# Slowing down in recovery of phytoplankton community due to recurrent heatwaves

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# Introduction

Climate change is an urgent global challenge characterized by significant alterations in weather patterns, rising global temperatures, and increased frequency of extreme weather events (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), including in freshwater ecosystems (Woolway *et al.* 2021, 2022). The projected increase in HWs frequency and intensity is growing concerns, as evidence of the detrimental effects of HWs on freshwater populations, communities, and ecosystems keeps accululating (Correa-Araneda *et al.* 2020; Mouthon & Daufresne 2006; Polazzo *et al.* 2022; Woodward *et al.* 2016).

HWs have been shown to negatively affect multiple dimension of functional and compositional stability, such as resistance, recovery and temporal stability in freshwater ecosystems (Polazzo *et al.* 2023; Ross *et al.* 2021). Additionally, HWs may detrmine abrupt shift in aquatic ecosystems (Bertani *et al.* 2016; Meunier *et al.* 2024; Turner *et al.* 2020; Wernberg *et al.* 2016).

However, the evidence for HW – driven abrupt shift or collapse are limited in freshwater systems (Bertani *et al.* 2016; Filiz *et al.* 2020; Polazzo *et al.* 2022). Scarse evidence for collapse may relate to the fact that most studies analyse a single HW event (Polazzo *et al.* 2022). A single HW event, although detrimental, may not be sufficient to determine an abruput shift in freshwater systems. Indeed, freshwater organism experience large daily and seasonal temperature fluctuations, and are thus adapted to large environmental fluctuations (REF). Yet, since HWs are projected to become more frequent in the future, concern is rising about whether and how natural communities can face multiple recurring HWs and keep performing their functions.

There is currently a lack of knowledge and understanding about how recurrent HWs can influence community and ecosystem stability. The few avaible studies considering recurrent HWs have focused on other aspects of HW’s effects of freshwater organisms (Hermann *et al.* 2023, 2024).

There are two major stability-related ecological framework that are commonly applied to systems facing repeated perturbations: critical slowing down and community rescue.

Critical slowing down is a mechanism relayed to the abrupt shift in funcitoning or composition communities and ecosystems show when experiencing a gradually increasing pressure or when exposed to repeated perturbations (Veraart *et al.* 2012). The exact shifting point is notoriously difficult to predict (REF). Therefore, the focus has shifted to deducing processes from patterns. This involves identifying observable signals in measurable aspects of a biological system that indicate changes in the underlying processes, which may results in alterations of the system’s structure and function. In the last two decades, the phenomenon known as clitical slowing down has been indicated as a possible early warning signals (EWSs) of an approching abrubt shift, derived from dynamic systems theory (REF). The theory suggests that early warning signals could be based on the idea that recovery rates from disturbances tend to zero as a system approaches a transition point. However, there is limited evidence to support this occurring in complex systems.

Oppositely, community rescue happens when ecological or evolutionary processes restore recovery under stressful conditions that would have been detrimental to the community in its original form, thereby preventing community or ecosystem functioning from collapsing in a worsening environment. Although empirical examples of community rescue are scarce, it is considered a key mechanism that enhances stress resistance in communities and helps maintain aggregate community properties, such as biomass, under stressful conditions.

It is possible that either critical slowing down or community rescue may occur in communities that have been exposed to recurrent HWs. A community may sustain and / or recover following a first HW. However, the recovery following a subsequent HW may be impaired, and additional HWs may impede further recovery as a result of accumulating stress, driving the community to collapse. It is also possible to envisage a community rescue. A first HW may result in a decline in community functioning, which may be recovered through a compositional change that promotes stress-tolerant species (Fugère *et al.* 2020). This new community composition might then be more resistant to a following HW, and thus determine higher community stability. The same process may occur as a result of adaptation or evolutionary processes, where more resistant genotypes are selected and result in a more resistant and stable community to subsequent HWs. Thus, both framework are plausible and have been documented in communities undergoing repeated perturbations (Fugère *et al.* 2020; Veraart *et al.* 2012). Critically though, the result in terms of community stability would be opposite, where community rescue is stabilising and critical slowing down is destabilising.

It is crucial to emphasise that a recent meta-analysis has demonstrated that critical transitions are not commonly observed in empirical data (Hillebrand et al., 2020). Nevertheless, it is not necessary for a community to collapse in order to show a slowing down in recovery. It can be anticipated that repeated HWs may result in a reduction in a community’s recovery capacity, even though community collapse is not reached.

With this article we wanted to test which of these two major ecological frameworks is more likely to appear in a phytoplankton community exposed to recurring HWs.

For this we used data coming from an outdoor pond mesocosm experiment (2021 Spain) where a semi-natural phytoplankton community was exposed to three subsequent heatwaves of increasing intensity, and the change in the short term recovery after each HW was assessed.

