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Sensitivity of phytoplankton, zooplankton and macroinvertebrates to hydrogen peroxide treatments of cyanobacterial blooms

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\textbf{ABSTRACT}

Addition of hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) is a promising method to acutely suppress cyanobacterial blooms in lakes. However, a reliable H\textsubscript{2}O\textsubscript{2} risk assessment to identify potential effects on non-target species is currently hampered by a lack of appropriate ecotoxicity data. The aim of the present study was therefore to quantify the responses of a wide diversity of freshwater phytoplankton, zooplankton and macroinvertebrates to H\textsubscript{2}O\textsubscript{2} treatments of cyanobacterial blooms. To this end, we applied a multifaceted approach. First, we investigated the 24-h toxicity of H\textsubscript{2}O\textsubscript{2} to three cyanobacteria (\textit{Planktothrix agardhii}, \textit{Microcystis aeruginosa}, \textit{Anabaena} sp.) and 23 non-target species (six green algae, eight zooplankton and nine macroinvertebrate taxa), using EC\textsubscript{50} values based on photosynthetic yield for phytoplankton and LC\textsubscript{50} values based on mortality for the other organisms. The most sensitive species included all three cyanobacterial taxa, but also the rotifer \textit{Brachionus calyciflorus} and the cladocerans \textit{Ceriodaphnia dubia} and \textit{Daphnia pulex}. Next, the EC\textsubscript{50} and LC\textsubscript{50} values obtained from the laboratory toxicity tests were used to construct a species sensitivity distribution (SSD) for H\textsubscript{2}O\textsubscript{2}. Finally, the species predicted to be at risk by the SSD were compared with the responses of phytoplankton, zooplankton and macroinvertebrates to two whole-lake treatments with H\textsubscript{2}O\textsubscript{2}. The predictions of the laboratory-based SSD matched well with the responses of the different taxa to H\textsubscript{2}O\textsubscript{2} in the lake. The first lake treatment, with a relatively low H\textsubscript{2}O\textsubscript{2} concentration and short residence time, successfully suppressed cyanobacteria without major effects on non-target species. The second lake treatment had a higher H\textsubscript{2}O\textsubscript{2} concentration with a longer residence time, which resulted in partial suppression of cyanobacteria, but also in a major collapse of rotifers and decreased abundance of small cladocerans. Our results thus revealed a trade-off between the successful suppression of cyanobacteria at the expense of adverse effects on part of the zooplankton community. This delicate balance strongly depends on the applied H\textsubscript{2}O\textsubscript{2} dosage and may affect the decision whether to treat a lake or not.

1. Introduction

Cyanobacterial blooms threaten the water quality of lakes and reservoirs across the globe (O’Neil \textit{et al.}, 2012; Huisman \textit{et al.}, 2018). Decay of cyanobacterial blooms may cause oxygen depletion, with detrimental effects on many aquatic organisms (Rabalais \textit{et al.}, 2010). Moreover, several bloom-forming cyanobacteria produce potent toxins that may affect human and ecosystem health (e.g., Svircev \textit{et al.}, 2019; Chorus and Welker 2021), and can cause severe economic damage with implications for drinking water production, agriculture, fisheries and recreation (Dodds \textit{et al.}, 2009; Qin \textit{et al.}, 2010; Bulerjahn \textit{et al.}, 2016).

Several methods have been developed to prevent and suppress cyanobacterial blooms (Ibelings \textit{et al.}, 2016). Reduction of external nutrient loading is the preferred approach for the restoration of lakes and prevention of harmful algal blooms (Conley \textit{et al.}, 2009; Fastner \textit{et al.}, 2016). Yet, reducing the nutrient input is often a long-term effort...
The use of 

concerning 

Meinertz et al., 2008; Smit et al., 2008; Matthijs et al., 2012; Reich et al., 2018; Wang et al., 2019). Second, in contrast to many other chemical treatments, 

addition of hydrogen peroxide (H

selectively kills cyanobacteria within one or two days, while the 

effects on non-target organisms, to safeguard biodiversity, food-web 
structure and ecosystem integrity (Geist and Hawkins, 2016; Sumudu E.F.J. Weenink et al. bathing waters are closed for recreation because of toxic blooms. In 2018; Wang et al., 2019). Hence, a low dosage of 

H

dosage should not induce adverse effects on target and non-target organisms responded to two different levels of 

H

O

-2 dosage ranged from 2 mg L

1 to 10 mg L

-1 (Matthijs et al., 2012; Cory et al., 2016; Weenink et al., 2021). The use of H

2 against cyanobacterial blooms has therefore been investigated extensively in controlled laboratory experiments and field incubations (e.g., Lürling et al., 2014; Yang et al., 2018; Piel et al., 2020; Lusty and Gohler 2020; Sandrini et al., 2020; Spoof et al., 2020), and has been applied in several natural waters (Matthijs et al., 2012; Burson et al., 2014; Huang and Zimba 2020; Weenink et al., 2021; Piel et al., 2021). The H

2 dosage is a critical issue in applications to real ecosystems. On the one hand, the dosage should be high enough to effectively suppress the harmful bloom. To this end, the H

