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

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ABSTRACT

Addition of hydrogen peroxide (H_2O_2) is a promising method to acutely suppress cyanobacterial blooms in lakes. However, a reliable H_2O_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_2O_2 treatments of cyanobacterial blooms. To this end, we applied a multifaceted approach. First, we investigated the 24-h toxicity of H_2O_2 to three cyanobacteria (*Planktothrix agardhii, Microcystis aeruginosa, Anabaena* sp.) and 23 non-target species (six green algae, eight zooplankton and nine macroinvertebrate taxa), using EC_{50} values based on photosynthetic yield for phytoplankton and LC_{50} values based on mortality for the other organisms. The most sensitive species included all three cyanobacterial taxa, but also the rotifer *Brachionus calyciflores* and the cladocerans *Ceriodaphnia dubia* and *Daphnia pulex*. Next, the EC_{50} and LC_{50} values obtained from the laboratory toxicity tests were used to construct a species sensitivity distribution (SSD) for H_2O_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_2O_2. The predictions of the laboratory-based SSD matched well with the responses of the different taxa to H_2O_2 in the lake. The first lake treatment, with a relatively low H_2O_2 concentration and short residence time, successfully suppressed cyanobacteria without major effects on non-target species. The second lake treatment had a higher H_2O_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_2O_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 et al., 2012; Huisman et al., 2018). Decay of cyanobacterial blooms may cause oxygen depletion, with detrimental effects on many aquatic organisms (Rabalais et al., 2010). Moreover, several bloom-forming cyanobacteria produce potent toxins that may affect human and ecosystem health (e.g., Svirčev *et al.*, 2019; Chorus and Welker 2021), and can cause severe economic damage with implications for drinking water production, agriculture, fisheries and recreation (Dodds et al., 2009; Qin et al., 2010; Bullerjahn et al., 2016).

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

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(Gulati and van Donk, 2002), while in some instances a fast and immediate elimination of cyanobacterial blooms is required, e.g., when the provision of drinking water is in danger, livestock is threatened, or bathing waters are closed for recreation because of toxic blooms. In those emergency cases, addition of hydrogen peroxide (H_2O_2) is a promising method to acutely suppress cyanobacterial blooms (Matthijs et al., 2012; Huang and Zimba 2020; Sukenik and Kaplan 2021).

The use of H_2O_2 has two major advantages. First, cyanobacteria are more sensitive to H_2O_2 and display much lower H_2O_2 degradation rates than most eukaryotic algae (Drábková et al., 2007; Barrington and Ghadouani 2008; Weenink et al., 2021). Hence, a low dosage of H_2O_2 selectively kills cyanobacteria within one or two days, while the eukaryotic phytoplankton community remains largely unaffected and may even increase in abundance and diversity after a cyanobacterial bloom has been largely suppressed (Weenink et al., 2015; Yang et al., 2018; Wang et al., 2019). Second, in contrast to many other chemical treatments, H_2O_2 addition leaves no long-term chemical traces behind in the environment (Matthijs et al., 2016). Instead, the added H_2O_2 breaks down into water and oxygen typically within a few hours to days, depending on the biological and chemical characteristics of the lake and the applied H_2O_2 concentration (Matthijs et al., 2012; Cory et al., 2016; Weenink et al., 2021). The use of H_2O_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 Gobler 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_2O_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_2O_2 dosage ranged from 2 mg L^{-1} (Matthijs et al., 2012) to 10 mg L^{-1} (Huang and Zimba 2020) in treatments of cyanobacterial blooms in ponds and lakes. H_2O_2 has also been used to eradicate a highly toxic bloom of the dinoflagellate *Alexandrium ostenfeldii* (Burson et al., 2014) and a fish-killing bloom of the haptophyte *Prymnesium parvum* (Wagstaff et al., 2021). Since dinoflagellates and haptophytes are less sensitive to H_2O_2 than cyanobacteria, these two lake treatments required a higher H_2O_2 dosage of 40 to 50 mg L^{-1}.

On the other hand, the H_2O_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_2O_2 is commonly applied to protect fish against infections by parasites, bacteria and fungi, which has shown that many fish species can tolerate H_2O_2 concentrations of 200 to 1500 mg L^{-1} (e.g., Rach et al., 1997; Avendaño-Herrera et al., 2006). Therefore, it is unlikely that the much lower H_2O_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_2O_2 on other non-target organisms. Some zooplankton taxa including the cladocerans *Daphnia carinata, Moina* sp., *Ceriodaphnia dubia,* and the rotifer *Brachionus* sp. appeared to be sensitive to H_2O_2 concentrations in the range from 1 to 10 mg L^{-1} H_2O_2 (Meinertz et al., 2008; Smit et al., 2008; Matthijs et al., 2012; Reichwaldt et al., 2012; Sinha et al., 2018; Yang et al., 2018). Knowledge concerning H_2O_2 toxicity to freshwater macroinvertebrates is even scarcer, but studies of marine invertebrates have shown large interspecific variation in H_2O_2 sensitivity and some species appear sensitive to concentrations < 10 mg L^{-1} (Smit et al., 2008; Friedman et al., 2018). Hence, the few available toxicity data suggest that adverse effects on non-target species cannot be ruled out in H_2O_2 treatments of lakes. This knowledge gap limits our understanding of the potential environmental impacts of these lake treatments, and an ecological risk assessment is thus urgently needed before H_2O_2 can be routinely applied to combat cyanobacterial blooms.