# Materials and Methods

## Mesocosm experiment

We performed an outdoor mesocosm experiment at thefacilities of the IMDEA Water Institute (Alcalá de Henares, Madrid, Spain) between April and July 2021. The 8 mesocosms used in this study were filled with 40 cm of sediments and 850 L of water from an artificial lagoon. The biological community of the mesocosms was composed of phytoplankton, zooplankton, and macroinvertebrates, and was allowed to establish and homogenize among experimental units for 2 months prior to the start of the experiment. Four of the test mesocosms were used to simulate the HW scenario (n = 4): three repeated HWs (Figure 1); while four mesocosms were kept at ambient temperature for the whole experimental duration and were used as controls. 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, which meant that the HW absolute temperature progressively increased from the first to the third HW, as the water temperature in the control mesocosm warmed as result of seasonality.

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 place 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 and biomass quantification

The phytoplankton community was sampled on days -4, 3, 10, 15, 24, 30, and 38 to the start of the first HW. Samplings were performed by taking depth-integrated water samples with a polyvinyl chloride (PVC) tube (six sub-samples per mesocosm mixed in a bucket). Next, for phytoplankton samples, 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 perfomed 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 measuredusing 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 specific weight of water = 1.

## Ecosystem functioning proxies

We measured chlorophyll – *a* (chl *a*) as proxy for primary productivity, as it represents the living part of the phytoplankton biomass. Chl *a* was measured on days -4, 3, 7, 10, 15, 24, 30, and 38 in situ by using a portable multi-meter (YSI Pro DSS 626,973–01). We also measured dissolved oxygen (DO) during the experimental period. Oxygen is essential to all aerobic organisms, and its dynamics in freshwater involve 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 38in situ by using a portable multi-meter (YSI Pro DSS 626,973–01).

## Statistical analysis

To investigate the effect of heatwave treatment on oxygen levels in 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, time, and their interaction as fixed effects, with mesocosm identifier as a random effect to account for the repeated measures within each mesocosm. The model diagnostics were performed using the “check\_model” function from the performance package (Lüdecke *et al.* 2020) to ensure the assumptions of the LMM were met. When an interaction effect between HW and time was found we performed a post-hoc comparisons across different days using the “emmeans” package (Lenth *et al.* 2024) to perform estimated marginal means (EMMs) analysis.

The same approach was used to analyse the effects of the HWs on chl *a.*

To quantify whether there was a slowing down in recovery after each HW, we first calculated the difference in DO or chlorophyll-a concentration between control and HW mesocosms. We then used the value of the slope of the linear regression between two subsequent time points (during and after a HW) as a measurement of recovery. For that, we calculated the slope between day 3 and 10 (during and after the first HW), 15 and 24 (during and after the second HW), and 30 and 38 (during and after the third HW). A positive slope was interpreted as sign of recovery, whereas a negative slope as a sing or missing recovery. In case of critical slowing down, we would expect the slope of the recovery to become increasingly less positive, and perhaps eventually negative. In case of rescue, we expect the slope to become close to zero after the first HW, as this would suggest a smaller effect of the second and third HW determined by a change in the community, that may have shifted towards a more resistant composition.

To investigate the effects of the HWs on 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.

# Results

During the experiment, water temperatures ranged between 13.1 and 27.6 °C (Amb), and 13.2 and 35.7 °C (HW) (Fig. 1). Throughout the whole experiment the average ambient water temperature was 20.18 °C and ranged between 13 °C and 29.64 °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 24.19°C, and ranged between 13.21°C and 35.73°C.

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Dissolved oxygen declined sharply after the first HW but recovered quicky returning to values higher than the control between the first and the second HW (Fig. 2). The second HW determined a similar decreased in DO, which was however completely recovered, and between the second and third HWs, although with a less steep recovery trajectory, DO returned to control levels. The third HW drastically decreased DO during its course and on and after the last HW (day 38), DO further declined, showing no sign of recovery (Fig. 2). The trend was also confirmed by the calculated slope of the recovery, which became less and less positive after the first two HWs, and eventually turned negative after the third HW (Fig. 2).

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Figure 1. Dissolved oxygen dynamics over time. The red areas show the three heatwaves, whereas the horizontal dashed line at 0 represent the mean DO in the control mesocosms.

