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RESEARCH ARTICLE

Dominance and toxicity without lethality: Exploring biomass, cyanometabolites, and *Daphnia* responses across Cyanobacterial strains

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Abstract

Cyanobacteria are cosmopolitan, important components of biomass with a remarkable ability to synthesize a diverse array of bioactive compounds, some of which may have toxic effects on other organisms and ecosystems. In this study, filamentous cyanobacterial strains isolated predominantly from temperate European freshwaters (*Aphanizomenon*, *Chrysosporum*, *Cuspidothrix*, *Dolichospermum*, *Planktothrix*, *Raphidiopsis*), with a subset from Arctic waters (*Microcoleus*, *Phormidesmis*), were qualitatively screened for commonly studied cyanometabolites, using immunoassays and chromatographic techniques. We also assessed the effects of culture extracts and filtrates on the survival of *Daphnia magna* and *Daphnia pulicaria*. The results indicated a broad capacity among tested strains to produce toxic and bioactive compounds. Specifically, we detected anabaenopeptins, anatoxin-a,

Abbreviations: APs, anabaenopeptins; ATX-a, anatoxin-a; cyanoHABs, cyanobacterial harmful algal blooms; CYN, cylindrospermopsin; ELISA, enzyme-linked immunosorbent assay; EPS, extracellular polymeric substances; GLMM, generalized linear mixed model; HPLC-DAD, high performance liquid chromatography coupled with diode array UV detection; LC-MS, liquid chromatography coupled with mass spectrometry; MCs, microcystins; NOD, nodularin; PCR, polymerase chain reaction; STXs, saxitoxins; TRFIA, noncompetitive time resolved fluorescence immunoassay.

cylindrospermopsin, microcystins, and saxitoxins. Although numerous strains showed the potential to produce a variety of toxic and bioactive compounds and reached high biomass after 40 days of incubation, their extracts and filtrates frequently had negligible negative effects on the survival of *Daphnia*. In contrast, significant lethality was observed in response to planktic *Limnothrix* sp. and glacial *Microcoelus autumnalis* that tested negative for the studied compounds, as well as for an *Aphanizomenon klebahnii* strain in which only anabaenopeptins were detected. We also observed a positive relationship between the biomass used to prepare cyanobacterial extracts and *Daphnia* survival, and this result raises the question of whether there is a trade-off between the growth rates of strains and their toxicities. Our study extends the current knowledge of the geographical distribution of toxic cyanobacteria and suggests that some of them may also produce other, yet unidentified toxic compounds.

KEYWORDS
aquatic ecosystems, cyanobacterial blooms, cyanotoxins, eutrophication, extremophiles, HPLC-MS, toxicity assays

INTRODUCTION

Phylogenetically, cyanobacteria are one of the oldest, widespread, and diverse autotrophic organisms worldwide. They were among the first organisms to shape environmental conditions since the Precambrian, initiating biogeochemical transformations in both the hydrosphere and atmosphere (Demoulin et al., 2019). They have been evolving and diversifying for more than 3 billion years on Earth, to become either widely distributed opportunistic species or narrowly ranged stenotypic specialists. To date, these prokaryotes can be found in different types of ecosystems, from marine and freshwater environments to terrestrial habitats, from geothermal waters to the surface of ice. Many species also have sub-cosmopolitan distribution (Padisák, 2003). Due to numerous adaptations such as the production of extracellular polymeric substances, ability to desiccate, ability to survive freeze–thaw events, etc. (Carey et al., 2012; Chen et al., 2021; Dokulil & Teubner, 2000; Kvíderová et al., 2019), cyanobacteria often dominate in different ecosystems (Amarouche-Yala et al., 2014; Rybak et al., 2024; Vincent, 2000). Although these organisms have played pivotal roles as primary producers and early colonizers during primary succession from the deep geological past to the present (Zawierucha, 2024), their excessive proliferation can be harmful to ecosystems.

Accelerated anthropogenic eutrophication and ongoing climate change are altering the biogeochemistry of aquatic ecosystems, making them increasingly susceptible to harmful cyanobacterial blooms (also referred to as cyanoHABs; Huisman et al., 2018; Lürling et al., 2017). Although the blooms occur most frequently and intensively in eutrophic waters in summer (Jöhnk et al., 2008), there have been increasing reports of the mass occurrences of cyanobacteria in the cold season (Babanazarova et al., 2013; Coffer et al., 2020; Lindholm et al., 1989; Wejnerowski et al., 2018, 2024) and in oligotrophic waters (Cederwall & Cott, 2025; Reinl et al., 2021). Cyanobacterial blooms cause deterioration of water quality, formation of anoxic zones, biodiversity loss, and trophic network disruption at the macro- and microscale (Havens, 2008; Paerl & Otten, 2013; Wilk-Woźniak, 2019). This disruption results in a decline in ecosystem service quality and negative impacts on human health, the economy, and tourism (Carmichael & Boyer, 2016; Hamilton et al., 2013). Given the long-term trends in the intensity, duration, and scale of cyanoHABs (Vieira-Lanero et al., 2022; Zong et al., 2019) as well as climate change perspectives (Intergovernmental Panel on Climate Change, 2023), it is predicted that cyanobacterial bloom events will be more intense in the future and may favor toxic genotypes in cyanobacterial community structure (Mantzouki et al., 2018; Walls et al., 2018). According to previous estimates (Chorus, 2001; National Rivers Authority, 1990), toxic cyanoHABs can account for 25% to 75% of blooms.

The cyanotoxins frequently detected in water bodies are microcystins (MCs), nodularins (NODs), anatoxin-a (ATX-a) variants, beta-methylamino-L-alanine, saxitoxins (STXs), and cylindrospermopsins (CYNs)—some sharing similar, while others having distinct, modes of action (Codd et al., 2017) and all exerting diverse toxic effects on biota (Krienitz et al., 2003; Svirčev et al., 2016). Other bioactive secondary metabolites of cyanobacteria, not considered toxins, can also have toxic effects or at least inhibitory activity, for example, anabaenopeptins (APs; Spoof et al., 2016) or lipopolysaccharides (Gągała-Borowska et al., 2022). So far, global monitoring of cyanobacteria in the environment has shown that numerous cyanobacterial species from different genera can produce harmful compounds. For example, cytotoxic alkaloids such as CYN can be produced by certain species of *Anabaena*, *Aphanizomenon*, *Chrysosporum*, *Dolichospermum*, *Lyngbya*, *Oscillatoria*, *Raphidiopsis*, and *Umezakia*, and hepatotoxins such as MCs and NODs are even more widespread (Bernard et al., 2017). Nevertheless, there are still gaps in knowledge, and an important aspect is the identification of the species that can produce certain toxic/bioactive metabolites. Much of the research has been focused on urbanized, polluted areas or located near scientific centers. In contrast, a plethora of habitats around the world, especially the cryospheric ecosystems, remain largely terra incognita with respect to cyanotoxins and other toxic compounds. Notably, cyanobacteria can be toxic in both thermophilic (Krienitz et al., 2003) and psychrophilic environments (Chrapusta et al., 2015; Jungblut et al., 2018; Kleinteich et al., 2018). Although new species of cyanobacteria and their morphotypes are still being described at low and high latitudes (Kaštovský, 2024), their toxin profiles are usually unknown. The toxic potential of some species may also be underestimated due to the small number of tested strains. Some reports demonstrated the potential of cyanobacteria to produce other unknown metabolites, potentially responsible for toxicity observed in various bioassays (Davidović et al., 2023; Falfushynska et al., 2021; Hrouzek et al., 2016; Tokodi et al., 2018).

Polar and high mountain regions are undergoing rapid changes, including glacier retreat, which might lead to increased release of the toxic substances accumulated on glacial surfaces and pose a risk to the health of people relying on glacial meltwater for drinking (Beard et al., 2022). Waterbodies on glacial ice surface (known as cryoconite holes) and in glacier forefields (ponds and lakes) can accumulate various contaminants (Clason et al., 2023). Glaciers also are sources of both organic and inorganic pollutants, including biohazards (Buda et al., 2024; Makowska-Zawierucha et al., 2022). Cyanobacteria play a crucial role in glacier and glacier-adjacent habitats as ecosystem engineers (Rozwalak et al., 2022; Wejnerowski et al., 2023). More thorough investigations of cyanobacterial toxicity will yield insights into the evolutionary connection between the presence of certain toxic cyanometabolites and the diversity of cyanobacteria and the relation among toxin presence and habitat quality, ecological stressors, and biological activity/toxicity of specific cyanometabolites. All of them call for more comprehensive studies on toxic cyanobacteria.

This study aimed to characterize commonly studied cyanotoxins and other bioactive metabolites in filamentous cyanobacteria from a wide range of ecosystems, including strains from natural freshwater and brackish ecosystems and artificially heated lakes, and benthic, mat-forming glacial cyanobacteria from cryoconite holes. The studied strains included temperate isolates of planktic, bloom-forming, and potentially toxic cyanobacteria, representing both native and expansive taxa. We hypothesized that the toxicity of cyanobacteria extends beyond species commonly known to be toxic and that some strains classified as potentially nontoxic based on standard analytical assays exert harmful effects on aquatic organisms. To test this, 72 cyanobacterial strains were analyzed for the presence of various toxic cyanometabolites using immunoassays and chromatographic techniques. In addition to toxin detection, the toxicity of the studied strains was assessed using a short-term survival bioassay with *Daphnia magna* (Straus, 1820) and *D. pulicaria* (Forbes, 1893) as indicator species to link analytical detections with organismal responses. Specifically, we measured toxicity of extracts and filtrates to discriminate between the toxicity of substances released extracellularly by living cells and total toxicity (including intracellularly stored and released metabolites). We also quantified chlorophyll *a* concentration at harvest to test whether toxicity to *Daphnia* scaled with cyanobacterial biomass in extracts and filtrates. Based on a multifaceted toxicological approach, our study provides new, detailed insight into the occurrence of toxic cyanometabolites across a broad diversity of cyanobacteria taxa originating mainly from temperate European habitats, with a subset from Arctic environments.