This study aims to quantify the responses of a wide diversity of freshwater phytoplankton, zooplankton and aquatic macroinvertebrates to H_2O_2 treatments of cyanobacterial blooms. We first investigated the acute 24-h toxicity of H_2O_2 to three target cyanobacteria and 23 non-target species (six green algae, eight zooplankton and nine macroinvertebrate taxa) under controlled laboratory conditions. The decrease in photosynthetic yield was used as proxy for the H_2O_2 sensitivity of cyanobacteria and green algae, whereas we monitored mortality for the other species. Using the obtained effect concentrations, a species sensitivity distribution (SSD) was constructed for ecological risk assessment of the applied H_2O_2 dosage. Subsequently, the results of these laboratory toxicity tests were compared with responses of phytoplankton, zooplankton and macroinvertebrates to two whole-lake treatments, which provided a unique opportunity to assess how both target and non-target organisms responded to two different levels of H_2O_2 exposure in the field.

2. Materials and methods

2.1. Test organisms

Phytoplankton test species included the cyanobacteria *Anabaena* PCC 7938, *Microcystis aeruginosa* PCC 7806, and *Planktothrix agardhii* PCC 7811 and the green algae *Ankistrodesmus falcatus* SAG 202–9, *Chlamydomonas reinhardtii* SAG 77.8, *Chlorella sorokiniana* SAG 211–8k, *Desmodesmus armatus* SAG 276–4e, *Kirchneriella contorta* SAG 11.81 and *Monoraphidium griffithii* SAG 202–13. Cells were grown under axenic conditions and samples for toxicity experiments were taken during exponential growth.

Zooplankton test species included the cladocerans *Daphnia pulex, Daphnia magna* and *Ceriodaphnia dubia,* the rotifer *Brachionus calyciflorus*, the ostracod *Heterocypris incongruens,* the ciliate *Tetrahymena thermophila*, 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. incongruens,* 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 *Ephemera danica* and *Baetidae* sp. (late instar larvae), the hemipteran *Sigara striata*, the mysid *Limnomysis benedeni*, 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_2O_2 stock solution were added to the experimental replicates to achieve the desired range of H_2O_2 concentrations (see Table S1). Actual H_2O_2 concentrations were measured in triplicate water samples taken immediately after H_2O_2 addition. The H_2O_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_2O_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_2O_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, Kennebunk, 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 H_2O_2 concentration, three of which were used for measuring the H_2O_2 concentration and three for determination of the photosynthetic yield after 24 h of H_2O_2 exposure (expressed as percentage of the control without H_2O_2).

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 F_v/F_m (i.e., the maximum quantum yield of PSII electron transport) was calculated as:

$$F_v/F_m = (F_m - F_0)/F_m \quad (1)$$

where F_m is the maximum fluorescence in the dark following a saturating light pulse and F_0 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. calyciflorus, 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 H_2O_2 concentration, except for *B. calyciflorus* 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 H_2O_2, 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 km^2; average depth = 7 m) were treated with H_2O_2 on 19 June and 7 August 2018. Diluted H_2O_2 was homogeneously distributed into the lake by a specially designed boat equiped 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 H_2O_2 injection rate. The intended H_2O_2 concentration was 2.5 mg L^{-1} H_2O_2 to minimize potential effects on non-target species. During and after the treatments, H_2O_2 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 Hillebrand 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) swiped 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 H_2O_2 concentrations and the corresponding 24-h effect data to a logistic response model according to Haanstra et al. (1985) and Forfait-Dubuc et al. (2012). From these concentration-response relationships, the 24-h LC_{50} and EC_{50} values with accompanying 95% confidence intervals were calculated with IBM SPSS Statistic 23. The LC_{50} and EC_{50} 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 H_2O_2 than the green algae (Fig. 1a; Fig. S1). The EC_{50} values for the cyanobacteria ranged from 1.39 mg L^{-1}