The LMM found a significant interaction between HW and time, suggesting a time dependent effect of the HWs (Table 1).

| **term** | **estimate** | **std.error** | **statistic** | **df** | **p.value** |
| --- | --- | --- | --- | --- | --- |
| (Intercept) | 2.435 | 0.039 | 62.613 | 26.146 | 0.000 |
| HW | 0.060 | 0.055 | 1.090 | 26.146 | 0.286 |
| Day | 0.003 | 0.002 | 1.957 | 46.000 | 0.056 |
| HW:Day | -0.007 | 0.002 | -2.689 | 46.000 | **0.010** |

The post-hoc analysis showed that there was a significant difference in DO between control mesocosms and mesocosms undergoing HWs from day 24 onward. After day 24, the DO concentration was always significantly lower in mesocosms experiencing the HWs (Table 2).

| **contrast** | **Day** | **estimate** | **SE** | **df** | **t.ratio** | **p.value** | **p\_value** |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Control - HW | -4 | -0.087 | 0.063 | 35.683 | -1.381 | 0.176 | 0.176 |
| Control - HW | 3 | -0.040 | 0.050 | 19.228 | -0.802 | 0.432 | 0.432 |
| Control - HW | 10 | 0.007 | 0.040 | 8.677 | 0.182 | 0.860 | 0.86 |
| Control - HW | 15 | 0.041 | 0.036 | 6.142 | 1.122 | 0.304 | 0.304 |
| Control - HW | 24 | 0.101 | 0.041 | 9.486 | 2.490 | 0.033 | **0.033** |
| Control - HW | 30 | 0.142 | 0.049 | 18.925 | 2.869 | 0.010 | **0.010** |
| Control - HW | 38 | 0.195 | 0.065 | 37.548 | 3.023 | 0.004 | **0.004** |

Chlorophyll - *a* showed a slight decline after the first two HWs. Though, after the third HW, chlorophyll – *a* concentration abruptly declined in mesocosms experiencing the HWs compared to control levels (Fig. 3). This trend was highlighted also by the calculated slopes of recovery. After the first and second HWs, the slope was slightly negative. Yet, after the third HW, the slope became more negative, decreasing its value by roughly one order of magnitude.

The LMM analysis showed that the HW treatment had a significant effect on chlorophyll- a concentration (Table 3).

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| **term** | **estimate** | **std.error** | **statistic** | **df** | **p.value** |
| --- | --- | --- | --- | --- | --- |
| (Intercept) | 0.657 | 0.214 | 3.071 | 25.799 | 0.005 |
| HW | -0.650 | 0.303 | -2.148 | 25.799 | **0.041** |
| Day | 0.011 | 0.010 | 1.108 | 46.000 | 0.274 |
| HW:Day | -0.020 | 0.014 | -1.440 | 46.000 | 0.157 |

The relative biomass of different phytoplankton groups in mesocosms undergoing the HWs treatment showed a gradual turnover across the experiment, and became gradually more and more dissimilar to control mesocosms (Fig. 4). The increased compositional dissimilarity was confirmed by the PERMANOVA analysis, which highlighted significantly different compositions between control and HW mesocosms on day 10, 30, and 38 (Table 4).

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| **Day** | **F** | **R2** | **p\_value** |
| --- | --- | --- | --- |
| -4 | 0.891 | 0.129 | 0.367 |
| 3 | 3.008 | 0.334 | 0.067 |
| 10 | 3.127 | 0.343 | **0.027** |
| 15 | 1.698 | 0.221 | 0.129 |
| 24 | 0.737 | 0.109 | 0.691 |
| 30 | 3.065 | 0.338 | **0.032** |
| 38 | 2.429 | 0.288 | **0.026** |

# Discussion

Critical slowing down can appear even far away from the critical transition (van Nes & Scheffer 2007)

# References

Bates, D., Mächler, M., Bolker, B.M. & Walker, S.C. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, 67.

Bertani, I., Primicerio, R. & Rossetti, G. (2016). Extreme Climatic Event Triggers a Lake Regime Shift that Propagates Across Multiple Trophic Levels. *Ecosystems*, 19.

Correa-Araneda, F., Tonin, A.M., Pérez, J., Álvarez, K., López-Rojo, N., Díaz, A., *et al.* (2020). Extreme climate events can slow down litter breakdown in streams. *Aquatic Sciences*, 82, 1–7.

Filiz, N., Işkın, U., Beklioğlu, M., Öğlü, B., Cao, Y., Davidson, T.A., *et al.* (2020). Phytoplankton Community Response to Nutrients, Temperatures, and a Heat Wave in Shallow Lakes: An Experimental Approach. *Water*, 12, 3394.

Fischer, E.M., Sippel, S. & Knutti, R. (2021). Increasing probability of record-shattering climate extremes. *Nat. Clim. Chang.*, 11, 689–695.

Fugère, V., Hébert, M.P., da Costa, N.B., Xu, C.C.Y., Barrett, R.D.H., Beisner, B.E., *et al.* (2020). Community rescue in experimental phytoplankton communities facing severe herbicide pollution. *Nature Ecology and Evolution*, 4, 578–588.

Hermann, M., Jansen, R., van de Glind, J., Peeters, E.T.H.M. & Van den Brink, P.J. (2022). A transportable temperature and heatwave control device (TENTACLE) for laboratory and field simulations of different climate change scenarios in aquatic micro- and mesocosms. *HardwareX*, 11, e00307.