2 dosage ranged from 2 mg L

1 to 10 mg L

-1 (Huang and Zimba 2020) in treatments of cyanobacterial blooms in ponds and lakes. H

2 has also been used to eradicate a highly toxic bloom of the diatom flagellate 

Alexandrium ostenfeldii (Burson et al., 2014) and a fish-killing bloom of the haptophyte 

Phytotheckia parvm (Wagstaff et al., 2021). Since di- 

nofflagellates and haptophytes are less sensitive to H

2 than cyanobacteria, these two lake treatments required a higher H

2 dosage of 40 to 50 mg L

-1. On the other hand, the H

2 dosage should not induce adverse effects on non-target organisms, to safeguard biodiversity, food-web structure and ecosystem integrity (Geist and Hawkins, 2016; Sumudumali and Jayawardana, 2021). In aquaculture, H

2 is commonly applied to protect fish against infections by parasites, bacteria and fungi, which has shown that many fish species can tolerate H

2 concentrations of 200 to 1500 mg L

-1 (e.g., Rach et al., 1997; Avendano-Herrera et al., 2006). Therefore, it is unlikely that the much lower H

2 concentrations used for lake treatments of cyanobacterial blooms will have direct negative effects on fish populations. Much less is known, however, about potential effects of H

2 on other non-target organisms. Some zooplankton taxa including the cladocerans 

Daphnia pulex, 

Daphnia magna and Ceriodaphnia dubia, the rotifer 

Brachionus calyciflorus, the ostracod 

Heterocylops incongruus, the ciliate 

Tetrahymena thermophila, the calanoid copepods (unidentified species) and cyclopoid copepods (unidentified species). For 

D. pulex and 

D. magna, newly released neonates (<24 h old) originating from maximum eight-week old cultures were used. For 

C. dubia, 

B. calyciflorus, 

H. incongruus, and 

T. thermophila, individuals <24 h old were used. Copepods were collected from the field, and included both copepodite and adult stages. The tested macroinvertebrate species included first instar larvae of the dipteran 

Chironomus riparius and the trichopteran 

Limnephilus lunatus and adults of the two oligochaete worms 

Limnodrilus hoffmeisteri and 

Lumbriculus variegatus cultured under laboratory conditions. The mayflies 

Ephemerida danica and Baetidae sp. (late instar larvae), the hemip- 

teran 

Sigara striata, the mayfly 

Limnomyx benedent, and the amphipod 

Gammarus pulex were collected from the field. Toxicity tests with field- 
collected organisms were performed within 7 days after sampling. The origins of the species and further experimental details are described in the Supporting Information (Table S1 and Supplementary Methods). 2.2. Laboratory toxicity experiments At the start of the toxicity tests, predefined volumes of 

H

2 stock solution were added to the experimental replicates to achieve the desired range of 

H

2 concentrations (see Table S1). Actual 

H

2 concentrations were measured in triplicate water samples taken immediately after 

H

2 addition. The 

H

2 concentration was analyzed by mixing 100 μl sample with 100 μl p-nitrophenyl boronic acid reagent (Sigma) according to Lu et al. (2011). The 

H

2-dependent formation of di-nitrophenol was quantified by absorbance at 405 nm measured with a microplate fluorescence reader (SPECTROstar nano, BMG Labtech). This method is able to detect 

H

2 concentrations of 3.125 μM (0.106 mg L

-1) and higher (Lu et al., 2011). For the phytoplankton toxicity tests, 12-well plates (Corning Incorporated, Corning, USA) were inoculated with phytoplankton cultures at a final biovolume concentration of 0.59 ± 0.04 mm

3 mL

-1 (average ± SD, n = 35), as quantified with a Casy 1 TTT cell counter (OLS OMNI Life
Science, Bremen, Germany). We ran six replicates per H₂O₂ concentration, three of which were for measuring the H₂O₂ concentration and three for determination of the photosynthetic yield after 24 h of H₂O₂ exposure (expressed as percentage of the control without H₂O₂).

Phytoplankton was dark adapted for 10 min before the photosynthetic yield was determined with a Mini-PAM-2 fluorometer according to the manufacturer’s instructions (Walz, Effeltrich, Germany), with the sensor mounted just above the wells. The maximum photosynthetic yield Fv/Fm (i.e., the maximum quantum yield of PSII electron transport) was calculated as:

\[ F_v/F_m = (F_m - F_o)/F_m \]  

where \( F_m \) is the maximum fluorescence in the dark following a saturating light pulse and \( F_o \) is the minimum fluorescence (Maxwell and Johnson, 2000).

Toxicity tests with zooplankton and macroinvertebrates were performed following guidelines 202 and 235 of the Organization for Economic Cooperation and Development (OECD, 2004, 2011) with some modifications. All tests with macroinvertebrates, *D. magna* and *D. pulex* were performed in 6-well plates (Corning Incorporated, Kennebunk, USA) using 10 mL of ADaM medium (Klüttgen et al., 1994) for the two *Daphnia* species, 10 mL of Dutch Standard Water (DSW; NEN 6503, 1980) for the cultured macroinvertebrate taxa, and 10 mL of filtered (1.2 μm pore size) field-collected water for the field-collected macroinvertebrate taxa. Toxicity tests with *B. calciflorus*, *C. dubia*, *H. incongruens*, and *T. thermophila* were performed according to the Standard Operating Procedures (SOP) provided by the supplier with slight modifications (Table S1). Tests were performed with four replicates per H₂O₂ concentration, except for *B. calciflorus* and *H. incongruens* where we used eight replicates in accordance with the supplier’s instructions. Five individuals were added to each replicate well, except for *C. riparius* where ten individuals were added following de Baat et al. (2012).