MATERIALS AND METHODS

Cyanobacteria

We studied strains comprising bloom-forming filamentous cyanobacteria from the temperate zone (*Aphanizomenon*, *Chrysosporum*, *Cuspidothrix*, *Dolichospermum*, *Planktothrix*, *Raphidiopsis*) and a small subset of cyanobacteria from Arctic glaciers (*Microcoleus*, *Phormidesmis*) held in the culture collection at Adam Mickiewicz University in Poznań. The cultures were monocyanobacterial, each originally obtained from a single trichome that was transferred several times in a microcapillary (inlet diameter ~10 μm) through drops of medium containing imipenem antibiotic solution ($10\,\mu g \cdot mL^{-1}$). Prior to the study, all strains were reisolated using microcapillaries. This procedural control was applied to ensure the homogeneity of the biological material. Microscopic observations, trichome isolations, and photographic documentation were conducted using an inverted microscope Leica DM IL LED (Leica Microsystems, Wetzlar, Germany) and a digital camera Jenoptik Gryphax NAOS (Jenoptik

Optical Systems, Jena, Germany). The identification of strains was based on morphological (Hindák, 2008; Komárek, 2013; Komárek & Anagnostidis, 2005) and molecular (16S rRNA gene sequencing and maximum likelihood phylogenic) approaches (Appendix S1). Glacial strains were identified earlier in Wejnerowski et al. (2023).

Cultures of temperate strains were incubated in glass vials using WC medium (Guillard & Lorenzen, 1972) as the growth medium. They were maintained in a culture room under a defined temperature (20 ± 0.5°C), photoperiod (16:8 h light:dark cycle), and light intensity (23 μmol photons $\cdot$ m^{-2} $\cdot$ s^{-1}). Glacial cyanobacteria were grown in a cold room at 4 ± 0.5°C, at a 12:12 h light:dark cycle, and under 24 μmol photons $\cdot$ m^{-2} $\cdot$ s^{-1}.

Detection of cyanometabolites

The biomass of 21-day-old cultures of each strain, concentrated by centrifugation from ~50 mL to volumes of 2 mL each, was analyzed for the presence of 14 toxic cyanometabolites. Detection of APs, ATX-a, and STXs was conducted with enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer protocols (Eurofins Abraxis, Warminster, United States; Product No. 520070, 520060, 520040, 52255B, respectively). Cylindrospermopsin (CYN), MC (variants: dmMC-RR, MC-RR, MC-YR, dmMC-LR, MC-LR, MC-LY, MC-LW, MC-LF), and NOD identification were performed using high-performance liquid chromatography with diode array detection (HPLC-DAD) and mass spectrometry (LC-MS). Identification of CYNs, MCs, and NODs we based on comparing retention times and absorption spectra of particular compounds (HPLC-DAD) or their retention times and the observed *m/z* values (LC-MS) to the standards. Cylindrospermopsin and NOD standards were acquired from the National Research Council (Halifax, Canada); ATX-a and MCs were compared to in-house standards prepared from cyanobacterial strain ANA123 (ATX-a), NIES107 (MCs), and PCC7820 (MCs) originating from the Finnish Environment Institute (Helsinki, Finland), National Institute for Environmental Studies (Tsukuba, Japan), and Pasteur Culture Collection of Cyanobacteria (Paris, France), respectively. Cyanobacterial samples prepared for HPLC were also used to conduct the noncompetitive time resolved fluorescence immunoassay (TRFIA), which detects both microcystin and nodularin (Akter et al., 2016). Sample preparation for the ELISA assay, chromatography, the TRFIA assay, and the corresponding analyses were performed following the procedure of Wejnerowski et al. (2024). Strains that were ATX-a-positive and a few ATX-a-negative isolates (negative control) revealed by ELISA were also checked for ATX-a using the above-mentioned chromatographic methods.

Daphnia survival tests

As model indicators of cyanobacterial toxicity, we used *Daphnia magna* clone BDeM2 and *D. pulicaria* clone DpBrH. Stock cultures were maintained in glass jars filled with 500 mL of filtered and conditioned water from Lake Kakskerranjärvi (southwestern Finland; 60°21′54.25″ N, 22°14′7.971″ E). Cultures were incubated in a phytotron chamber at 20°C and light intensity of ~20 μmol photons $\cdot$ m^{-2} $\cdot$ s^{-1}. They were refreshed weekly with aerated lake filtrate. The newly prepared food suspension (green alga *Tetradesmus obliquus*; strain SAG 276-3a) was supplied to cultures three times a week at a concentration far exceeding the level limiting early growth.

Incubation of cyanobacteria for the *Daphnia* survival test started from inoculation of 5 mL of 20-day-old stock culture of each strain separated into 175-cm^2 cell culture flasks (Nunc™ EasYFlask™, Thermo Scientific, San Jose, California, United States) containing 500 mL of WC medium. The incubation of strains lasted 40 days, and after that, 100-mL samples were collected and filtered on GF/C grade filters (Whatman, Maidstone, United Kingdom). Chlorophyll *a* was extracted, and its concentration was measured according to method ISO10260:1992 (International Organization for Standardization, 1992). The remaining culture volume of each strain was used in *Daphnia* survival tests, which involved testing the effect of filtered extracts and filtrates from cyanobacterial cultures on *Daphnia* survival. Preparation of filtered extracts involved triple freeze-thawing and sonication for 15 min (Sonorex RK156, Banelin electronics, Berlin, Germany) of cyanobacterial culture (100 mL). Subsequently, extracts were filtered through GF/C filters using a glass vacuum filtration kit. Filtrates were obtained by filtration of cyanobacterial volume (100 mL) through GF/C filters without the sonication and freezing processes. The prepared filtered extracts and filtrates were maintained at −20°C until analysis.

Survival tests were conducted on *Daphnia* juveniles (not older than ~48 h) originating from stocks containing up to ~150 reproducing females. The experimental setup was the same for each studied *Daphnia* clone and was as follows: five replicates of a given clone (each replicate composed of 10 randomly selected individuals) were exposed for 48 h to either 7 mL of filtered extract or 7 mL of filtrate of each cyanobacterial strain in glass vials. Filtered extracts and filtrates from up to seven strains of cyanobacteria were tested at one time. In each round, we used controls that constituted five replicates of each *Daphnia* clone exposed to 7 mL of freshly prepared WC medium that cyanobacteria were cultured in. The WC medium used in controls was always fresh (i.e., ~24 h since autoclaving). Before each test, filtrated extracts and filtrates of cyanobacteria and the control medium were aerated by air bubbling for 15 min. Incubation of experimental samples was carried out in the same phytotron chamber as what *Daphnia* stocks were cultured in, and the samples were not fed during the test. After exposure, the numbers of living and dead daphnids were counted in all vials using a stereoscopic microscope. In total, 15,400 *Daphnia* newborns were used (2 *Daphnia* clones ×2 types of tested liquids ×5 replicates ×10 individuals within a replicate ×72 cyanobacterial strains plus 10 controls ×5 replicates ×10 individuals within a replicate ×2 *Daphnia* clones). Based on the recorded data, the percentage survival of each *Daphnia* was calculated.

Statistical analyses

The chlorophyll *a* data were used as a proxy for cyanobacteria biomass at the end of incubation. The generalized linear mixed models (GLMMs) were used to compare *Daphnia* survival in response to cyanobacterial extracts and filtrates. Separate models for *D. magna* and *D. pulicaria* were built, each time including *Daphnia* survival as the response variable. Both models included treatment (extract vs. filtrate) in interaction with cyanobacterial taxon and treatment in interaction with the biomass (chlorophyll *a* was used to prepare extract/filtrate) as explanatory terms. The strain of cyanobacterium was included as the random intercept in each model. Such model parameterization allowed the comparison of *Daphnia* survival between extracts and filtrates depending on cyanobacterial taxon and the testing of the relationship between biomass (chlorophyll *a* used to prepare extract/filtrate) and *Daphnia* survival depending on treatment (extract vs. filtrate). The models were fit with the beta error distribution and logit link function. For post hoc comparisons of *Daphnia* survival between extracts and filtrates, estimated marginal means with Tukey's multiplicity adjustment implemented via the emmeans ver. 1.10.6 R package (Lenth, 2023) were used. Because control groups exhibited minimal variability and were therefore excluded from the GLMMs, we conducted additional complementary analysis explicitly comparing each treatment with its matched control, separately for each *Daphnia* clone and for each strain × treatment combination. *Daphnia* survival was tested against the clone-specific control using one-sided exact binomial tests. The *p*-values were adjusted for multiple testing using the Benjamini–Hochberg false discovery rate (FDR) procedure. All analyses were conducted using the R software version 4.2.2 (R Core Team, 2022) and RStudio version 2023.12.1 (R Studio Team, 2024).

RESULTS

Cyanometabolites detected in the strains

Of 72 investigated strains, 35 were determined to produce at least one of the toxic/bioactive metabolites investigated. Anabaenopeptins were detected by ELISA in *Aphanizomenon gracile* (12 strains), *A. klebahnii* (two strains), and *Planktothrix agardhii* (14 strains). Anatoxin-a was only observed in *Cuspidothrix issatschenkoi* (two strains) and was confirmed by ELISA, HPLC-DAD, and LC-MS. Chromatographic methods showed that CYN was only present in *A. gracile* (five strains). Microcystins were found only in *P. agardhii* (nine strains), and this result was confirmed by TRFIA, HPLC-DAD, and LC-MS analyses. The latter two techniques allowed the identification of MC homologs: dmMC-RR was present in all MC-positive *P. agardhii* strains dmMC-LR was present only in two, whereas MC-RR was confirmed only for one strain. Other variants of MC were not detected in the studied set of *P. agardhii* strains. The TRFIA and chromatography showed that none of the 72 strains was NOD positive. Saxitoxin was detected by ELISA in *A. gracile* (one strain). Detailed data from the toxicological screening are in Appendix S2.

Cyanobacterial growth

The concentration of chlorophyll *a* measured in 40-day-old cultures ranged from 10.1 to 979.6 $\mu g \cdot L^{-1}$ (Figure 1a). The biomass of more than 500 $\mu g \cdot L^{-1}$ of chlorophyll *a* was detected in 15 strains comprising five species (*Aphanizomenon gracile*, *A. klebahnii*, *Cuspidothrix issatschenkoi*, *Planktothrix agardhii*, and *Raphidiopsis raciborskii*). Among the strains with biomass of more than 500 $\mu g \cdot L^{-1}$ were those producing different studied cyanometabolites, including anabaenopeptins, anatoxin-a, CYN, and MCs. No studied metabolites were detected in any of the polar strains (*Microcoleus*, *Phormidesmis*). *Microcoleus autumnalis* reached a higher biomass (chlorophyll *a* of 187.2 $\mu g \cdot L^{-1}$) than *Phormidesmis priestleyi* strains, for which chlorophyll *a* ranged from 17.0 to 42.9 $\mu g \cdot L^{-1}$.

