 3**. Results of the LMM analysing the effects of HW and time on chlorophyll *- a.*

| **effect** | **term** | **estimate** | **2.5%** | **97.5%** | **p-value** |
| --- | --- | --- | --- | --- | --- |
| fixed | (Intercept) | 0.657 | 0.252 | 1.062 | 0.005 |
| **fixed** | **TreatmentHW** | **-0.650** | **-1.222** | **-0.078** | **0.041** |
| fixed | Day | 0.011 | -0.008 | 0.029 | 0.274 |
| fixed | TreatmentHW:Day | -0.020 | -0.046 | 0.007 | 0.157 |

**Table 4**. Results of the LMM analysing the effects of HW and time on phytoplankton biomass*.*

| **effect** | **term** | **estimate** | **2.5%** | **97.5%** | **p.value** |
| --- | --- | --- | --- | --- | --- |
| fixed | (Intercept) | 2.445 | 2.229 | 2.662 | 0.000 |
| fixed | TreatmentHW | -0.180 | -0.487 | 0.126 | 0.263 |
| fixed | Day | 0.002 | -0.008 | 0.012 | 0.752 |
| fixed | TreatmentHW:Day | -0.013 | -0.027 | 0.001 | 0.085 |

**Table 5**. Results of the post-hoc test (estimated marginal means (EMMs) analysis) of the LMM assessing the effects of HWs and time on phytoplankton biomass.

| **contrast** | **Day** | **estimate** | **SE** | **df** | **t.ratio** | **p.value** |
| --- | --- | --- | --- | --- | --- | --- |
| Control - HW | -4 | 0.129 | 0.183 | 37.643 | 0.702 | 0.487 |
| Control - HW | 3 | 0.219 | 0.143 | 20.521 | 1.531 | 0.141 |
| **Control - HW** | **10** | **0.310** | **0.113** | **8.935** | **2.736** | **0.023** |
| **Control - HW** | **15** | **0.375** | **0.103** | **6.155** | **3.636** | **0.010** |
| **Control - HW** | **24** | **0.491** | **0.116** | **9.827** | **4.230** | **0.002** |
| **Control - HW** | **30** | **0.569** | **0.143** | **20.192** | **3.993** | **0.001** |
| **Control - HW** | **38** | **0.673** | **0.188** | **39.467** | **3.571** | **0.001** |



**Table 6**. Results of the SIMPER analysing the effects of the HWs on phytoplankton taxa biomass over the course of the experiment. The column “Average” represents the average contribution of each taxon to the overall dissimilarity between groups. “sd” is the standard deviation of the contributions of each species to the dissimilarity. “ratio” is the ratio of the average contribution to the standard deviation (average/sd). This indicates the consistency of the species' contribution to the dissimilarity. Higher ratios suggest more consistent contributions. “ava” is the average biomass of each species in the group A (i.e. HW). “avb” is the average biomass of each species in group B (i.e. Control). “cumsum” is the cumulative sum of the contributions of species to the overall dissimilarity, expressed as a fraction. This shows the cumulative proportion of the total dissimilarity accounted for by the species up to that row in the table. P-value are resulting from permutation test.