After 24 h of exposure to H₂O₂, the number of surviving individuals per well was counted, except for the ciliate *T. thermophila* where the endpoint of the toxicity test was the turnover of provided substrate into biomass according to the supplier’s instructions (Table S1). Substrate concentration was determined by measuring the optical density (OD) at 440 nm with a microplate fluorescence reader (SPECTROstar nano, BMG Labtech).

### 2.3. Lake treatments

Cyanobacterial blooms in Lake Oosterduinse Meer (52° 16′ 55″ N, 4° 30′ 28″ E; surface area = 0.3 km²; average depth = 7 m) were treated with H₂O₂ on 19 June and 7 August 2018. Diluted H₂O₂ was homogeneously distributed into the lake by a specially designed boat equipped with a ‘water harrow’ (sensu Matthijs et al., 2012). This is a tubular injection system, that was attached on a manifold extending 2 m on each side of the boat and consisted of tubes with outlet valves that can be positioned at various depths up to 5 m (Piel et al., 2021). The boat slowly moved back and forth across the lake, using a computer-controlled system integrating the cruise track and cruise speed to calculate the required H₂O₂ injection rate. The intended H₂O₂ concentration was 2.5 mg L⁻¹ H₂O₂ to minimize potential effects on non-target species. During and after the treatments, H₂O₂ concentrations in the water were measured throughout the entire lake at multiple time points and at depths up to 5 m using Quantofix indicator sticks (Macherey-Nagel, Düren, Germany).

The weather during the first lake treatment was mostly cloudy but without rain (daily mean temperature 18.5 °C; daily sunshine 4.8 h; daily windspeed 4.6 m s⁻¹; daily precipitation 0 mm; water temperature at 0.5 m depth 18.0 °C). It was mostly sunny and warm, with a little bit of rain, during the second lake treatment (daily mean temperature 26.2 °C; daily sunshine 10.0 h; daily windspeed 2.6 m s⁻¹; daily precipitation 20 mm; water temperature at 0.5 m depth 27.0 °C).

### 2.4. Sampling of biota

Phytoplankton was sampled on an approximately biweekly basis between 14 June and 30 August 2018 at three sampling locations in the lake, with more intense sampling during the two lake treatments. Integrated water samples were taken from 0 to 6 m depth with a flexible PVC water hose (10 m length, 5 cm width). Phytoplankton samples were preserved with 0.4% Lugol’s iodine solution and stored in the dark at 4 °C until microscopic analysis. Phytoplankton was counted and identified to species level using an inverted microscope with a 1 mL counting chamber (Utermöhl,1958). Biovolumes of the phytoplankton were calculated from cell numbers and cellular geometry following Hillbrand et al. (1999).

Zooplankton was sampled during the same time period using the same water hose at five locations in the open water of the lake. At each location, the zooplankton sample was concentrated by filtering 10 L of an integrated water sample over a 41 μm mesh size. The concentrated samples were preserved with a 1% alkaline Lugol’s iodine solution, and stored in the dark at 4 °C until microscopic analysis. Zooplankton were identified to the lowest possible taxonomic level and counted with tubular and Bogorov counting chambers (Hydro-Bios, Kiel Germany).

Macroinvertebrates were sampled six days before and six days after the second lake treatment, at four locations in the littoral zone of Lake Oosterduinse Meer. At each location, one macroinvertebrate sample was taken from the macrophyte vegetation and one from the sediment using a dip net (0.3 m wide, 500 μm mesh size) swept over a length of 2.5 m. Macroinvertebrates were sorted and preserved in 70% ethanol, except oligochaete worms (96% ethanol) and water mites (50% glycerin, 20% acetic acid, 30% aqua dest.). Organisms were identified to the lowest possible taxonomic level and counted.

### 2.5. Data analysis

Concentration-response relationships were constructed by fitting the actual H₂O₂ concentrations and the corresponding 24-h effect data to a logistic response model according to Haanstra et al. (1985) and Forfaït-Dubuc et al. (2012). From these concentration-response relationships, the 24-h LC₅₀ and EC₅₀ values with accompanying 95% confidence intervals were calculated with IBM SPSS Statistic 23. The LC₅₀ and EC₅₀ values of the 26 species were used to construct an SSD, using an online SSD Generator (Posthuma et al., 2002; US EPA, 2016).

A two-sample t-test was used to compare population densities of each species before and after the lake treatments. For phytoplankton and zooplankton, we compared population densities on the first and second day before with those on the first and second day after each treatment. For macroinvertebrates we used the same approach, but with population densities sampled six days before and six days after the second lake treatment. Furthermore, the Shannon diversity index (Shannon, 1949) and dominance index (Krebs, 1989) of the macroinvertebrate community were calculated before and after the second lake treatment. Statistical analyses and index calculations were performed using PAST (Hammer et al., 2001).