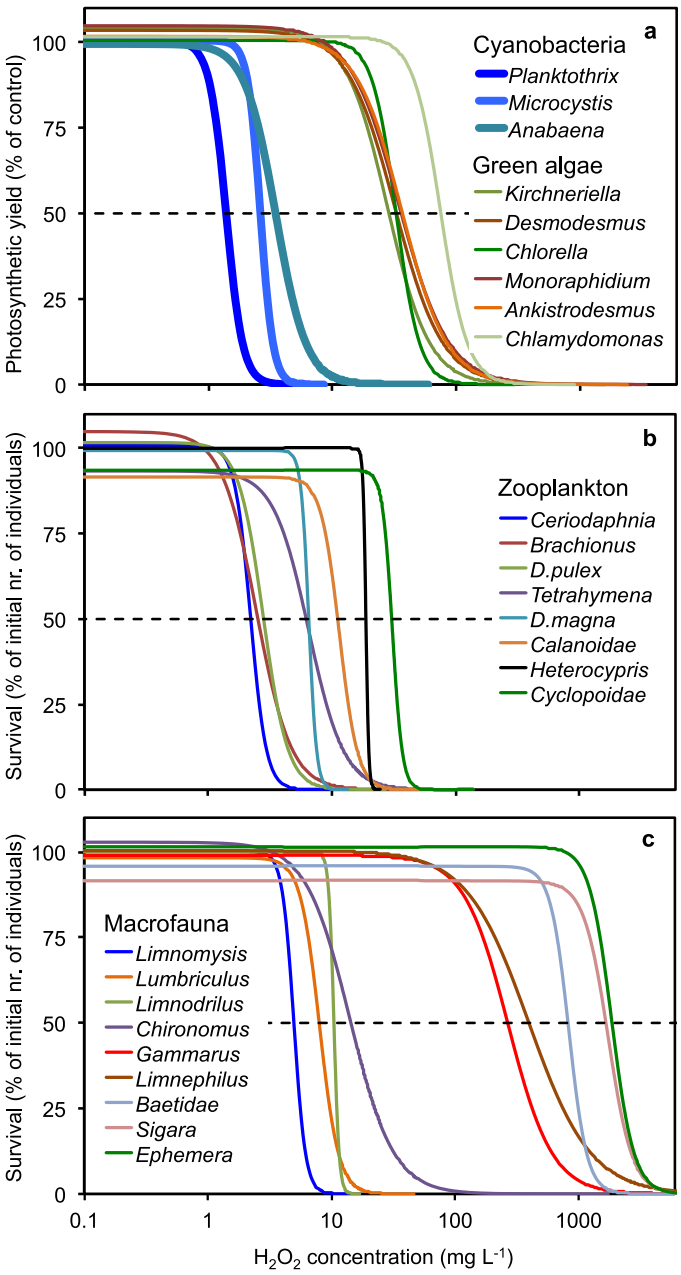

Fig. 1. Concentration-response relationships after 24 h of H_2O_2 exposure in laboratory toxicity tests. The panels show logistic models of (**a**) the photosynthetic yield of nine phytoplankton taxa (as % of control), and the survival of (**b**) eight zooplankton taxa and (**c**) nine macroinvertebrate taxa (as % of initial number of individuals). Underlying data and model fits are presented in Figs. S1-S3.

H_2O_2 for *Planktothrix agardhii* to 3.49 mg L^{-1} H_2O_2 for *Anabaena* sp. (Table 1). The EC_{50} values for the investigated green algae were approximately 20 times higher, ranging from 27.7 to 74.4 mg L^{-1} H_2O_2.

The zooplankton species also showed interspecific variation in H_2O_2 sensitivity (Fig. 1b; Fig. S2). The most sensitive taxa were the rotifer *B. calyciflorus* and the cladocerans *C. dubia* and *D. pulex* with LC_{50} values ranging from 2.2 to 2.8 mg L^{-1} H_2O_2 (Table 1). The ciliate *T. thermophilla* and cladoceran *D. magna* showed higher LC_{50} values of ~6.5 mg L^{-1} H_2O_2. Calanoid and cyclopoid copepods and the ostracod *H. incongruens* were the least sensitive zooplankton species with LC_{50} values ranging from 11.5 to 24.2 mg L^{-1} H_2O_2.

Macroinvertebrates showed large interspecific differences in H_2O_2 sensitivity as well (Fig. 1c; Fig. S3). The mysid shrimp *Limnomysis benedeni* was the most sensitive species with a LC_{50} value of 5.0 mg L^{-1} H_2O_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_2O_2 (Table 1). The other five macroinvertebrate species were much less sensitive to H_2O_2, with LC_{50} values ranging from 269 to 1850 mg L^{-1} H_2O_2.

Table 1
Calculated EC_{50} and LC_{50} values after 24 h of exposure to H_2O_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_2O_2) for the phytoplankton taxa, and 24 h LC_{50} values ± 95% confidence intervals (in mg L^{-1} H_2O_2) for zooplankton and macrofauna taxa.

TAXON	EC_{50} or LC_{50} (mg L^{-1})
PHYTOPLANKTON	EC_{50}
Cyanobacteria	
Planktothrix agardhii	1.39 ± 0.30
Microcystis aeruginosa	2.62 ± 0.15
Anabaena sp.	3.49 ± 0.63
Green algae	
Kirchneriella contorta	27.7 ± 4.5
Desmodesmus armatus	31.9 ± 5.2
Chlorella sorokiniana	32.6 ± 4.1
Monoraphidium griffithii	34.4 ± 6.4
Ankistrodesmus falcatus	36.2 ± 3.7
Chlamydomonas reinhardtii	74.4 ± 5.8
ZOOPLANKTON	**LC_{50}**
Ceriodaphnia dubia	2.20 ± 0.14
Brachionus calyciflorus	2.45 ± 0.40
Daphnia pulex	2.79 ± 0.41
Tetrahymena thermophila	6.50 ± 0.55
Daphnia magna	6.51 ± 0.19
Calanoid copepoda	11.5 ± 1.1
Heterocypris incongruens	18.9 ± 0.3
Cyclopoid copepoda	30.8 ± 3.1
MACROINVERTEBRATES	**LC_{50}**
Limnomysis benedeni	4.97 ± 0.32
Lumbriculus variegatus	8.02 ± 0.84
Limnodrilus hoffmeisteri	10.5 ± 0.6
Chironomus riparius	14.1 ± 2.2
Gammarus pulex	269 ± 57
Limnephilus lunatus	394 ± 107
Baetidae sp.	833 ± 111
Sigara striata	1734 ± 200
Ephemera danica	1850 ± 362

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_2O_2 concentration. During the first lake treatment, H_2O_2 fluctuated between 0.2 and 2.7 mg L^{-1} for the first 5.5 hr after the start of the H_2O_2 addition (Fig. 3a), and then decreased to concentrations below the detection limit after 6.5 hr. The average H_2O_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 cyanobacterium *P. agardhii* was likely to be affected.