| **Taxonomic groups** | **average** | **sd** | **ratio** | **ava** | **avb** | **cumsum** | **p-value** |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Day -4** |  |  |  |  |  |  |  |
| Charophyta | 0.206 | 0.171 | 1.207 | 293.749 | 99.457 | 0.371 | 0.401 |
| Dinophyta | 0.133 | 0.131 | 1.013 | 228.716 | 42.859 | 0.611 | 0.083 |
| Cryptophyta | 0.133 | 0.104 | 1.273 | 215.925 | 206.102 | 0.850 | 0.319 |
| Bacillariophyta | 0.072 | 0.036 | 2.011 | 109.057 | 35.663 | 0.980 | 0.177 |
| Chlorophyta | 0.007 | 0.006 | 1.157 | 14.110 | 2.809 | 0.992 | 0.728 |
| Euglenophyta | 0.003 | 0.004 | 0.695 | 6.179 | 0.373 | 0.997 | 0.428 |
| Cyanobacteria | 0.002 | 0.002 | 0.768 | 0.559 | 1.160 | 1.000 | 0.875 |
| Synurophyta | 0.000 | 0.000 |  | 0.000 | 0.000 | 1.000 | 0.001 |
| **Day 3** |  |  |  |  |  |  |  |
| Cryptophyta | 0.513 | 0.289 | 1.779 | 75.579 | 230.067 | 0.741 | 0.051 |
| Dinophyta | 0.076 | 0.091 | 0.834 | 8.011 | 21.363 | 0.850 | 0.332 |
| Chlorophyta | 0.047 | 0.081 | 0.584 | 16.870 | 1.239 | 0.919 | 0.908 |
| **Charophyta** | **0.042** | **0.062** | **0.682** | **0.342** | **17.338** | **0.980** | **0.037** |
| **Cyanobacteria** | **0.007** | **0.010** | **0.642** | **0.000** | **1.684** | **0.990** | **0.001** |
| Euglenophyta | 0.005 | 0.006 | 0.766 | 1.439 | 0.804 | 0.997 | 0.944 |
| Bacillariophyta | 0.002 | 0.003 | 0.705 | 0.186 | 0.747 | 1.000 | 0.370 |
| Synurophyta | 0.000 | 0.000 |  | 0.000 | 0.000 | 1.000 | 0.001 |
| **Day 10** |  |  |  |  |  |  |  |
| **Charophyta** | **0.272** | **0.237** | **1.147** | **32.657** | **135.963** | **0.407** | **0.031** |
| Dinophyta | 0.148 | 0.121 | 1.224 | 9.747 | 65.023 | 0.628 | 0.053 |
| **Cryptophyta** | **0.146** | **0.108** | **1.347** | **13.064** | **45.836** | **0.846** | **0.001** |
| **Bacillariophyta** | **0.072** | **0.078** | **0.926** | **5.035** | **34.741** | **0.954** | **0.001** |
| Chlorophyta | 0.023 | 0.025 | 0.911 | 3.339 | 11.008 | 0.988 | 0.184 |
| Cyanobacteria | 0.005 | 0.007 | 0.803 | 0.550 | 2.366 | 0.996 | 0.946 |
| Euglenophyta | 0.003 | 0.004 | 0.713 | 1.014 | 0.765 | 1.000 | 0.975 |
| Synurophyta | 0.000 | 0.000 |  | 0.000 | 0.000 | 1.000 | 0.001 |
| **Day 15** |  |  |  |  |  |  |  |
| Cryptophyta | 0.430 | 0.249 | 1.729 | 68.053 | 307.782 | 0.687 | 0.120 |
| Charophyta | 0.091 | 0.089 | 1.021 | 46.209 | 26.590 | 0.832 | 0.949 |
| Dinophyta | 0.063 | 0.077 | 0.808 | 10.681 | 29.374 | 0.932 | 0.062 |
| Chlorophyta | 0.030 | 0.024 | 1.232 | 8.345 | 15.295 | 0.980 | 0.403 |
| Bacillariophyta | 0.009 | 0.008 | 1.069 | 0.773 | 4.510 | 0.993 | 0.113 |
| Euglenophyta | 0.003 | 0.004 | 0.626 | 1.057 | 0.000 | 0.998 | 0.893 |
| **Cyanobacteria** | **0.001** | **0.001** | **2.037** | **0.002** | **0.639** | **1.000** | **0.001** |
| Synurophyta | 0.000 | 0.000 |  | 0.000 | 0.000 | 1.000 | 0.001 |