Hermann, M., Peeters, E.T.H.M. & Van den Brink, P.J. (2023). Heatwaves, elevated temperatures, and a pesticide cause interactive effects on multi-trophic levels of a freshwater ecosystem. *Environmental Pollution*, 327, 121498.

Hermann, M., Polazzo, F., Cherta, L., Crettaz-Minaglia, M., García-Astillero, A., Peeters, E.T.H.M., *et al.* (2024). Combined stress of an insecticide and heatwaves or elevated temperature induce community and food web effects in a Mediterranean freshwater ecosystem. *Water Research*, 260, 121903.

Hillebrand, H., Dürselen, C.D., Kirschtel, D., Pollingher, U. & Zohary, T. (1999). Biovolume calculation for pelagic and benthic microalgae. *Journal of Phycology*, 35.

Intergovernmental Panel on Climate Change (IPCC). (2023). *Climate Change 2022 – Impacts, Adaptation and Vulnerability: Working Group II Contribution to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge.

Lenth, R.V., Bolker, B., Buerkner, P., Giné-Vázquez, I., Herve, M., Jung, M., *et al.* (2024). emmeans: Estimated Marginal Means, aka Least-Squares Means.

Lüdecke, D., Makowski, D. & Waggoner, P. (2020). performance: Assessment of Regression Models Performance. R package version 0.4.4. https://CRAN.R-project.org/package=performance. *CRAN.R*.

Meunier, Z.D., Hacker, S.D. & Menge, B.A. (2024). Regime shifts in rocky intertidal communities associated with a marine heatwave and disease outbreak. *Nat Ecol Evol*, 1–13.

Mouthon, J. & Daufresne, M. (2006). Effects of the 2003 heatwave and climatic warming on mollusc communities of the Saône: A large lowland river and of its two main tributaries (France). *Global Change Biology*, 12, 441–449.

van Nes, E.H. & Scheffer, M. (2007). Slow Recovery from Perturbations as a Generic Indicator of a Nearby Catastrophic Shift. *The American Naturalist*, 169, 738–747.

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., Mcglinn, D., *et al.* (2019). vegan: Community Ecology Package. R package version 2.4-2. *Community ecology package*, 2.5-6.

Perkins, S.E., Alexander, L.V. & Nairn, J.R. (2012). Increasing frequency, intensity and duration of observed global heatwaves and warm spells. *Geophysical Research Letters*, 39, 1–5.

Polazzo, F., Hermann, M., Crettaz-Minaglia, M. & Rico, A. (2023). Impacts of extreme climatic events on trophic network complexity and multidimensional stability. *Ecology*, 104, e3951.

Polazzo, F., Roth, S.K., Hermann, M., Mangold-Döring, A., Rico, A., Sobek, A., *et al.* (2022). Combined effects of heatwaves and micropollutants on freshwater ecosystems: Towards an integrated assessment of extreme events in multiple stressors research. *Global Change Biology*, 28, 1248–1267.

Ross, S.R.P. ‐J., García Molinos, J., Okuda, A., Johnstone, J., Atsumi, K., Futamura, R., *et al.* (2021). Predators mitigate the destabilising effects of heatwaves on multitrophic stream communities. *Global Change Biology*, 1–14.

Schneider, C.A., Rasband, W.S. & Eliceiri, K.W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*, 9.

Sun, J. & Liu, D. (2003). Geometric models for calculating cell biovolume and surface area for phytoplankton. *Journal of Plankton Research*, 25.

Turner, M.G., Calder, W.J., Cumming, G.S., Hughes, T.P., Jentsch, A., LaDeau, S.L., *et al.* (2020). Climate change, ecosystems and abrupt change: science priorities. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 375, 20190105.

Veraart, A.J., Faassen, E.J., Dakos, V., van Nes, E.H., Lürling, M. & Scheffer, M. (2012). Recovery rates reflect distance to a tipping point in a living system. *Nature*, 481, 357–359.

Wernberg, T., Bennett, S., Babcock, R.C., de Bettignies, T., Cure, K., Depczynski, M., *et al.* (2016). Climate-driven regime shift of a temperate marine ecosystem. *Science*, 353, 169–172.

Woodward, G., Bonada, N., Brown, L.E., Death, R.G., Durance, I., Gray, C., *et al.* (2016). The effects of climatic fluctuations and extreme events on running water ecosystems. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 371.

Woolway, R.I., Albergel, C., Frölicher, T.L. & Perroud, M. (2022). Severe Lake Heatwaves Attributable to Human‐Induced Global Warming. *Geophysical Research Letters*, 49, 1–10.

Woolway, R.I., Jennings, E., Shatwell, T., Golub, M., Pierson, D.C. & Maberly, S.C. (2021). Lake heatwaves under climate change. *Nature*, 589, 402–407.