### 3. Results

#### 3.1. Laboratory toxicity experiments

In the control treatments, photosynthetic yield of the phytoplankton species after 24 h was at least 90% of the initial value. Similarly, survival of zooplankton and macroinvertebrate taxa after 24 h was at least 90% and for most species no mortality was observed in the control treatment. These controls confirm the validity of the laboratory toxicity experiments.

The phytoplankton toxicity tests showed that the cyanobacterial species were more sensitive to H₂O₂ than the green algae (Fig. 1a; Fig. S1). The EC₅₀ values for the cyanobacteria ranged from 1.39 mg L⁻¹.
Calculated EC$_{50}$ and LC$_{50}$ values after 24 h of exposure to H$_2$O$_2$ for phytoplankton (9 taxa), zooplankton (8 taxa) and macrofauna (9 taxa). The table shows 24 h EC$_{50}$ values ± 95% confidence intervals (in mg L$^{-1}$ H$_2$O$_2$) for the phytoplankton taxa, and 24 h LC$_{50}$ values ± 95% confidence intervals (in mg L$^{-1}$ H$_2$O$_2$) for zooplankton and macrofauna taxa.

### Table 1

<table>
<thead>
<tr>
<th>TAXON</th>
<th>EC$<em>{50}$ or LC$</em>{50}$ (mg L$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHYTOPLANKTON</td>
<td></td>
</tr>
<tr>
<td>Cyanobacteria</td>
<td></td>
</tr>
<tr>
<td>Planktothrix agardhii</td>
<td>1.39 ± 0.30</td>
</tr>
<tr>
<td>Microcystis aeruginosa</td>
<td>2.62 ± 0.15</td>
</tr>
<tr>
<td>Anabaena sp.</td>
<td>3.49 ± 0.63</td>
</tr>
<tr>
<td>Green algae</td>
<td></td>
</tr>
<tr>
<td>Kirchneriella contorta</td>
<td>27.7 ± 4.5</td>
</tr>
<tr>
<td>Desmodesmus armatus</td>
<td>31.9 ± 5.2</td>
</tr>
<tr>
<td>Chlorococcum sorokiniana</td>
<td>32.6 ± 4.1</td>
</tr>
<tr>
<td>Monoraphidium griffithii</td>
<td>34.4 ± 6.4</td>
</tr>
<tr>
<td>Ankistrodesmus falcatus</td>
<td>36.2 ± 3.7</td>
</tr>
<tr>
<td>Chlamydomonas reinhardtii</td>
<td>74.4 ± 5.8</td>
</tr>
<tr>
<td>ZOOPLANKTON</td>
<td></td>
</tr>
<tr>
<td>Ceriodaphnia dubia</td>
<td>2.20 ± 0.14</td>
</tr>
<tr>
<td>Brachionus calyciflorus</td>
<td>2.45 ± 0.40</td>
</tr>
<tr>
<td>Daphnia pulex</td>
<td>2.79 ± 0.41</td>
</tr>
<tr>
<td>Tetrahymena thermophila</td>
<td>6.50 ± 0.55</td>
</tr>
<tr>
<td>Daphnia magna</td>
<td>6.51 ± 0.19</td>
</tr>
<tr>
<td>Calanoid copepoda</td>
<td>11.5 ± 1.1</td>
</tr>
<tr>
<td>Heterocyclops incongruenus</td>
<td>18.9 ± 0.3</td>
</tr>
<tr>
<td>Cyclopoid copepoda</td>
<td>30.8 ± 3.1</td>
</tr>
<tr>
<td>MACROINVERTEBRATES</td>
<td></td>
</tr>
<tr>
<td>Lefuymysis benedeni</td>
<td>1.97 ± 0.32</td>
</tr>
<tr>
<td>Anabaena sp.</td>
<td>8.02 ± 0.84</td>
</tr>
<tr>
<td>Limnadius hoffmeisteri</td>
<td>10.5 ± 0.6</td>
</tr>
<tr>
<td>Anabaena sp.</td>
<td>14.1 ± 2.2</td>
</tr>
<tr>
<td>Gammarus pulex</td>
<td>26.9 ± 57</td>
</tr>
<tr>
<td>Limnephilus lunatus</td>
<td>394 ± 107</td>
</tr>
<tr>
<td>Baetidae sp.</td>
<td>833 ± 111</td>
</tr>
<tr>
<td>Sigara sriata</td>
<td>1734 ± 200</td>
</tr>
<tr>
<td>Ephemera danica</td>
<td>1850 ± 362</td>
</tr>
</tbody>
</table>

**benedeni** was the most sensitive species with a LC$_{50}$ value of 5.0 mg L$^{-1}$ H$_2$O$_2$, while the two annelid worms and the midge Chironomus riparius had LC$_{50}$ values in the range of 8.0 to 14.1 mg L$^{-1}$ H$_2$O$_2$ (Table 1). The other five macroinvertebrate species were much less sensitive to H$_2$O$_2$, with LC$_{50}$ values ranging from 269 to 1850 mg L$^{-1}$ H$_2$O$_2$.