During the second lake treatment the H_2O_2 concentration fluctuated between 1.7 and 3.7 mg L^{-1} for at least 13 hr (Fig. 3b), although the added H_2O_2 was degraded after 24 hr (data not shown). The average H_2O_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_2O_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).

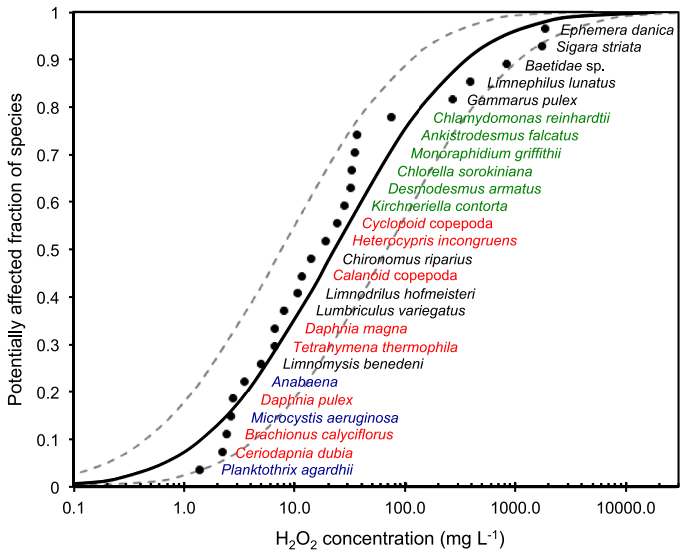

Fig. 2. Species sensitivity distribution as function of the H_2O_2 concentration, based on 24-h EC_{50} values for three cyanobacteria (blue) and six green algae (green) and 24-h LC_{50} values for eight zooplankton (red) and nine macroinvertebrate taxa (black). Dashed lines represent the 95% confidence interval.

3.3. Phytoplankton responses to lake treatments

Before the first H_2O_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. 4i).

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 H_2O_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