Daphnia survival in response to cyanobacterial extracts and filtrates

Average survival of *Daphnia* in control trials was 100%, except in trial 4 of *D. pulicaria*, trial 8 of *D. magna*, and

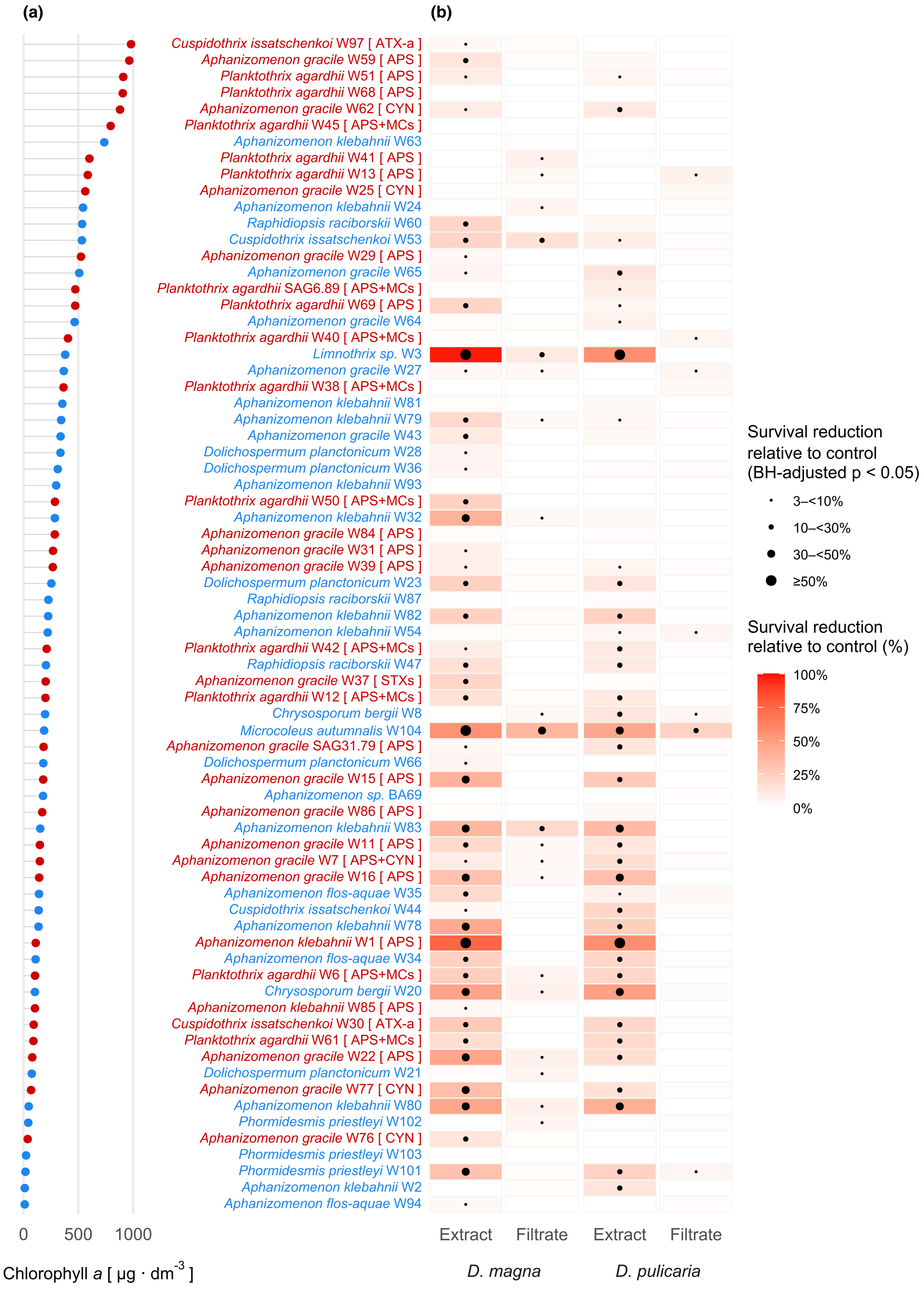

FIGURE 1 Chlorophyll *a* concentration in 40-day-old cyanobacterial cultures (a) and (b) mean reduction of *Daphnia* survival after 48 h exposure to extracts or filtrates of individual cyanobacterial strains, shown relative to the within-clone control. Strains with detected toxic/bioactive cyanometabolites are marked in red, while strains in which no toxic/bioactive cyanometabolites were detected are marked in blue. Heat map tiles depict survival reduction relative to the within-clone control. Black dots mark treatments with a statistically significant effect of extract or filtrate versus the within-clone control (exact one-sample binomial test, BH-FDR, adjusted $p < 0.05$), while dot size encodes effect magnitude (3%–10%, 10%–30%, 30%–50%, ≥50%) absolute survival reduction relative to the within-clone control. APs, anabaenopeptins; ATX-a, anatoxin-a; CYN, cylindrospermopsin; MCs, microcystins; STXs, saxitoxins.

TABLE 1 Results of the generalized linear mixed model to study the effects of treatment (extract vs. filtrate) in different cyanobacterial taxa (*Aphanizomenon flos-aquae*, *A. gracile*, *A. klebahnii*, *Aphanizomenon* sp., *Chrysosporum bergii*, *Cuspidothrix issatschenkoi*, *Dolichospermum planctonicum*, *Limnothrix* sp., *Microcoleus autumnalis*, *Phormidium priestleyi*, *Planktothrix agardhii*, *Raphidiopsis raciborskii*) and cyanobacterial biomass (chlorophyll *a*, μg · L^{-1}; biomass, from which the extract and filtrate were prepared) on the survival of *Daphnia magna* and *D. pulicaria*.

Model term	df_{num}	df_{denom}	F	p
Daphnia magna				
Treatment	1	692	61.82	<0.001
Cyanobacterial taxon	11	692	5.24	<0.001
Cyanobacterial biomass	1	692	11.30	<0.001
Treatment × Cyanobacterial taxon	11	692	5.16	<0.001
Treatment × Cyanobacterial biomass	1	692	24.49	<0.001
D. pulicaria				
Treatment	1	692	67.20	<0.001
Cyanobacterial taxon	11	692	10.17	<0.001
Cyanobacterial biomass	1	692	18.64	<0.001
Treatment × Cyanobacterial taxon	11	692	6.77	<0.001
Treatment × Cyanobacterial biomass	1	692	19.62	<0.001

Note: The model was fitted with beta error distribution and logit link function and included the strain of cyanobacterium as a random effect. Numerator and denominator degrees of freedom (df_{num}, df_{denom}), *F*-statistics, and *p*-values are shown.

trial 10 of *D. pulicaria*, in which it reached 98%, 98%, and 96%, respectively. Binomial tests comparing treatments to clone-specific controls showed that only a minority of extracts and even fewer filtrates caused a marked, biologically meaningful (≥30%) reduction in *Daphnia* survival (Figure 1b). Reductions >50% relative to control were observed for extracts of *Limnothrix* sp. W3 and *Aphanizomena klebahnii* W1 in both *Daphnia* clones and for extracts of *Microcoleus autumnalis* W104 in *D. magna*. In *D. magna*, 30%–50% survival reductions were detected for extracts of 10 strains comprising *A. gracile*, *A. klebahnii*, *Chrysosporum bergii*, and *Phormidesmis priestleyi*, whereas only one filtrate (*M. autumnalis*) reached this range. In *D. pulicaria*, 30%–50% survival reductions were observed for extracts from five strains comprising *A. gracile*, *A. klebahnii*, *Ch. bergii*, and *M. autumnalis*, whereas no filtrates reached this threshold. Reductions of 10%–30% were detected for 19 extracts and three filtrates in *D. magna* and for 22 extracts and one filtrate in *D. pulicaria*. Reductions of *Daphnia* survival of 3%–10% were significant (BH-adjusted $p < 0.05$) for 19 extracts and 16 filtrates in *D. magna* and for eight extracts and six filtrates in *D. pulicaria*.

The GLMM revealed a significant effect of the treatment in interaction with cyanobacterial taxon on the survival of both *Daphnia* species (Table 1; model term: Treatment × Cyanobacterial taxon). The post hoc analyses showed that the exposure of both *D. magna* and *D. pulicaria* to the filtered extracts of *Aphanizomenon gracile*, *A. klebahnii*, *Cuspidothrix issatschenkoi*, *Limnothrix* sp., and *Planktothrix agardhii* resulted in lower survival of the *Daphnia* species compared to the filtrates obtained from these taxa (Table 2, Figure 2). A greater reduction in *Daphnia* survival due to extracts was also observed for *Raphidiophsis raciborskii* in relation to *D. magna* and for *Chrysosporum bergii* and *Microcoleus autumnalis* in relation to *D. pulicaria*. Survival of both *Daphnia* species was significantly affected by the interaction between the treatment and the cyanobacterial biomass, from which the extract/filtrate was prepared (Table 1; model term: Treatment × Cyanobacterial biomass). A positive relationship between *Daphnia* survival and chlorophyll *a* content in cyanobacterial extracts was observed for both *D. magna* ($\beta = 0.0020$, 95% confidence interval, CI = 0.0012–0.0028) and *D. pulicaria* ($\beta = 0.0017$, 95% CI = 0.00112–0.0022; Table 1, Figure 3). In contrast, there was no significant relationship between survival and chlorophyll *a* concentration in the cyanobacterial filtrates for either *D. magna* ($\beta = 0.0001$, 95% CI = −0.0005–0.0008) or *D. pulicaria* ($\beta = 1.09 \times 10^{-6}$, 95% CI = −0.0004–0.0004; Table 1, Figure 3).