#### 3.2. Predictions based on the SSD

The SSD displays the LC$_{50}$ and EC$_{50}$ values of the species in increasing order (Fig. 2). The SSD can be used to estimate the fraction of species potentially affected by a given H$_2$O$_2$ concentration. During the first lake treatment, H$_2$O$_2$ fluctuated between 0.2 and 2.7 mg L$^{-1}$ for the first 5.5 hr after the start of the H$_2$O$_2$ addition (Fig. 3a), and then decreased to concentrations below the detection limit after 6.5 hr. The average H$_2$O$_2$ concentration (± SD) over the first 5.5 hrs was 1.13 ± 0.78 mg L$^{-1}$ (n = 9 time points), which is plotted on the SSD (Fig. 3c).

Accordingly, the potentially affected fraction of species was estimated at 8 ± 5%, and of our laboratory species only the cyano bacterium *P. agardhii* was likely to be affected.

During the second lake treatment the H$_2$O$_2$ concentration fluctuated between 1.7 and 3.7 mg L$^{-1}$ for at least 13 hr (Fig. 3b), although the added H$_2$O$_2$ was degraded after 24 hr (data not shown). The average H$_2$O$_2$ concentration (± SD) during the first 5.5 hrs was 2.19 ± 0.39 mg L$^{-1}$ (n = 11), which is also plotted on the SSD (Fig. 3c). Hence, during the second treatment, the lake was exposed to slightly higher H$_2$O$_2$ concentrations and for a longer duration than during the first treatment. Consequently, a larger fraction of the species pool was potentially affected by the second lake treatment (14 ± 2%), including the cyanobacteria *P. agardhii* and *M. aeruginosa* but also non-target species such as the cladoceran *C. dubia* and rotifer *B. calyciflorus* (Fig. 3c).
3.3. Phytoplankton responses to lake treatments

Before the first \( \text{H}_2\text{O}_2 \) treatment, the lake was covered by a cyanobacterial bloom dominated by *Aphanizomenon klebahnii* (86.9% of total phytoplankton biovolume) with a smaller contribution by *Microcystis* spp. (1.9%) (Fig. 4). In response to the first lake treatment, the total cyanobacterial biovolume decreased by 85.1% within two days (Fig. 4a), primarily due to a major collapse of the *A. klebahnii* population (Fig. 4c). Subsequently, *A. klebahnii* was completely eradicated and did not return. The cyanobacterium *Microcystis* spp. also declined significantly, by 35.9%, within two days after the first lake treatment (Fig. 4f).

The total eukaryotic phytoplankton biovolume and most eukaryotic taxa were not significantly affected by the first lake treatment (Fig. 4; Fig. S2). The dinoflagellate *C. hirundinella* decreased slightly but significantly during the first days after the treatment and increased again a few days later (Fig. 4f).

Before the second \( \text{H}_2\text{O}_2 \) treatment, the lake suffered from another cyanobacterial bloom co-dominated by *Planktothrix agardhii* (26.5%) and *Dolichospermum flos-aquae* (25.7%), with a smaller contribution by *Microcystis* spp. (2.5%) and several other cyanobacterial taxa (4.8%) (Fig. 4). In response to the second lake treatment, the total cyanobacterial biovolume and the cyanobacterial taxa *D. flos-aquae* and *Microcystis* spp. decreased significantly. In particular, *D. flos-aquae* collapsed by 96.8% within two days and was permanently eradicated four days after the lake treatment (Fig. 4g). The cyanobacterium...
**P. agardhii** did not decrease significantly, however, and returned within several weeks (Fig. 4e).

The total eukaryotic biovolume decreased significantly after the second lake treatment (Fig. 4b). The two abundant dinoflagellate taxa *C. furcoides* and *C. hirundinella* declined just prior to the second lake treatment, and, similar to the green algae, were not significantly affected by the lake treatment itself (Fig. 4d,f,j). The diatom *A. granulata* declined significantly within two days after the second treatment but recovered in the subsequent weeks (Fig. 4h).

### 3.4. Zooplankton responses to lake treatments

The zooplankton community consisted of large populations of rotifers and smaller populations of copepods and cladocerans (Fig. 5a; Table S3). The first lake treatment did not significantly affect the total number of zooplankton individuals (Fig. 5a), nor any of the zooplankton taxa (Fig. 5b-j).

Before the second lake treatment, zooplankton was much more abundant than before the first lake treatment and largely dominated by
rotifers (94.9% of the zooplankton individuals) (Fig. 5a). In response to the second lake treatment, the zooplankton abundance declined steeply and significantly, primarily due to an 82.0% collapse of the rotifer community. The most abundant rotifer *Keratella cochlearis* and also *Pompholyx* sp. vanished almost completely after the second lake treatment, and did not recover in the subsequent weeks (Fig. 5b and c). The rotifer *Trichocerca* sp. also declined significantly, but maintained a lower stable population during the subsequent period (Fig. 5d), whereas *Keratella quadrata* was not significantly affected by the second lake treatment (Fig. 5e). The cladoceran *Sididae* sp. decreased significantly but still maintained ~70% of its population size (Fig. 5f), while *Daphnia* sp. did not respond significantly to the second lake treatment (Fig. 5g).