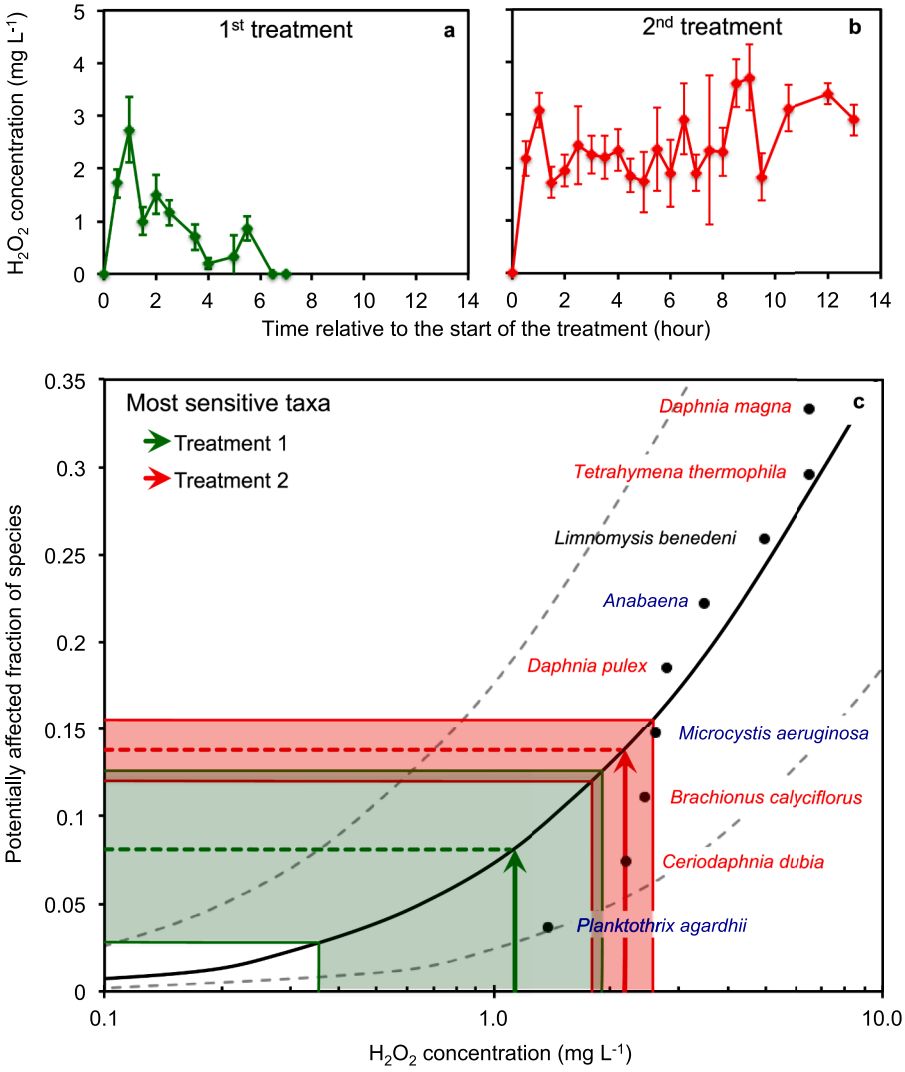

Fig. 3. H_2O_2 concentrations during the two treatments of Lake Oosterduinse Meer projected on the species sensitivity distribution. (**a,b**) H_2O_2 concentration in the upper 5 m of the lake (n = 2 - 44 spatially distributed measurements ± SE per time point) during the first several hours after (**a**) the first lake treatment (19 June 2018) and (**b**) the second lake treatment (7 August 2018). (**c**) Lower range of the species sensitivity distribution with the most sensitive phytoplankton, zooplankton and macroinvertebrate taxa. Vertical arrows and shaded areas indicate the average H_2O_2 concentration and its SD during the first 5.5 h after the first lake treatment (green arrow and shaded area) and the second lake treatment (red arrow and shaded area). Horizontal shaded areas show the corresponding potentially affected fraction of species.