DISCUSSION

Nearly half of the studied strains of cyanobacteria were observed to produce toxic/bioactive metabolites. Our results mostly corroborate existing literature reports demonstrating that (1) *Aphanizomenon gracile* is capable of producing APs, CYN, and STXs, (2) *Cuspidothrix issatschenkoi* has the potential to produce ATX-a, and (3) *Planktothrix agardhii* has the ability to produce APs and MCs (Table 3). However, we have observed that *A. klebahnii* can produce APs, which to our knowledge has not been detected in this species. So far, this ability has only been documented in other species of this genus (Table 3). Producers of APs can also be found in *Aphanizomenon* occurring in waterbodies experiencing brackish water intrusions (Overlingė et al., 2024). Consistent with this pattern, we detected three AP-positive strains of *A. gracile* (W15, W16, W22) isolated from the Baltic coastal lakes Wicko, Kopań, and

TABLE 2 Results of post hoc tests comparing the survival of *D. magna* and *D. pulicaria* between the treatments (extract vs. filtrate) for different cyanobacterial taxa.

	D. magna				***D. pulicaria***			
Taxon	**Extract**	**Filtrate**	***t***	***p***	**Extract**	**Filtrate**	***t***	***p***
Aphanizomenon flos-aquae	0.94 (0.87–0.97)	0.97 (0.93–0.99)	−1.66	0.09	0.96 (0.93–0.98)	0.95 (0.92–0.97)	0.46	0.644
Aphanizomenon gracile	0.90 (0.87–0.92)	0.96 (0.95–0.97)	−7.07	**<0.001**	0.93 (0.91–0.94)	0.96 (0.95–0.97)	−3.87	**<0.001**
Aphanizomenon klebahnii	0.89 (0.85–0.92)	0.96 (0.94–0.97)	−5.12	**<0.001**	0.91 (0.89–0.93)	0.96 (0.95–0.97)	−4.29	**<0.001**
Aphanizomenon sp.	0.98 (0.91–0.99)	0.97 (0.89–0.99)	0.38	0.706	0.97 (0.92–0.99)	0.95 (0.89–0.98)	0.64	0.519
Chrysosporum bergii	0.91 (0.80–0.96)	0.95 (0.87–0.98)	−1.15	0.250	0.68 (0.52–0.80)	0.95 (0.90–0.97)	−4.57	**<0.001**
Cuspidothrix issatschenkoi	0.88 (0.78–0.93)	0.96 (0.92–0.98)	−3.39	**<0.001**	0.83 (0.75–0.89)	0.96 (0.93–0.97)	−4.36	**<0.001**
Dolichospermum planctonicum	0.94 (0.90–0.97)	0.97 (0.94–0.98)	−1.78	0.075	0.96 (0.93–0.97)	0.96 (0.94–0.97)	−0.30	0.765
Limnothrix sp.	0.03 (0.009–0.12)	0.88 (0.67–0.97)	−8.04	**<0.001**	0.17 (0.08–0.35)	0.96 (0.91–0.99)	−7.10	**<0.001**
Microcoleus autumnalis	0.51 (0.23–0.79)	0.60 (0.29–0.84)	−0.54	0.589	0.28 (0.13–0.50)	0.83 (0.66–0.93)	−3.80	**<0.001**
Phormidesmis priestleyi	0.97 (0.92–0.99)	0.96 (0.92–0.98)	0.29	0.775	0.96 (0.93–0.98)	0.95 (0.92–0.97)	0.40	0.685
Planktothrix agardhii	0.90 (0.86–0.93)	0.96 (0.95–0.98)	−5.43	**<0.001**	0.93 (0.90–0.94)	0.96 (0.94–0.97)	−3.14	**0.001**
Raphidiopsis raciborskii	0.91 (0.82–0.96)	0.97 (0.94–0.99)	−2.76	**0.005**	0.93 (0.89–0.96)	0.96 (0.94–0.98)	−1.60	0.110

Note: Shown is the mean survival rate together with the corresponding 95% confidence interval (in parentheses), *t*-statistics, and *p*-values. Significant differences are highlighted in bold.

Sarbsko (Northern Poland), all characterized by brackish conditions (Woszczyk & Schubert, 2021).

Although most of the tested species were already identified as producers of the detected toxins/metabolites (Table 3), many of them had not yet been observed in the investigated region (Appendix S1). Importantly, this is evidence for the presence of ATX-a-producing strains of *Cuspidothrix issatschenkoi* and an STX-producing strain of *Aphanizomenon gracile*. We also detected CYN-producing *A. gracile* strains in four new lakes, confirming the potential of this species to produce this toxin. Collectively, these data support the occurrence of toxin-producing cyanobacteria in lowland Central European water bodies.

Based on the chlorophyll *a* concentrations determined in this study, the biomass achieved by most of the strains in the simulated laboratory blooms exceeded the Alert Level 2 threshold ($12\,\mu g \cdot L^{-1}$) as defined in the World Health Organization guideline (2024) as well as the alert levels specified in the Joint Research Centre Technical Report (Sanseverino et al., 2023) for the monitoring and management of cyanobacteria in recreational waters. Considering the concentration of chlorophyll *a* in 40-day-old cultures and the results of cyanometabolite detection, it is hard to question earlier estimations (Chorus, 2001; National Rivers Authority, 1990) that toxic cyanobacteria can constitute a considerable fraction of blooms. Among the strains with the highest biomasses reached within the incubation period were the strains in which APs (*Aphanizomenon gracile*, *Planktothrix agardhii*), ATX-a (*Cuspidothrix issatschenkoi*), CYN (*A. gracile*), and MCs (*P. agardhii*) were detected. A higher growth potential of toxic genotypes compared to potentially non-toxic genotypes has been observed but for coccoid bloom-forming cyanobacterium *Microcystis* (Davis et al., 2009). The fact that toxin-producing cyanobacteria grow more rapidly than their competitors that do not produce toxins is alarming.

Despite the relatively high number of strains producing both substantial biomass and toxic metabolites, the average survival of *Daphnia* in most cyanobacterial extracts and filtrates exceeded 70%. Only three cyanobacterial candidates had marked, biologically meaningful negative effects: the planktic *Limnothrix* sp. W3 and *Aphanizomenon klebahnii* W1, which had extracts that noticeably reduced the survival of both *Daphnia* species, and the glacial *Microcoleus autumnalis* W104, which had extracts that reduced the survival of *D. pulicaria*. In the case of *D. magna*, survival was markedly reduced in response to both the extract and filtrate of *M. autumnalis*, and the measured trait exhibited a distinctly wide 95% confidence interval. Unexpectedly, both *Limnothrix* sp. and *M. autumnalis* were free of the studied metabolites, whereas *A. klebahnii* produced just APs—compounds also present in several other non-lethal strains to *Daphnia*.

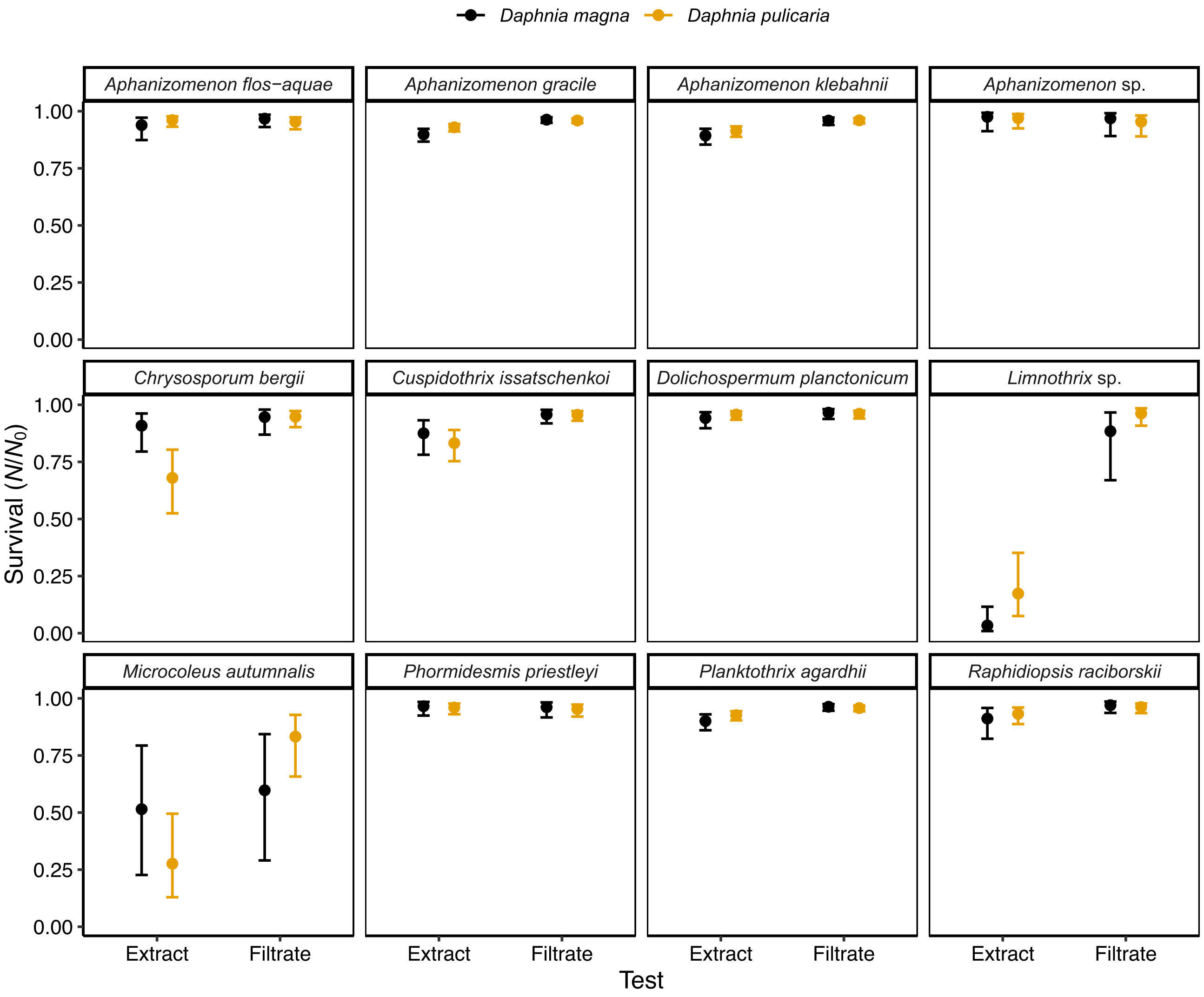

FIGURE 2 Mean (±95% confidence interval) survival of *Daphnia magna* and *D. pulicaria* in extract and filtrate as a function of cyanobacterial taxon.