Copepod nauplii and calanoid copepods were not significantly affected (Fig. 5h and i), whereas cyclopoid copepods decreased significantly after the second lake treatment but subsequently recovered (Fig. 5j).

3.5. Macroinvertebrate responses to the second lake treatment

Macroinvertebrates in both the vegetation and the sediment were less affected by the lake treatment than the zooplankton (Fig. 6a,b; Table S4). Both the total number of Chironomidae and the most abundant chironomid taxa were not significantly different before and after the second lake treatment (Fig. 6c; Fig. S4). Numbers of the mysid *Limnomysis benedenii* in the vegetation were similar before and after the
lake treatment, but their numbers in the sediment were significantly lower after the treatment (Fig. 6d). Numbers of Trichoptera, Ephemeroptera, Hemiptera, Isopoda, Hirudinea and Gastropoda were not significantly different before and after the treatment (Fig. 6e-j). Likewise, the Shannon diversity index of the macroinvertebrates was not significantly affected by the lake treatment (Fig. 6k), and neither was the dominance index (Fig. S5).

4. Discussion

4.1. Comparing the $H_2O_2$ sensitivity of aquatic organisms in laboratory and field

This multifaceted study was motivated by the need to quantify the sensitivity of a wide diversity of phytoplankton, zooplankton and macroinvertebrates to $H_2O_2$ treatments of cyanobacterial blooms. To this end, we generated laboratory toxicity data for 26 taxa, which allowed the construction of an SSD. Such SSDs are nowadays predominantly applied in environmental risk assessment, where they serve as the basis...
for the derivation of environmental quality standards. Posthuma et al. (2019), for example, derived SSDs based on acute and chronic ecotoxicity test data for more than 12,000 different compounds.

Only a limited number of studies, however, evaluated if laboratory-derived SSDs matched with the responses of species in the field (e.g., Posthuma and de Zwart, 2012). Therefore, our study provided a unique opportunity to evaluate if the species identified to be at risk by the laboratory-based SSD indeed matched with the field responses of phytoplankton, zooplankton and macroinvertebrate taxa to H\textsubscript{2}O\textsubscript{2} exposure during two whole-lake treatments. A good agreement between laboratory and field observations is not self-evident, since laboratory conditions do not necessarily match field conditions (e.g., in terms of concentration and a longer H\textsubscript{2}O\textsubscript{2} exposure during two whole-lake treatments. A good agreement between laboratory and field observations is not self-evident, since laboratory conditions do not necessarily match field conditions (e.g., in terms of temperature, light conditions and nutrient concentrations). Nonetheless, the present comparison revealed that the taxa identified to be at risk by the SSD matched very well with the organisms that declined in abundance in response to the lake treatments. Our results thus confirm the predictive power of SSDs and support the applicability of SSDs in real world environmental impact assessments.

4.2. Phytoplankton

In line with the high H\textsubscript{2}O\textsubscript{2} sensitivity of cyanobacteria in the laboratory toxicity experiments, the dominant cyanobacteria A. klebsi and D. flos-aquae disappeared after the first and second lake treatment, respectively, and both species did not return. However, P. agardhii did not decline significantly after the second lake treatment, despite its low EC\textsubscript{50} value, and increased ten days later. This contrasts with the field study by Matthijs et al. (2012), where the P. agardhii abundance declined quickly after H\textsubscript{2}O\textsubscript{2} addition and remained low for about seven weeks. A possible explanation for the persistence of P. agardhii in the present study could be that part of the population survived the H\textsubscript{2}O\textsubscript{2} treatment in deeper water layers. Furthermore, P. agardhii blooms may vary in their H\textsubscript{2}O\textsubscript{2} sensitivity due to genetic variation, as observed in laboratory studies with P. rubescens (Lärling et al., 2020).

The H\textsubscript{2}O\textsubscript{2} sensitivity of green algae measured in the laboratory was much lower than that of the cyanobacteria, which aligns with previous studies (Drábková et al., 2007; Sinha et al., 2018; Weenink et al., 2021) as well as with our lake treatments where green algae were not affected. Yet, the abundances of the dinoflagellate C. hilarundinella and diatom A. granulata declined significantly after the first and second lake treatment, respectively, although both eukaryotic species showed high resilience and their numbers increased again within days. In a mesocosm study with a H\textsubscript{2}O\textsubscript{2} concentration of 10 mg L\textsuperscript{-1}, eukaryotic phytoplankton was also affected (Santos et al., 2021). Hence, these results confirm that eukaryotic phytoplankton are generally less sensitive to H\textsubscript{2}O\textsubscript{2} than bloom-forming cyanobacteria, although some eukaryotic taxa appear more sensitive than the green algae investigated in our laboratory experiments.

