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

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 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 (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 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 abrubut 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 these perturbations and keep performing their functions.

There is currently a lack of knowledge and understing about how multiple 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 orgsnisms (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 both critical slowing down and community rescue could 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 could 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 separatedone another by 7 days of ambient temperature. In the HWs treatment, the temperature was +8Cabove the control temperature. During the experiment, the mean recorded temperature inthe ambient control treatment was 19.7C (±3.29C), whereas in the HWs treatment it was 24.3 (±5.06C).

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).

## Phytoplankton sampling and biomass quantification

The phytoplankton community was sampled on days -4, 2, 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 and counts were assessed in 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, 1, 2, 3, 7, 10, 17, 24, 31, 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, 1, 2, 3, 7, 10, 17, 24, 31, and 38 in 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 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

Dissolved oxygen declined sharply after the first HW, but recovered quicky returning to values higher than the control (Fig. 2). The second HW determined a similar decreased in DO, which was however completely recovered, and in between the second and third HWs, DO returned to control levels. The third heatwave strongly decreased DO during its course and slowed down its recovery in the post HW phase so that it never got back to control levels (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 effect of time, and an interaction between HW and time, suggesting a time dependent effect of the HWs (Table 1).

| **term** | **estimate** | **std.error** | **statistic** | **df** | **p.value** |
| --- | --- | --- | --- | --- | --- |
| (Intercept) | 2.440 | 0.026 | 92.609 | 19.538 | 0.000 |
| HW | -0.008 | 0.026 | -0.297 | 19.538 | 0.769 |
| Time | 0.003 | 0.001 | 2.518 | 94.000 | **0.013** |
| HW:Time | 0.003 | 0.001 | 2.605 | 94.000 | **0.011** |

The post-hoc analysis 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** |
| --- | --- | --- | --- | --- | --- | --- |
| Control - HW | -4 | -0.038 | 0.059 | 28.871 | -0.652 | 0.519 |
| Control - HW | 1 | -0.010 | 0.051 | 17.640 | -0.195 | 0.848 |
| Control - HW | 2 | -0.004 | 0.050 | 15.917 | -0.086 | 0.933 |
| Control - HW | 3 | 0.001 | 0.048 | 14.365 | 0.029 | 0.977 |
| Control - HW | 10 | 0.041 | 0.041 | 7.606 | 1.003 | 0.347 |
| Control - HW | 15 | 0.070 | 0.039 | 6.065 | 1.794 | 0.122 |
| Control - HW | 16 | 0.075 | 0.039 | 6.004 | 1.945 | 0.1 |
| Control - HW | 17 | 0.081 | 0.039 | 6.018 | 2.091 | 0.081 |
| Control - HW | 24 | 0.121 | 0.042 | 8.450 | 2.863 | **0.02** |
| Control - HW | 29 | 0.150 | 0.048 | 13.491 | 3.135 | **0.008** |
| Control - HW | 30 | 0.155 | 0.049 | 14.942 | 3.168 | **0.006** |
| Control - HW | 31 | 0.161 | 0.050 | 16.560 | 3.195 | **0.005** |
| Control - HW | 38 | 0.201 | 0.061 | 32.699 | 3.277 | **0.002** |

Chlorophyll - *a* showed a slight decline and relative slight recovery 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).

The LMM analysis showed that the HW treatment had a significant effect on chlorophyll- a concentration (Table 3). The post-hoc proved that the HW treatment significantly reduced chlorophyll- a concentration from the first HW onwards, with an increasingly larger estimate as time progressed (Table 4).

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| **term** | **estimate** | **std.error** | **statistic** | **df** | **p.value** |
| --- | --- | --- | --- | --- | --- |
| (Intercept) | 0.371 | 0.034 | 11.057 | 16.359 | 0.000 |
| Treatment1 | 0.073 | 0.034 | 2.181 | 16.359 | **0.044** |
| Day | 0.001 | 0.001 | 0.862 | 94.000 | 0.391 |
| Treatment1:Day | 0.003 | 0.001 | 1.947 | 94.000 | 0.055 |

| **contrast** | **Day** | **estimate** | **SE** | **df** | **t.ratio** | **p\_value** |
| --- | --- | --- | --- | --- | --- | --- |
| Control - HW | -4 | 0.126 | 0.074 | 23.493 | 1.699 | 0.103 |
| Control - HW | 1 | 0.151 | 0.065 | 14.923 | 2.313 | **0.035** |
| Control - HW | 2 | 0.156 | 0.064 | 13.621 | 2.449 | **0.029** |
| Control - HW | 3 | 0.161 | 0.062 | 12.446 | 2.589 | **0.023** |
| Control - HW | 10 | 0.197 | 0.054 | 7.268 | 3.625 | **0.008** |
| Control - HW | 15 | 0.222 | 0.052 | 6.052 | 4.285 | **0.005** |
| Control - HW | 16 | 0.228 | 0.052 | 6.003 | 4.392 | **0.005** |
| Control - HW | 17 | 0.233 | 0.052 | 6.015 | 4.488 | **0.004** |
| Control - HW | 24 | 0.268 | 0.056 | 7.926 | 4.827 | **0.001** |
| Control - HW | 29 | 0.294 | 0.061 | 11.784 | 4.775 | **>0.001** |
| Control - HW | 30 | 0.299 | 0.063 | 12.883 | 4.746 | **>0.001** |
| Control - HW | 31 | 0.304 | 0.064 | 14.106 | 4.713 | **>0.001** |
| Control - HW | 38 | 0.339 | 0.077 | 26.481 | 4.422 | **>0.001** |

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 5).

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| **Day** | **F** | **R2** | **p\_value** |
| --- | --- | --- | --- |
| -4 | 0.891 | 0.129 | 0.367 |
| 2 | 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** |

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