The results for these three strains may suggest their ability to synthetize other, unknown compounds that are harmful to *Daphnia*. Findings for *Limnothrix* sp. and *M. autumnalis* support the hypothesis that cyanobacterial toxicity extends beyond traditionally recognized toxic species. This hypothesis suggests that strains diagnosed as non-toxic by standard assays may still exert harmful effects on aquatic organisms, especially in cryospheric ecosystems, wherein such impacts have been poorly studied to date. Thereby, classifying strains as "toxic" or "potentially non-toxic" based solely on the presence or absence of target metabolites can be an oversimplification.

The common feature of *Limnothrix* sp. and *Microcoleus autumnalis* and the formation of excessive amounts of extracellular polymeric substances (EPSs; Figure 4) to form irregular gelatinous aggregates at the bottom (*Limnothrix* sp.) or carpet-like mats covering the bottom and walls of culture containers (*M. autumnalis*; Appendix S1). The presence of EPSs can be highly beneficial for cyanobacteria growth (Deng et al., 2025). Although the EPSs themselves are rather non-toxic (More et al., 2014), they can interact with toxic cyanometabolites (Gan et al., 2012) and absorb them from water (Mohamed et al., 2023). It is possible that sonication of the biomass during extract preparation led to the release of unknown cyanobacterial toxic compounds adsorbed in the EPSs, thus revealing the toxicity of these strains to *Daphnia*. The case of *Limnothrix* sp. provides plausible support for this assumption, as only the extract of this strain induced severe mortality in both *D. magna* and *D. pulicaria*.

Our results did not indicate the presence of known toxins in the glacial cyanobacteria. However, the observed strong reductions in *Daphnia* survival caused by *Microcoleus autumnalis* extracts and filtrates and by the extract of a single strain of *Phormidesmis* highlight the need for further assessment of other glacial cyanobacteria in the context of water safety, especially since cyanotoxins have previously been detected in polar

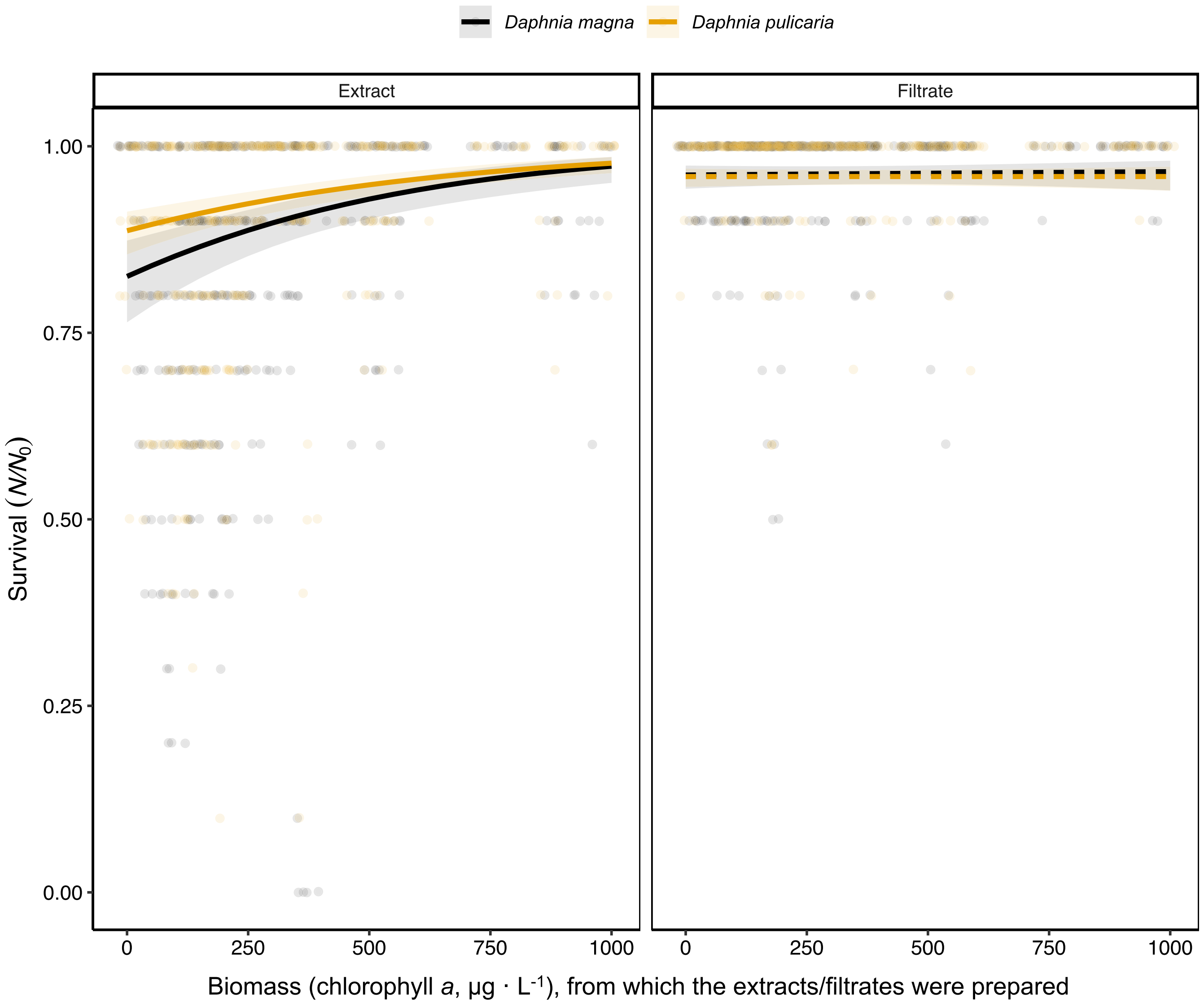

FIGURE 3 The relationship between the chlorophyll *a* concentration (from which the extracts/filtrates were prepared) and the survival of *Daphnia magna* and *D. pulicaria* as a function of treatment (extract vs. filtrate). Significant relationships are shown as solid lines, while the shaded areas indicate 95% confidence intervals. Each point represents the mean survival (N/N_0) of *Daphnia* exposed to extracts or filtrates from a single experimental vessel.

samples (Chrapusta et al., 2015; Jungblut et al., 2018; Kleinteich et al., 2018).

A higher biomass of cyanobacteria increases the potential for higher concentrations of toxins (Visser et al., 2016) and can cause more pronounced toxic effects. However, we observed a counterintuitive positive correlation between the biomass at harvest and *Daphnia* survival in extract bioassays. Although most of the high-biomass strains were capable of producing known toxic or bioactive compounds, their extracts did not markedly reduce *Daphnia* survival. Considered alongside substantial among-strain (and within-species) variation in biomass under the same initial nutrient-rich conditions, our findings may reflect inherent, strain-specific life-history strategies shaped by native environments. More specifically, our findings suggest a possible trade-off between growth rate and investment in toxic metabolites—compounds that can deter consumers but are costly to produce, with costs manifesting as reduced growth (Lei et al., 2024). Conceptually, the observed pattern aligns with growth-defense frameworks in plant ecology (e.g., Coley's Resource Availability Hypothesis; Coley et al., 1985), which posit that taxa from resource-limited environments have slower growth with greater investment in defenses, whereas those from resource-rich environments exhibit faster growth with lower defensive investment. We note that most studied strains originated

TABLE 3 Toxicological screening of cyanobacterial taxa, summarizing the number of strains in which specific cyanometabolites were detected in this study using ELISA (APs, ATX-a, STXs), TRFIA (MCs/NOD), and HPLC-DAD/LC-MS (ATX-a, CYN, MCs, NOD).

Number of studied strains within a taxon	Number of strains with detected cyanometabolites					
	APs	ATX-a	CYN	MCs	NOD	STXs
Aphanizomenon flos-aquae (3 strains)	a	d	hi			
Aphanizomenon gracile (21 strains)	12 b		5 j			1 p
A. klebahnii (14 strains)	2					
Aphanizomenon sp. (1 strain)						
Chrysosporum bergii (2 strains)			kl			
Cuspidothrix issatschenkoi (4 strains)		2 e				r
Dolichospermum planctonicum (5 strains)		f				
Limnothrix sp. (1 strains)						
Microcoelus autumnalis (1 strain)						
Phormidesmus priestleyi (3 strains)						
Planktothrix agardhii (14 strains)	14 c	g	m	9 o		
Raphidiopsis raciborskii (3 strains)	b		n			st

Note: Cells containing numbers indicate detections in this study. Lowercase letters in gray-filled boxes denote taxa for which metabolite production has been reported previously in the literature. [a]Murakami et al. (2000), [b]Falfushynska et al. (2021), [c]Monteiro et al. (2021), [d]Rapala et al. (1993), [e]Ballot, Fastner, Lentz, and Wiedner (2010), [f]Park et al. (1993), [g]Sivonen et al. (1989), [h]Preußel et al. (2006), [i]Fastner et al. (2007), [j]Kokociński et al. (2013), [k]Schembri et al. (2001), [l]Adamski et al. (2020), [m]Mohamed and Bakr (2018), [n]Hawkins et al. (1985), [o]Akcaalan et al. (2006), [p]Ballot, Fastner, and Wiedner (2010), [r]Nogueira et al. (2004), [s]Lagos et al. (1999), [t]Bernard et al. (2017).

from eutrophic temperate waters and were incubated under the same nutrient-rich medium. Moreover, culture monitoring revealed no organoleptic signs of aging (e.g., yellowing or whitening of the culture biomass or subjective changes in odor) nor any physicochemical indicators such as pH shifts and nutrient limitation (Appendix S3). Additionally, cultures with the highest biomass often contained a relatively low level of phaeophytin, a degradation product of chlorophyll *a*. Therefore, we suspect that the allocation patterns predicted by Coley's framework may also operate in cyanobacteria even without experimentally imposed resource limitation, suggesting that strain-level tradeoffs between growth and toxicity reflect inherent evolutionary allocation strategies. Since increased toxin production also requires nutrient investment (Van de Waal et al., 2009), reduced allocation to toxin synthesis could serve as a resource-conserving strategy under non-stressful conditions. Further studies are needed to quantify toxin dynamics in the studied strains and record cyanobacterial growth curves to test the potential trade-off between growth rate and toxicity and to determine whether faster growing strains producing ATX-a, CYN, MCs, and STXs may also reach the stationary-phase earlier—a stage during which toxin production typically declines (Harland et al., 2013, 2015; Sivonen, 1996).