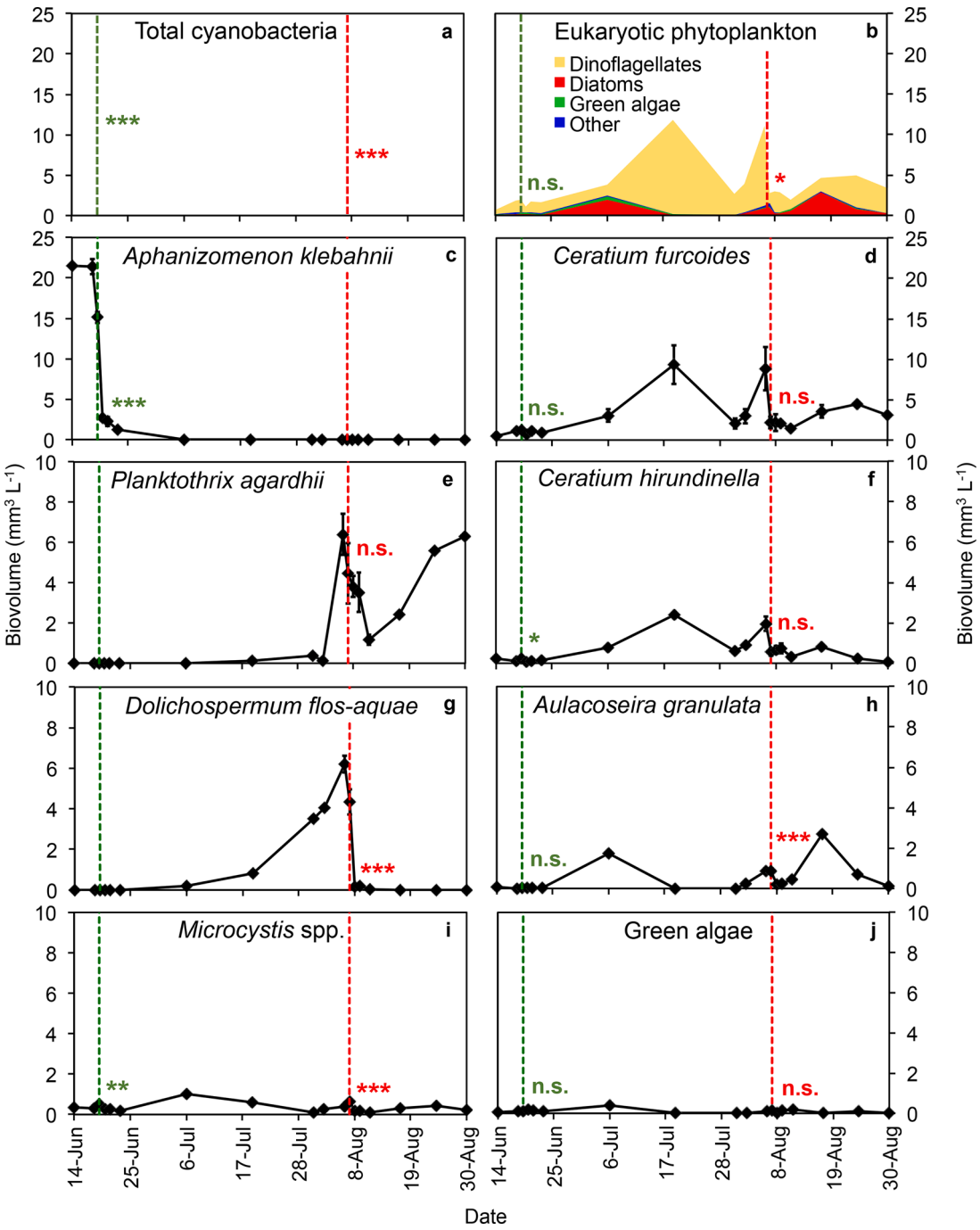

Fig. 4. Phytoplankton community dynamics in the lake. Left panels show the most abundant cyanobacterial taxa: (**a**) total cyanobacteria, (**c**) *Aphanizomenon klebahnii,* (**e**) *Planktothrix agardhii*, (**g**) *Dolichospermum flos-aquae* and (**i**) *Microcystis* spp. Right panels show the most abundant eukaryotic phytoplankton taxa: (**b**) total eukaryotic phytoplankton, (**d**) dinoflagellate *Ceratium furcoides*, (**f**) dinoflagellate *Ceratium hirundinella*, (**h**) diatom *Aulacoseira granulata* and (**j**) total green algae (multiple taxa). Values represent averages ± SE ($n = 3$). Green and red vertical lines show the timing of the first and second lake treatment, respectively. Significant differences between population abundances before and after the lake treatments are indicated as * = $p < 0.05$, ** = $p < 0.01$, and *** = $p < 0.001$; n.s. = not significant.

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

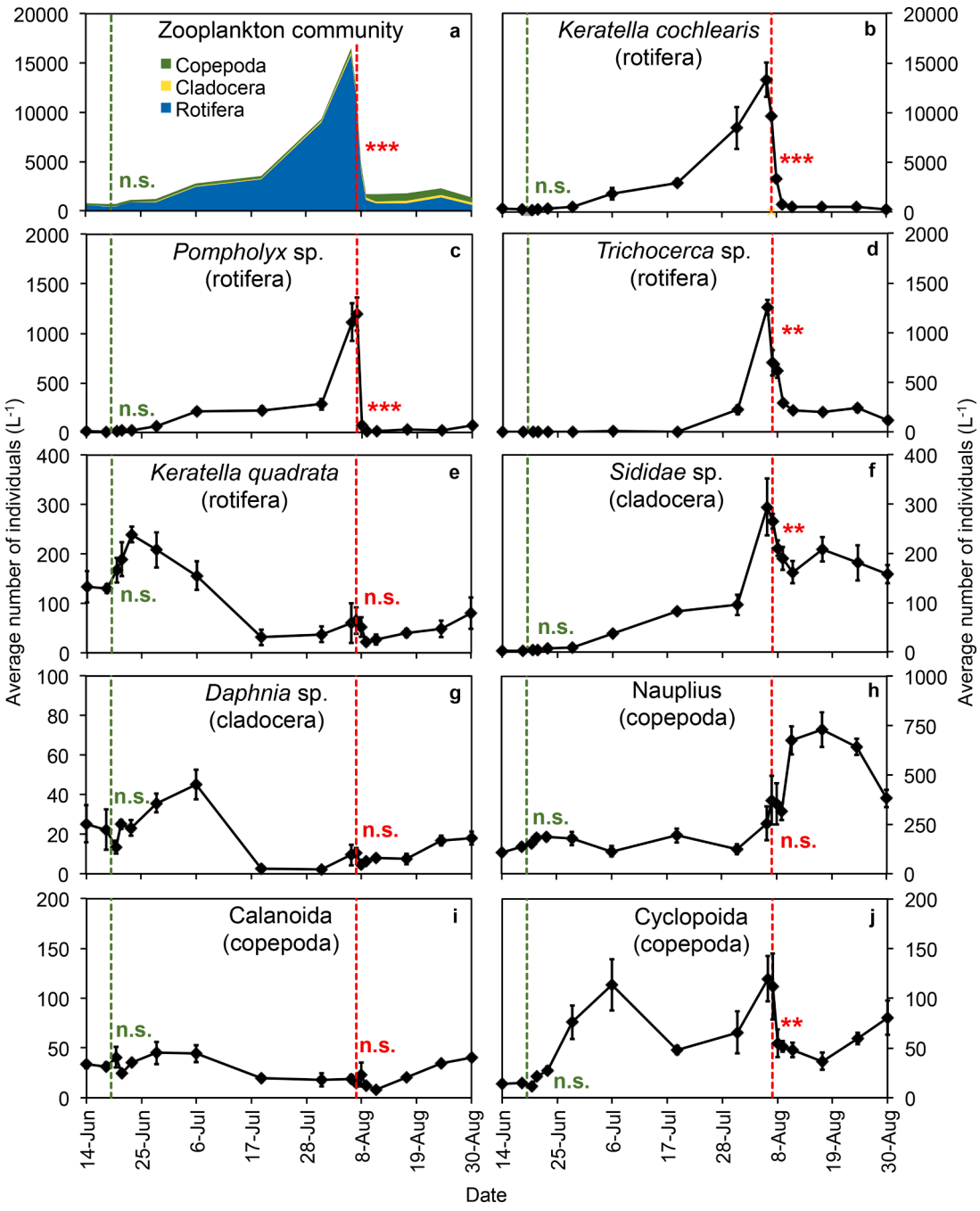

Fig. 5. Zooplankton community dynamics in the lake, for (**a**) total zooplankton, the rotifers (**b**) *Keratella cochlearis,* (**c**) *Pompholyx* sp., (**d**) *Trichocerca* sp., (**e**) *Keratella quadrata*, the cladocerans (**f**) *Sididae* sp., (**g**) *Daphnia* sp., and (**h**) copepod nauplii, (**i**) calanoid copepods, and (**j**) cyclopoid copepods. Values represent averages ± SE ($n = 5$). Green and red vertical lines show the timing of the first and second lake treatment, respectively. Significant differences between population abundances before and after the lake treatments are indicated as ** = $p < 0.01$, and *** = $p < 0.001$; n.s. = not significant.