4.3. Zooplankton

Zooplankton was rather sensitive to H\textsubscript{2}O\textsubscript{2}, although there were clear species-specific differences. The rotifer B. calyciflorus had a similar LC\textsubscript{50} value as the EC\textsubscript{50} values of the investigated cyanobacteria and the LC\textsubscript{50} value of the marine rotifer B. plicatilis studied by Smit et al. (2008). The lake treatments confirmed these laboratory results. In the first lake treatment the average H\textsubscript{2}O\textsubscript{2} concentration of 1.13 mg L\textsuperscript{-1} was below the LC\textsubscript{50} value for B. calyciflorus and had no significant effect on rotifer abundances. In the second lake treatment the average H\textsubscript{2}O\textsubscript{2} concentration of 2.19 mg L\textsuperscript{-1} was in the same range as the LC\textsubscript{50} value for B. calyciflorus. Moreover, the exposure time during the first lake treatment was only ~5.5 h, while H\textsubscript{2}O\textsubscript{2} remained in the water for at least 13 h during the second lake treatment. This combination of a higher H\textsubscript{2}O\textsubscript{2} concentration and a longer H\textsubscript{2}O\textsubscript{2} residence time during the second lake treatment was probably responsible for the major collapse of the rotifer populations, including K. cochlearis, Pompholyx spp. and Trichocerca spp.

Similarly, Sinha et al. (2018) showed that the abundance of Brachionus spp. in experimental ponds was not affected by exposure to 2.5 mg L\textsuperscript{-1} H\textsubscript{2}O\textsubscript{2}, whereas Brachionus spp. declined significantly when treated with 4.0 mg L\textsuperscript{-1} H\textsubscript{2}O\textsubscript{2}.

The relatively small cladocerans C. dubia and D. pulex showed a similar H\textsubscript{2}O\textsubscript{2} sensitivity as the rotifer B. calyciflorus, while the larger D. magna was somewhat less sensitive. Similarly, Reichwaldt et al. (2012) reported a low LC\textsubscript{50} value of 2 mg L\textsuperscript{-1} H\textsubscript{2}O\textsubscript{2} for the small cladoceran Moina sp. and a higher LC\textsubscript{50} of 5.6 mg L\textsuperscript{-1} H\textsubscript{2}O\textsubscript{2} for the larger D. carinata. This variation in H\textsubscript{2}O\textsubscript{2} sensitivity of cladocerans was also observed during the lake treatments, where small Sibidæ sp. decreased significantly after the second lake treatment, whereas abundances of the larger Daphnia sp. were not significantly affected. In addition to direct effects of H\textsubscript{2}O\textsubscript{2}, indirect effects of the lake treatments cannot be ruled out. For example, the decline of small cladocerans could also result from the decline of cyanobacteria as a food source (e.g., Urrutia-Cordero et al., 2015).

In total, however, our results and previous studies (e.g., Matthijs et al., 2012; Sinha et al., 2018; Yang et al., 2018) all indicate that cladoceran populations are negatively affected by H\textsubscript{2}O\textsubscript{2} concentrations in the range of 2 to 7 mg L\textsuperscript{-1}, albeit with considerable interspecific variation that might be size dependent.

Copepods and ostracods were the least H\textsubscript{2}O\textsubscript{2} sensitive zooplankton taxa in our laboratory tests, possibly because their body tissue is protected from H\textsubscript{2}O\textsubscript{2} exposure by an exoskeleton (copepods) or valves (ostracod). The LC\textsubscript{50} values for these taxa were far above the H\textsubscript{2}O\textsubscript{2} concentrations applied during the lake treatments, which did not affect the abundances of copepod nauplii and calanoid copepods. Contrary to expectation, however, cyclopoid copepods declined significantly after the second lake treatment. A possible explanation could be that cyclopoid copepods are efficient predators of rotifers (Brandl, 2005), and hence declined as an indirect effect of their dwindling prey populations. Another possibility is that the temporary decrease of cyclopoid copepods was part of their natural population dynamics, unrelated to the H\textsubscript{2}O\textsubscript{2} treatment. The latter explanation would be consistent with the lack of responses of copepods exposed to H\textsubscript{2}O\textsubscript{2} concentrations < 7 mg L\textsuperscript{-1} H\textsubscript{2}O\textsubscript{2} in other studies (Sinha et al., 2018; Yang et al., 2018). In the lake study of Burson et al. (2014), who applied 50 mg L\textsuperscript{-1} H\textsubscript{2}O\textsubscript{2}, zooplankton was almost completely wiped out, but many copepod nauplii survived the treatment. These observations all support the conclusion that copepods are generally less sensitive to H\textsubscript{2}O\textsubscript{2} than rotifers and cladocerans.

4.4. Aquatic macroinvertebrates

The survival of aquatic macroinvertebrates was less affected by H\textsubscript{2}O\textsubscript{2} than the photosynthetic yield of the cyanobacteria investigated in our laboratory experiments. Yet, the H\textsubscript{2}O\textsubscript{2} sensitivity of aquatic macroinvertebrates showed large interspecific variation, with LC\textsubscript{50} values ranging from 5 mg L\textsuperscript{-1} H\textsubscript{2}O\textsubscript{2} for the mayfly nymph E. danica. These results are consistent with the previous study of Smit et al. (2008), who found EC\textsubscript{50} values of 46 mg L\textsuperscript{-1} for the amphipod Corophium volutator and 188 mg L\textsuperscript{-1} for the fairy shrimp Artemia salina. In agreement with our laboratory experiments, L. benedeni was the only macroinvertebrate taxon with a significant negative response to the second lake treatment, whereas all other macroinvertebrates showed no response. Similarly, Matthijs et al. (2012) found no effect of a whole-lake treatment with 2 mg L\textsuperscript{-1} H\textsubscript{2}O\textsubscript{2} on macroinvertebrates, and no clear effects on chironomids, oligochaete worms and water mites (Acari) in lake mesocosms treated with up to 8 mg L\textsuperscript{-1} H\textsubscript{2}O\textsubscript{2}.