CONCLUSIONS

Toxicological screening revealed the widespread potential of bloom-forming cyanobacteria to produce various cyanometabolites, including APs (*Aphanizomenon gracile*, *A. klebahnii*, and *Planktothrix agardhii*), ATX-a (*Cuspidothrix issatschenkoi*), CYN (*A. gracile*), MCs (*P. agardhii*), and STXs (*A. gracile*). Several of these results expand the current understanding of the geographic distribution of toxic cyanobacteria.

In line with Roy-Lachapelle et al. (2021), future work will require verification of the ELISA-positive AP strains using LC-MS, along with comparative analyses of AP structure and activity across strains. The limitations of ELISA were particularly evident in the detection of ATX-a, as numerous strains yielded false-positive results near the lower detection limit. Only two strains with ELISA-detected concentrations above the upper detection threshold were confirmed to contain ATX-a via chromatographic analysis.

Toxin-producing strains tended to achieve higher biomass compared to potentially non-toxic strains, suggesting a possible ecological advantage. However, their toxicological impact on *Daphnia* survival was limited. *Daphnia* generally exhibited high resistance to cyanobacterial extracts and filtrates, even when prepared from dense biomass of confirmed toxin-producing

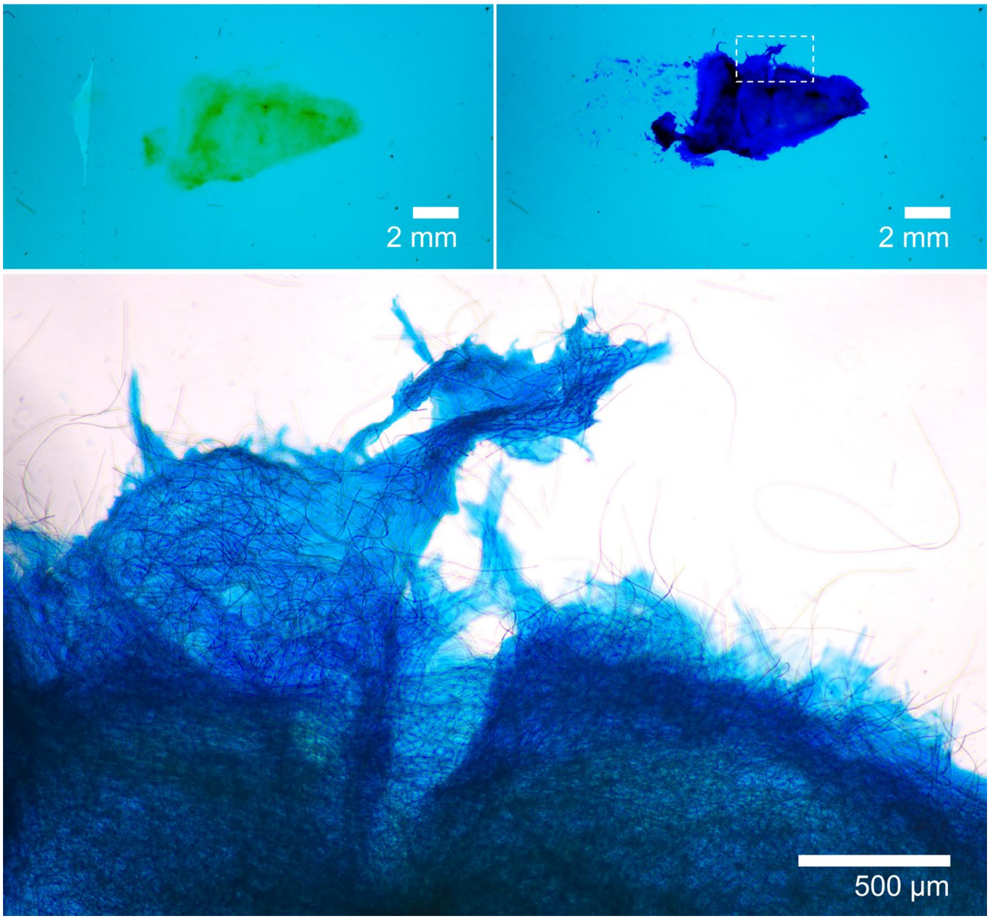

FIGURE 4 Mat fragments of *Limnothrix* sp. strain W3 before (upper left micrograph) and after staining with Alcian blue (upper right micrograph), showing EPS visible as blue layers around the trichomes. The bottom micrograph shows trichomes within the mat at higher magnification. The white dashed frame indicates the region magnified in the bottom panel. Evidence for EPS presence in *Microcoleus autumnalis* strain W104 with Alcian blue dye was demonstrated in Wejnerowski et al. (2023).

strains. Some strains that tested negative for studied cyanotoxins or bioactive compounds still caused significant reductions in *Daphnia* survival, indicating the possible presence of other unidentified toxic metabolites. Interestingly, we observed slow-growing cyanobacterial strains to be more toxic to *Daphnia* than the fast-growing ones, suggesting that cyanobacteria can utilize alternative strategies based on a trade-off between growth rate and toxicity—an allocation pattern conceptually analogous to that predicted by Coley's Resource Availability Hypothesis. These findings underscore the need for further research into such trade-offs and their implications for the ecological success and toxicity of bloom-forming cyanobacteria.

AUTHOR CONTRIBUTIONS

Łukasz Wejnerowski: Conceptualization (equal); data curation (equal); formal analysis (equal); funding acquisition (equal); investigation (equal); methodology (equal); project administration (equal); resources (equal); software (equal); supervision (equal); validation (equal); visualization (equal); writing – original draft (equal); writing – review and editing (equal). **Tamara Dulić:** Investigation (equal); writing – review and editing (equal). **Sultana Akter:** Data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); resources (equal); software (equal); validation (equal); writing – review and editing (equal). **Michał Rybak:** Data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); resources (equal); software (equal); validation (equal); writing – review and editing (equal). **Agnieszka Piasecka:** Investigation (equal); methodology (equal); resources (equal); validation (equal); writing – review and editing (equal). **Jakub Szymkowiak:** Data curation (equal); formal analysis (equal); methodology (equal); resources (equal); software (equal); visualization (equal); writing – review and editing (equal). **Oskar Kamiński:** Investigation (equal); writing – review and editing (equal). **Anna Czerepska:** Investigation (equal); writing – review and editing (equal). **Filip Pniewski:** Resources (equal); writing – review and editing (equal). **Zorica Svirčev:** Writing – review and editing (equal). **Ewa Poniecka:** Resources (equal); writing – review and editing (equal). **Krzysztof Zawierucha:** Resources (equal); writing – review and editing (equal). **Katarzyna Taylor:** Methodology (equal); resources (equal); writing

– review and editing (equal). **Marcin Krzysztof Dziuba:** Data curation (equal); formal analysis (equal); investigation (equal); methodology (equal); resources (equal); software (equal); writing – review and editing (equal). **Jussi Meriluoto:** Formal analysis (equal); investigation (equal); methodology (equal); resources (equal); software (equal); validation (equal); writing – review and editing (equal).

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DATA AVAILABILITY STATEMENT

Data are available in the manuscript and Supporting Information. More information could be provided upon request.

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REFERENCES

Adamski, M., Wołowski, K., Kamiński, A., & Hindáková, A. (2020). Cyanotoxin cylindrospermopsin producers and the catalytic decomposition process: A review. *Harmful Algae*, *98*, 101894. https://doi.org/10.1016/j.hal.2020.101894

Akcaalan, R., Young, F. M., Metcalf, J. S., Morrison, L. F., Albay, M., & Codd, G. A. (2006). Microcystin analysis in single filaments of *Planktothrix* spp. in laboratory cultures and environmental blooms. *Water Research*, *40*, 1583–1590. https://doi.org/10.1016/j.watres.2006.02.020

Akter, S., Vehniäinen, M., Spoof, L., Nybom, S., Meriluoto, J., & Lamminmäki, U. (2016). Broad-spectrum noncompetitive immunocomplex immunoassay for cyanobacterial peptide hepatotoxins (microcystins and nodularins). *Analytical Chemistry*, *88*, 10080–10087. https://doi.org/10.1021/acs.analchem.6b02470

Amarouche-Yala, S., Benouadah, A., Bentabet, A. E. O., & López-García, P. (2014). Morphological and phylogenetic diversity of thermophilic cyanobacteria in Algerian hot springs. *Extremophiles*, *18*, 1035–1047. https://doi.org/10.1007/s00792-014-0680-7

Babanazarova, O., Sidelev, S., & Schischeleva, S. (2013). The structure of winter phytoplankton in Lake Nero, Russia, a hypertrophic lake dominated by *Planktothrix*-like cyanobacteria. *Aquatic Biosystems*, *9*, 18. https://doi.org/10.1186/2046-9063-9-18

Ballot, A., Fastner, J., Lentz, M., & Wiedner, C. (2010). First report of anatoxin-a-producing cyanobacterium *Aphanizomenon issatschenkoi* in northeastern Germany. *Toxicon*, *56*, 964–971. https://doi.org/10.1016/j.toxicon.2010.06.021

Ballot, A., Fastner, J., & Wiedner, C. (2010). Paralytic shellfish poisoning toxin-producing cyanobacterium *Aphanizomenon gracile* in northeast Germany. *Applied and Environmental Microbiology*, *76*, 1173–1180. https://doi.org/10.1128/aem.02285-09

Beard, D. B., Clason, C. C., Rangecroft, S., Poniecka, E., Ward, K. J., & Blake, W. H. (2022). Anthropogenic contaminants in glacial environments II: Release and downstream consequences. *Progress in Physical Geography: Earth and Environment*, *46*, 790–808. https://doi.org/10.1177/03091333221127342

Bernard, C., Ballot, A., Thomazeau, S., Maloufi, S., Furey, A., Mankiewicz-Boczek, J., Pawlik-Skowrońska, B., Capelli, C., & Salmaso, N. (2017). Appendix 2: Cyanobacteria associated with the production of cyanotoxins. In J. Meriluoto, L. Spoof, & G. A. Codd (Eds.), *Handbook of cyanobacterial monitoring and cyanotoxin analysis* (pp. 501–525). John Wiley & Sons. https://doi.org/10.1002/9781119068761.app2