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 *K. 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

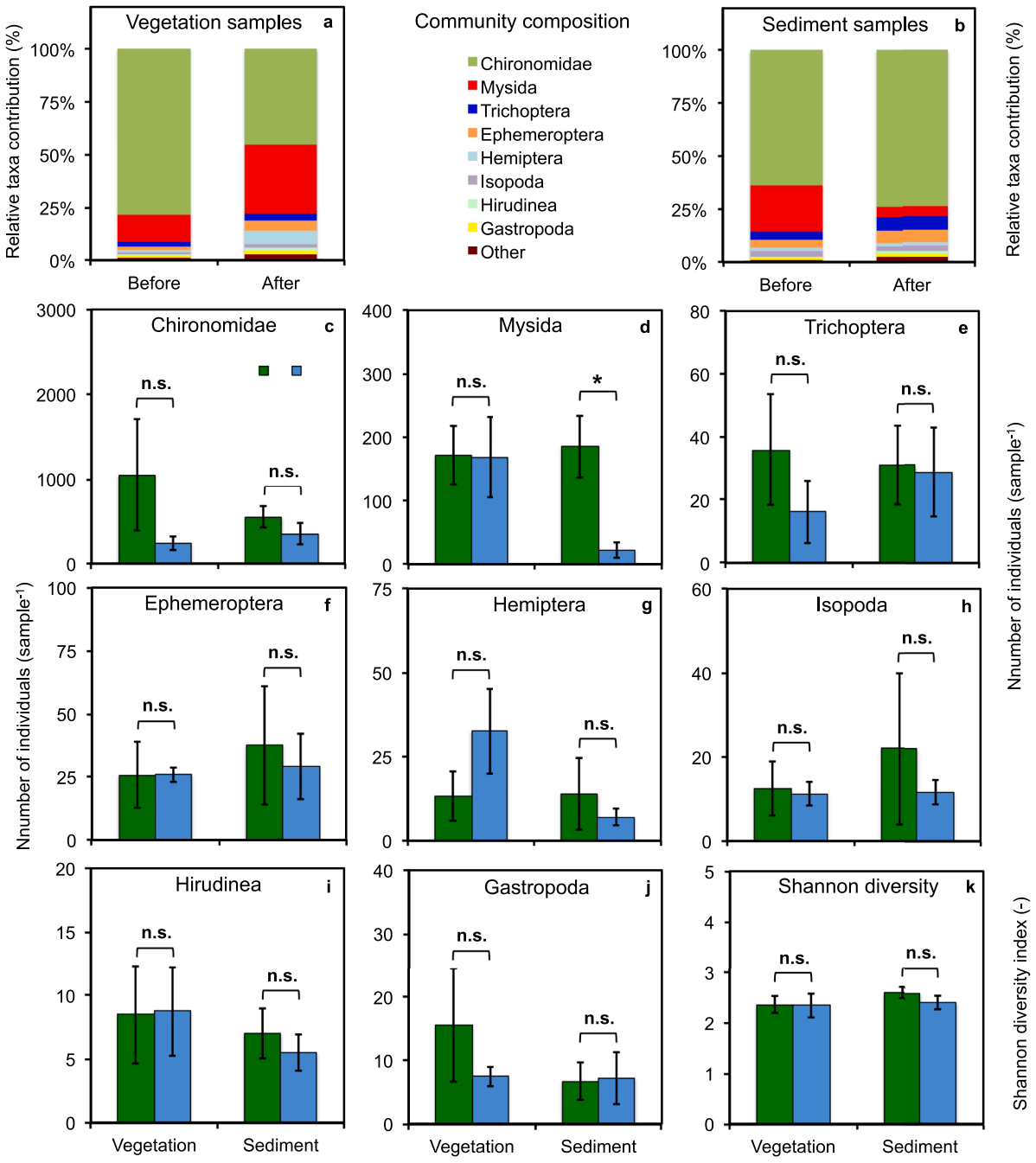

Fig. 6. Macroinvertebrate community before and after the second lake treatment. (**a,b**) Community composition of macroinvertebrates before and after the lake treatment, in (**a**) vegetation and (**b**) sediment. (**c-j**) Abundances of macroinvertebrate taxa in vegetation and sediment, before the treatment (green bars) and after the treatment (blue bars), for (**c**) Chironomidae, (**d**) Mysida, (**e**) Trichoptera, (**f**) Ephemeroptera, (**g**) Hemiptera, (**h**) Isopoda, (**i**) Hirudinea and (**j**) Gastropoda. (**k**) Shannon diversity index of macroinvertebrates. Values represent averages ± SE ($n = 4$). Significant differences between values before and after the lake treatment are indicated as * = $p < 0.05$; n.s. = not significant.

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_2O_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_2O_2 sensitivity of cyanobacteria in the laboratory toxicity experiments, the dominant cyanobacteria *A. klebahnii 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_{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_2O_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_2O_2 treatment in deeper water layers. Furthermore, *P. agardhii* blooms may vary in their H_2O_2 sensitivity due to genetic variation, as observed in laboratory studies with *P. rubescens* (Lürling et al., 2020).