| **Day 24** |  |  |  |  |  |  |  |
| Chlorophyta | 0.232 | 0.325 | 0.713 | 456.690 | 21.977 | 0.393 | 0.866 |
| Cryptophyta | 0.152 | 0.151 | 1.008 | 80.832 | 125.624 | 0.652 | 0.740 |
| Charophyta | 0.106 | 0.161 | 0.654 | 29.724 | 53.875 | 0.831 | 0.543 |
| Dinophyta | 0.049 | 0.050 | 0.990 | 6.142 | 23.099 | 0.915 | 0.078 |
| Euglenophyta | 0.029 | 0.037 | 0.785 | 23.128 | 1.110 | 0.964 | 0.365 |
| Bacillariophyta | 0.019 | 0.011 | 1.748 | 21.166 | 4.900 | 0.996 | 0.459 |
| Cyanobacteria | 0.003 | 0.003 | 0.966 | 1.106 | 0.848 | 1.000 | 0.619 |
| Synurophyta | 0.000 | 0.000 |  | 0.000 | 0.000 | 1.000 | 0.001 |
| **Day 30** |  |  |  |  |  |  |  |
| **Cryptophyta** | **0.332** | **0.264** | **1.256** | **31.725** | **165.021** | **0.452** | **0.001** |
| Chlorophyta | 0.180 | 0.169 | 1.066 | 41.734 | 99.096 | 0.697 | 0.933 |
| Charophyta | 0.110 | 0.158 | 0.696 | 12.816 | 59.473 | 0.847 | 0.306 |
| Dinophyta | 0.095 | 0.090 | 1.055 | 2.670 | 53.407 | 0.976 | 0.058 |
| Bacillariophyta | 0.010 | 0.009 | 1.016 | 5.289 | 3.337 | 0.989 | 0.936 |
| Cyanobacteria | 0.006 | 0.005 | 1.127 | 0.250 | 3.758 | 0.998 | 0.113 |
| Euglenophyta | 0.002 | 0.003 | 0.519 | 0.835 | 0.000 | 1.000 | 0.971 |
| Synurophyta | 0.000 | 0.000 |  | 0.000 | 0.000 | 1.000 | 0.001 |
| **Day 38** |  |  |  |  |  |  |  |
| **Cryptophyta** | **0.230** | **0.162** | **1.420** | **4.567** | **100.140** | **0.304** | **0.001** |
| Dinophyta | 0.173 | 0.194 | 0.895 | 3.471 | 167.298 | 0.533 | 0.147 |
| **Chlorophyta** | **0.130** | **0.166** | **0.783** | **3.894** | **80.553** | **0.704** | **0.039** |
| Charophyta | 0.127 | 0.055 | 2.308 | 32.507 | 93.918 | 0.872 | 0.967 |
| Bacillariophyta | 0.059 | 0.047 | 1.260 | 11.421 | 31.128 | 0.950 | 0.429 |
| Cyanobacteria | 0.027 | 0.024 | 1.147 | 5.863 | 14.455 | 0.986 | 0.967 |
| Euglenophyta | 0.011 | 0.010 | 1.004 | 5.281 | 3.097 | 1.000 | 0.929 |
| Synurophyta | 0.000 | 0.000 |  | 0.000 | 0.000 | 1.000 | 0.001 |

**Table 7**. Results of the LMM analysing the effects of HW and time on zooplankton biomass*.*

| **effect** | **term** | **estimate** | **2.5%** | **97.5%** | **p.value** |
| --- | --- | --- | --- | --- | --- |
| fixed | (Intercept) | 0.128 | 0.063 | 0.192 | 0.001 |
| fixed | TreatmentHW | 0.014 | -0.050 | 0.079 | 0.678 |
| **fixed** | **Day** | **0.006** | **0.004** | **0.009** | **>0.001** |
| fixed | TreatmentHW:Day | 0.000 | -0.003 | 0.002 | 0.816 |

**Table 8**. Results of the PERMANOVA analysing the effects of the HWs on zoolankton community composition in different days of the experiment.

| **Day** | **df\_model** | **df\_residual** | **F** | **R2** | **p\_value** |
| --- | --- | --- | --- | --- | --- |
| -4 | 1 | 6 | 0.241 | 0.039 | 0.670 |
| 10 | 1 | 6 | 0.547 | 0.084 | 0.732 |
| 24 | 1 | 6 | 1.228 | 0.170 | 0.194 |
| 38 | 1 | 6 | 0.713 | 0.106 | 0.609 |