However, some previous lake treatments have used higher H\textsubscript{2}O\textsubscript{2} concentrations of 40 to 50 mg L\textsuperscript{-1} (Burson et al., 2014; Wagstaff et al., 2021). Our laboratory toxicity tests warn that the impacts of such high H\textsubscript{2}O\textsubscript{2} dosages may kill several macrofaunal taxa, including mysid shrimps, annelid worms and chironomid larvae. This is corroborated by results of Burson et al. (2014), who found dead specimens of ragworms (Nereis diversicolor) and their Table 3 also points at a complete removal.
of the chironomid larvae. Yet, other macroinvertebrates including iso-
pods, snails and gammarid shrimps remained abundant and active

despite this very severe \( \text{H}_2\text{O}_2 \) treatment.

### 4.5. Caveats and limitations

As a first guideline, Matthijs et al. (2016) argued that the \( \text{H}_2\text{O}_2 \) concentration in lake treatments should remain below 5 mg \( \text{L}^{-1} \) to avoid effects on non-target species. However, our results show that 5 mg \( \text{L}^{-1} \) of \( \text{H}_2\text{O}_2 \) will have detrimental effects on rotifers and small cladocerans, and may even have negative effects on mysid shrimps. Since this preliminary guideline is not tenable, we propose a more detailed classification of \( \text{H}_2\text{O}_2 \) lake treatments (see next section).

This classification may require further modification tailored to each specific lake, since the \( \text{H}_2\text{O}_2 \) sensitivity of species and the efficacy of lake treatments is influenced by a plethora of biotic and abiotic factors. These confounding factors include temperature (Rach et al., 1997), light (Piel et al., 2020), nutrients (Sandrini et al., 2020), phytoplankton abund-

ances (Weenink et al., 2015) and protective mechanisms such as mucus-embedded colony formation (Gao et al., 2015). Moreover, not only the \( \text{H}_2\text{O}_2 \) concentration but also the duration of \( \text{H}_2\text{O}_2 \) exposure is of key importance (Smith et al., 2008). The exposure time can differ sub-

stantially between lake treatments, which may affect the damage to non-target species, as observed in the present study.

Furthermore, our study focused on direct effects of \( \text{H}_2\text{O}_2 \), but did not investigate indirect effects mediated by species interactions. Knocking out some of the species may have cascading effects throughout the food web. An example is the decline of cyclopoid copepods after the second lake treatment, which might be attributed to the \( \text{H}_2\text{O}_2 \)-driven suppres-

sion of their rotifer prey. Interspecific protection against \( \text{H}_2\text{O}_2 \) is another important species interaction. High abundances of green algae can rapidly degrade \( \text{H}_2\text{O}_2 \), thereby protecting cyanobacteria against oxidative stress, which hampers successful suppression of cyanobacterial blooms (Weenink et al., 2021). In addition, the presence of \( \text{H}_2\text{O}_2 \)-sca-

venging heterotrophic bacteria may protect some cyanobacterial strains (Smith et al., 2022).

### 5. Recommendations and conclusions

Based on our findings, we propose a classification of \( \text{H}_2\text{O}_2 \) lake treatments according to their effectivity to suppress cyanobacterial blooms and their expected effects on non-target species:

- **Mild treatments** (≤ 2 mg \( \text{L}^{-1} \)) are able to suppress some cyanobacte-

rial blooms, while avoiding effects on non-target species.

- **Moderate treatments** (2–4 mg \( \text{L}^{-1} \)) can suppress many cyanobacterial blooms, but are likely to have negative effects on some zoo plankton taxa (particularly rotifers and small cladocerans) while avoiding effects on macroinvertebrates.

- **Severe treatments** (4–10 mg \( \text{L}^{-1} \)) can suppress most cyanobacterial blooms, but will have negative effects on many zooplankton taxa (rotifers, cladocerans, ciliates) and some macroinvertebrates (mysid shrimps, annelid worms).

- **Very severe treatments** (10–100 mg \( \text{L}^{-1} \)) will eliminate most cyanobacterial blooms and several eukaryotic harmful algae, but will also suppress other eukaryotic phytoplankton taxa, most zooplankton, and several macroinvertebrates (mysid shrimps, annelid worms, chironomids).

The choice of the most adequate treatment is a management decision. In some lakes, adverse effects on non-target species should be avoided, while in other cases terminating the detrimental effects of a toxic cyanobacterial bloom may outweigh the possible negative effects of a lake treatment.

This classification provides a starting point to assess the likely effects of \( \text{H}_2\text{O}_2 \) lake treatments, but may require further modification tailored to the local lake conditions. Ultimately, the decision to treat a lake or not, should involve careful consideration of the delicate balance be-
tween the successful suppression of a toxic cyanobacterial bloom and the potential adverse effects on non-target species.

### 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

We shared most data in the Supplementary Tables, other data will be available on request.

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### Supplementary materials


### References