The H_2O_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. hirundinella* 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_2O_2 concentration of 10 mg L^{-1}, eukaryotic phytoplankton was also affected (Santos et al., 2021). Hence, these results confirm that eukaryotic phytoplankton are generally less sensitive to H_2O_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_2O_2, although there were clear species-specific differences. The rotifer *B. calyciflores* had a similar LC_{50} value as the EC_{50} values of the investigated cyanobacteria and the LC_{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_2O_2 concentration of 1.13 mg L^{-1} was below the LC_{50} value for *B. calyciflores* and had no significant effect on rotifer abundances. In the second lake treatment the average H_2O_2 concentration of 2.19 mg L^{-1} was in the same range as the LC_{50} value for *B. calyciflores*. Moreover, the exposure time during the first lake treatment was only ~5.5 h, while H_2O_2 remained in the water for at least 13 h during the second lake treatment. This combination of a higher H_2O_2 concentration and a longer H_2O_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^{-1} H_2O_2, whereas *Brachionus* spp. declined significantly when treated with 4.0 mg L^{-1} H_2O_2.

The relatively small cladocerans *C. dubia* and *D. pulex* showed a similar H_2O_2 sensitivity as the rotifer *B. calyciflores*, while the larger *D. magna* was somewhat less sensitive. Similarly, Reichwaldt et al. (2012) reported a low LC_{50} value of 2 mg L^{-1} H_2O_2 for the small cladoceran *Moina* sp. and a higher LC_{50} of 5.6 mg L^{-1} H_2O_2 for the larger *D. carinata*. This variation in H_2O_2 sensitivity of cladocerans was also observed during the lake treatments, where small *Sididae* 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_2O_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_2O_2 concentrations in the range of 2 to 7 mg L^{-1}, albeit with considerable interspecific variation that might be size dependent.

Copepods and ostracods were the least H_2O_2 sensitive zooplankton taxa in our laboratory tests, possibly because their body tissue is protected from H_2O_2 exposure by an exoskeleton (copepods) or valves (ostracod). The LC_{50} values for these taxa were far above the H_2O_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_2O_2 treatment. The latter explanation would be consistent with the lack of responses of copepods exposed to H_2O_2 concentrations < 7 mg L^{-1} H_2O_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^{-1} H_2O_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_2O_2 than rotifers and cladocerans.

4.4. Aquatic macroinvertebrates

The survival of aquatic macroinvertebrates was less affected by H_2O_2 than the photosynthetic yield of the cyanobacteria investigated in our laboratory experiments. Yet, the H_2O_2 sensitivity of aquatic macroinvertebrates showed large interspecific variation, with LC_{50} values ranging from 5 mg L^{-1} H_2O_2 for the mysid *L. benedeni* to more than 1800 mg L^{-1} H_2O_2 for the mayfly nymph *E. danica*. These results are consistent with the previous study of Smit et al. (2008), who found EC_{50} values of 46 mg L^{-1} for the amphipod *Corophium volutator* and 188 mg L^{-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^{-1} H_2O_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^{-1} H_2O_2.

However, some previous lake treatments have used higher H_2O_2 concentrations of 40 to 50 mg L^{-1} (Burson et al., 2014; Wagstaff et al., 2021). Our laboratory toxicity tests warn that the impacts of such high H_2O_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 isopods, snails and gammarid shrimps remained abundant and active despite this very severe H_2O_2 treatment.

4.5. Caveats and limitations

As a first guideline, Matthijs et al. (2016) argued that the H_2O_2 concentration in lake treatments should remain below 5 mg L^{-1} to avoid effects on non-target species. However, our results show that 5 mg L^{-1} of H_2O_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 H_2O_2 lake treatments (see next section).

This classification may require further modification tailored to each specific lake, since the H_2O_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 abundances (Weenink et al., 2015) and protective mechanisms such as mucus-embedded colony formation (Gao et al., 2015). Moreover, not only the H_2O_2 concentration but also the duration of H_2O_2 exposure is of key importance (Smit et al., 2008). The exposure time can differ substantially 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 H_2O_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 H_2O_2-driven suppression of their rotifer prey. Interspecific protection against H_2O_2 is another important species interaction. High abundances of green algae can rapidly degrade H_2O_2, thereby protecting cyanobacteria against oxidative stress, which hampers successful suppression of cyanobacterial blooms (Weenink et al., 2021). In addition, the presence of H_2O_2-scavenging heterotrophic bacteria may protect some cyanobacterial strains (Smith et al., 2022).

5. Recommendations and conclusions

Based on our findings, we propose a classification of H_2O_2 lake treatments according to their effectivity to suppress cyanobacterial blooms and their expected effects on non-target species:

- *Mild treatments* ($\leq$ 2 mg L^{-1}) are able to suppress some cyanobacterial blooms, while avoiding effects on non-target species.
- *Moderate treatments* (2–4 mg L^{-1}) can suppress many cyanobacterial blooms, but are likely to have negative effects on some zooplankton taxa (particularly rotifers and small cladocerans) while avoiding effects on macroinvertebrates.
- *Severe treatments* (4–10 mg 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 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 H_2O_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 between 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

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2022.119169.

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