Buda, J., Łokas, E., Błażej, S., Gorzkiewicz, K., Buda, K., Ambrosini, R., Franzetti, A., Pittino, F., Crosta, A., Klimaszyk, P., & Zawierucha, K. (2024). Unveiling threats to glacier biota: Bioaccumulation, mobility, and interactions of radioisotopes with key biological components. *Chemosphere*, *348*, 140738. https://doi.org/10.1016/j.chemosphere.2023.140738

Carey, C. C., Ibelings, B. W., Hoffmann, E. P., Hamilton, D. P., & Brookes, J. D. (2012). Eco-physiological adaptations that favour freshwater cyanobacteria in a changing climate. *Water Research*, *46*, 1394–1407. https://doi.org/10.1016/j.watres.2011.12.016

Carmichael, W. W., & Boyer, G. L. (2016). Health impacts from cyanobacteria harmful algae blooms: Implications for the North American Great Lakes. *Harmful Algae*, *54*, 194–212. https://doi.org/10.1016/j.hal.2016.02.002

Cederwall, J., & Cott, P. A. (2025). Rapidly increasing cyanobacteria blooms in the subarctic Great Slave Lake: Observations from Indigenous, local, and scientific knowledge. *Scientific Reports*, *15*, 24492. https://doi.org/10.1038/s41598-025-07432-5

Chen, M. Y., Teng, W. K., Zhao, L., Hu, C. X., Zhou, Y. K., Han, B. P., Song, L. R., & Shu, W. S. (2021). Comparative genomics reveals insights into cyanobacterial evolution and habitat adaptation. *The ISME Journal*, *15*, 211–227. https://doi.org/10.1038/s41396-020-00775-z

Chorus, I. (2001). Introduction: Cyanotoxins–research for environmental safety and human health. In I. Chorus (Ed.), *Cyanotoxins* (pp. 1–4). Springer. https://doi.org/10.1007/978-3-642-59514-1_1

Chrapusta, E., Węgrzyn, M., Zabaglo, K., Kaminski, A., Adamski, M., Wietrzyk, P., & Bialczyk, J. (2015). Microcystins and anatoxin-a in Arctic biocrust cyanobacterial communities. *Toxicon*, *101*, 35–40. https://doi.org/10.1016/j.toxicon.2015.04.016

Clason, C. C., Baccolo, G., Łokas, E., Owens, P. N., Wachniew, P., Millward, G. E., Taylor, A., Blake, W. H., Beard, D. B., Poniecka, E., Selmes, N., Bagshaw, E. A., Cook, J., Fyfe, R., Hay, M., Land, D., Takeuchi, N., Nastasi, M., Sisti, M., … Di Mauro, B. (2023). Global variability and controls on the accumulation of fallout radionuclides in cryoconite. *Science of the Total Environment*, *894*, 164902. https://doi.org/10.1016/j.scitotenv.2023.164902

Codd, G. A., Meriluoto, J., & Metcalf, J. S. (2017). Introduction: Cyanobacteria, cyanotoxins, their human impact, and risk management. In J. Meriluoto, L. Spoof, & G. A. Codd (Eds.), *Handbook of cyanobacterial monitoring and cyanotoxin analysis* (pp. 3–8). John Wiley & Sons. https://doi.org/10.1002/9781119068761.ch1

Coffer, M. M., Schaeffer, B. A., Darling, J. A., Urquhart, E. A., & Salls, W. B. (2020). Quantifying national and regional cyanobacterial occurrence in US lakes using satellite remote sensing. *Ecological Indicators*, *111*, 105976. https://doi.org/10.1016/j.ecolind.2019.105976

Coley, P. D., Bryant, J. P., & Chapin, F. S. (1985). Resource availability and plant antiherbivore defense. *Science*, *230*, 895–899. https://doi.org/10.1126/science.230.4728.895

Davidović, P., Blagojević, D., Meriluoto, J., Simeunović, J., & Svirčev, Z. (2023). Biotests in cyanobacterial toxicity assessment–efficient enough or not? *Biology*, *12*, 711. https://doi.org/10.3390/biology12050711

Davis, T. W., Berry, D. L., Boyer, G. L., & Gobler, C. J. (2009). The effects of temperature and nutrients on the growth and dynamics of toxic and non-toxic strains of *Microcystis* during cyanobacteria blooms. *Harmful Algae*, *8*, 715–725. https://doi.org/10.1016/j.hal.2009.02.004

Demoulin, C. F., Lara, Y. J., Cornet, L., François, C., Baurain, D., Wilmotte, A., & Javaux, E. J. (2019). Cyanobacteria evolution: Insight from the fossil record. *Free Radical Biology and Medicine*, *140*, 206–223. https://doi.org/10.1016/j.freeradbiomed.2019.05.007

Deng, K., Yang, R., Zhang, S., Fang, F., Huo, Y., Yan, P., Chen, Y., & Guo, J. (2025). Extracellular polymeric substances enhanced photosynthesis over respiration in *Microcystis aeruginosa*. *Harmful Algae*, *145*, 102843. https://doi.org/10.1016/j.hal.2025.102843

Dokulil, M. T., & Teubner, K. (2000). Cyanobacterial dominance in lakes. *Hydrobiologia*, *438*, 1–12. https://doi.org/10.1023/A:1004155810302

Falfushynska, H., Horyn, O., Osypenko, I., Rzymski, P., Wejnerowski, Ł., Dziuba, M. K., & Sokolova, I. M. (2021). Multibiomarker-based assessment of toxicity of central European strains of filamentous cyanobacteria *Aphanizomenon gracile* and *Raphidiopsis raciborskii* to zebrafish *Danio rerio*. *Water Research*, *194*, 116923. https://doi.org/10.1016/j.watres.2021.116923

Fastner, J., Rücker, J., Stüken, A., Preußel, K., Nixdorf, B., Chorus, I., Köhler, A., & Wiedner, C. (2007). Occurrence of the cyanobacterial toxin cylindrospermopsin in northeast Germany. *Environmental Toxicology*, *22*, 26–32. https://doi.org/10.1002/tox.20230

Gągała-Borowska, I., Karwaciak, I., Jaros, D., Ratajewski, M., Kokociński, M., Jurczak, T., Remlein, B., Rudnicka, K., Pułaski, Ł., & Mankiewicz-Boczek, J. (2022). Cyanobacterial cell-wall components as emerging environmental toxicants – Detection and holistic monitoring by cellular signaling biosensors. *Science of the Total Environment*, *807*, 150645. https://doi.org/10.1016/j.scitotenv.2021.150645

Gan, N., Xiao, Y., Zhu, L., Wu, Z., Liu, J., Hu, C., & Song, L. (2012). The role of microcystins in maintaining colonies of bloom-forming *Microcystis* spp. *Environmental Microbiology*, *14*, 730–742. https://doi.org/10.1111/j.1462-2920.2011.02624.x

Guillard, R. R. L., & Lorenzen, C. J. (1972). Yellow-green algae with chlorophyllide *c*. *Journal of Phycology*, *8*, 10–14. https://doi.org/10.1111/j.1529-8817.1972.tb03995.x

Hamilton, D. P., Wood, S. A., Dietrich, D. R., & Puddick, J. (2013). Costs of harmful blooms of freshwater cyanobacteria. In N. K. Sharma, A. K. Rai, & L. J. Stal (Eds.), *Cyanobacteria: An economic perspective* (pp. 247–256). John Wiley & Sons. https://doi.org/10.1002/9781118402238.ch15

Harland, F., Wood, S. A., Broady, P., Williamson, W., & Gaw, S. (2015). Changes in saxitoxin-production through growth phases in the metaphytic cyanobacterium *Scytonema* cf. *crispum*. *Toxicon*, *103*, 74–79. https://doi.org/10.1016/j.toxicon.2015.06.014

Harland, F. M. J., Wood, S. A., Moltchanova, E., Williamson, W. M., & Gaw, S. (2013). *Phormidium autumnale* growth and anatoxin-a production under iron and copper stress. *Toxins*, *5*, 2504–2521. https://doi.org/10.3390/toxins5122504

Havens, K. E. (2008). Cyanobacteria blooms: Effects on aquatic ecosystems. In H. K. Hudnell (Ed.), *Cyanobacterial harmful algal blooms: State of the science and research needs* (pp. 733–747). Springer. https://doi.org/10.1007/978-0-387-75865-7_33

Hawkins, P. R., Runnegar, M. T., Jackson, A. R., & Falconer, I. R. (1985). Severe hepatotoxicity caused by the tropical cyanobacterium (blue-green alga) *Cylindrospermopsis raciborskii* (Woloszynska) Seenaya and Subba Raju isolated from a domestic water supply reservoir. *Applied and Environmental Microbiology*, *50*, 1292–1295. https://doi.org/10.1128/aem.50.5.1292-1295.1985

Hindák, F. (2008). *Colour atlas of cyanophytes*. VEDA, Publishing House of the Slovak Academy of Sciences.

Hrouzek, P., Kapuścik, A., Vacek, J., Voráčová, K., Paichlová, J., Kosina, P., Voloshko, L., Ventura, S., & Kopecký, J. (2016). Cytotoxicity evaluation of large cyanobacterial strain set using selected human and murine *in vitro* cell models. *Ecotoxicology and Environmental Safety*, *124*, 177–185. https://doi.org/10.1016/j.ecoenv.2015.10.020

Huisman, J., Codd, G. A., Paerl, H. W., Ibelings, B. W., Verspagen, J. M. H., & Visser, P. M. (2018). Cyanobacterial blooms. *Nature Reviews Microbiology*, *16*, 471–483. https://doi.org/10.1038/s41579-018-0040-1

Intergovernmental Panel on Climate Change. (2023). Summary for policymakers. In Core Writing Team, H. Lee, & J. Romero (Eds.), *Climate change 2023: Synthesis report. Contribution of working groups I, II and III to the sixth assessment report of the intergovernmental panel on climate change* (pp. 1–34). IPCC. https://doi.org/10.59327/IPCC/AR6-9789291691647.001

International Organization for Standardization. (1992). *Water quality – measurement of biochemical parameters – spectrometric determination of the chlorophyll-*a *concentration* (ISO Standard No. 10260:1992). https://www.iso.org/standard/18300.html

Jöhnk, K. D., Huisman, J., Sharples, J., Sommeijer, B., Visser, P. M., & Stroom, J. M. (2008). Summer heatwaves promote blooms of harmful cyanobacteria. *Global Change Biology*, *14*, 495–512. https://doi.org/10.1111/j.1365-2486.2007.01510.x

Jungblut, A. D., Wilbraham, J., Banack, S. A., Metcalf, J. S., & Codd, G. A. (2018). Microcystins, BMAA and BMAA isomers in 100-year-old Antarctic cyanobacterial mats collected during captain R.F. Scott's Discovery Expedition. *European Journal of Phycology*, *53*, 115–121. https://doi.org/10.1080/09670262.2018.1442587

Kaštovský, J. (2024). Welcome to the jungle!: An overview of modern taxonomy of cyanobacteria. *Hydrobiologia*, *851*, 1063–1077. https://doi.org/10.1007/s10750-023-05356-7

Kleinteich, J., Puddick, J., Wood, S. A., Hildebrand, F., Laughinghouse, H. D., IV, Pearce, D. A., Dietrich, D. R., & Wilmotte, A. (2018). Toxic cyanobacteria in Svalbard: Chemical diversity of microcystins detected using a liquid chromatography mass spectrometry precursor ion screening method. *Toxins*, *10*, 147. https://doi.org/10.3390/toxins10040147

Kokociński, M., Mankiewicz-Boczek, J., Jurczak, T., Spoof, L., Meriluoto, J., Rejmonczyk, E., Hautala, H., Vehniäinen, M., Pawełczyk, J., & Soininen, J. (2013). *Aphanizomenon gracile* (Nostocales), a cylindrospermopsin producing cyanobacterium in polish lakes. *Environmental Science and Pollution Research*, *20*, 5243–5264. https://doi.org/10.1007/s11356-012-1426-7

Komárek, J. (2013). *Cyanoprokaryota: 3. Teil/3rd part: Heterocytous genera*. Springer Spektrum.

Komárek, J., & Anagnostidis, K. (2005). *Cyanoprocaryota. 2. Teil: Oscillatoriales*. Spektrum Akademischer Verlag.

Krienitz, L., Ballot, A., Kotut, K., Wiegand, C., Pütz, S., Metcalf, J. S., Codd, G. A., & Pflugmacher, S. (2003). Contribution of hot spring cyanobacteria to the mysterious deaths of lesser flamingos at Lake Bogoria, Kenya. *FEMS Microbiology Ecology*, *43*, 141–148. https://doi.org/10.1111/j.1574-6941.2003.tb01053.x

Kvíderová, J., Elster, J., & Komárek, J. (2019). Ecophysiology of cyanobacteria in the polar regions. In A. K. Mishra, D. N. Tiwari, & A. N. Rai (Eds.), *Cyanobacteria: From basic science to applications* (pp. 277–302). Academic Press. https://doi.org/10.1016/B978-0-12-814667-5.00014-3

Lagos, N., Onodera, H., Zagatto, P. A., Andrinolo, D., Azevedo, S. M. F. Q., & Oshima, Y. (1999). The first evidence of paralytic shellfish toxins in the freshwater cyanobacterium *Cylindrospermopsis raciborskii*, isolated from Brazil. *Toxicon*, *37*, 1359–1373. https://doi.org/10.1016/s0041-0101(99)00080-x

Lei, L., Liu, W., Chen, Z., Peng, L., Xiao, L. J., Han, B. P., & Neilan, B. A. (2024). Grazer-induced toxin production is energetically costly and significantly reduces growth of cylindrospermopsin-producing cyanobacteria. *Limnology and Oceanography*, *69*, 2929–2940. https://doi.org/10.1002/lno.12721

Lenth, R. V. (2023). *emmeans*: Estimated marginal means, aka least-squares means. R package version 1.8.5. https://cran.r-project.org/package=emmeans

Lindholm, T., Eriksson, J. E., & Meriluoto, J. A. O. (1989). Toxic cyanobacteria and water quality problems – Examples from a eutrophic lake on Åland, South West Finland. *Water Research*, *23*, 481–486. https://doi.org/10.1016/0043-1354(89)90139-5

Lürling, M., Van Oosterhout, F., & Faassen, E. (2017). Eutrophication and warming boost cyanobacterial biomass and microcystins. *Toxins*, *9*, 64. https://doi.org/10.3390/toxins9020064

Makowska-Zawierucha, N., Mokracka, J., Małecka, M., Balazy, P., Chełchowski, M., Ignatiuk, D., & Zawierucha, K. (2022). Quantification of class 1 integrons and characterization of the associated gene cassettes in the high Arctic – Interplay of humans and glaciers in shaping the aquatic resistome. *Ecological Indicators*, *145*, 109633. https://doi.org/10.1016/j.ecolind.2022.109633

Mantzouki, E., Lürling, M., Fastner, J., De Senerpont Domis, L., Wilk-Woźniak, E., Koreivienė, J., Seelen, L., Teurlincx, S., Verstijnen, Y., Krztoń, W., Walusiak, E., Karosienė, J., Kasperovičienė, J., Savadova, K., Vitonytė, I., Cillero-Castro, C., Budzyńska, A., Goldyn, R., Kozak, A., … Ibelings, B. W. (2018). Temperature effects explain continental scale distribution of cyanobacterial toxins. *Toxins*, *10*, 156. https://doi.org/10.3390/toxins10040156

Mohamed, Z. A., & Bakr, A. (2018). Concentrations of cylindrospermopsin toxin in water and tilapia fish of tropical fishponds in Egypt, and assessing their potential risk to human health. *Environmental Science and Pollution Research*, *25*, 36287–36297. https://doi.org/10.1007/s11356-018-3581-y

Mohamed, Z. A., Elnour, R. O., Alamri, S., & Hashem, M. (2023). Increased production of extracellular polysaccharides in *Arthrospira platensis* as a protective response against saxitoxin: Implications to outdoor mass production. *Algal Research*, *74*, 103184. https://doi.org/10.1016/j.algal.2023.103184

Monteiro, P. R., do Amaral, S. C., Siqueira, A. S., Xavier, L. P., & Santos, A. V. (2021). Anabaenopeptins: What we know so far. *Toxins*, *13*, 522. https://doi.org/10.3390/toxins13080522

More, T. T., Yadav, J. S. S., Yan, S., Tyagi, R. D., & Surampalli, R. Y. (2014). Extracellular polymeric substances of bacteria and their potential environmental applications. *Journal of Environmental Management*, *144*, 1–25. https://doi.org/10.1016/j.jenvman.2014.05.010

Murakami, M., Suzuki, S., Itou, Y., Kodani, S., & Ishida, K. (2000). New anabaenopeptins, potent carboxypeptidase-A inhibitors from the cyanobacterium *Aphanizomenon flos-aquae*. *Journal of Natural Products*, *63*, 1280–1282. https://doi.org/10.1021/np000120k

National Rivers Authority. (1990). *Toxic blue-green algae* (Water Quality Series No 2). https://archive.org/details/toxicbluegreenal0000grea

Nogueira, I. C. G., Pereira, P., Dias, E., Pflugmacher, S., Wiegand, C., Franca, S., & Vasconcelos, V. M. (2004). Accumulation of paralytic shellfish toxins (PST) from the cyanobacterium *Aphanizomenon issatschenkoi* by the cladoceran *Daphnia magna*. *Toxicon*, *44*, 773–780. https://doi.org/10.1016/j.toxicon.2004.08.006

Overlingė, D., Toruńska-Sitarz, A., Cegłowska, M., Szubert, K., & Mazur-Marzec, H. (2024). Phylogenetic and molecular characteristics of two *Aphanizomenon* strains from the Curonian Lagoon, southeastern Baltic Sea and their biological activities. *Scientific Reports*, *14*, 24686. https://doi.org/10.1038/s41598-024-76064-y

Padisák, J. (2003). Phytoplankton. In P. E. O'Sullivan & C. S. Reynolds (Eds.), *The lakes handbook: Limnology and limnetic ecology* (pp. 251–308). Blackwell Science. https://doi.org/10.1002/9780470999271.ch10

Paerl, H. W., & Otten, T. G. (2013). Harmful cyanobacterial blooms: Causes, consequences, and controls. *Microbial Ecology*, *65*, 995–1010. https://doi.org/10.1007/s00248-012-0159-y

Park, H. D., Watanabe, M. F., Harada, K. I., Nagai, H., Suzuki, M., Watanabe, M., & Hayashi, H. (1993). Hepatotoxin (microcystin) and neurotoxin (anatoxin-a) contained in natural blooms and strains of cyanobacteria from Japanese freshwaters. *Natural Toxins*, *1*, 353–360. https://doi.org/10.1002/nt.2620010606

Preußel, K., Stüken, A., Wiedner, C., Chorus, I., & Fastner, J. (2006). First report on cylindrospermopsin producing *Aphanizomenon flos-aquae* (cyanobacteria) isolated from two German lakes. *Toxicon*, *47*, 156–162. https://doi.org/10.1016/j.toxicon.2005.10.013

R Core Team. (2022). R: A language and environment for statistical computing. Version 4.2.2. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

R Studio Team. (2024). RStudio: Integrated Development Environment for R (Version 2023.12.1). Posit Software. https://posit.co/

Rapala, J., Sivonen, K., Luukkainen, R., & Niemelä, S. I. (1993). Anatoxin-a concentration in *anabaena* and *Aphanizomenon* under different environmental conditions and comparison of growth by toxic and non-toxic *anabaena*-strains – A laboratory study. *Journal of Applied Phycology*, *5*, 581–591. https://doi.org/10.1007/BF02184637

Reinl, K. L., Brookes, J. D., Carey, C. C., Harris, T. D., Ibelings, B. W., Morales-Williams, A. M., De Senerpont-Domis, L. N., Atkins, K. S., Isles, P. D. F., Mesman, J. P., North, R. L., Rudstam, L. G., Stelzer, J. A. A., Venkiteswaran, J. J., Yokota, K., & Zhan, Q. (2021). Cyanobacterial blooms in oligotrophic lakes: Shifting the high-nutrient paradigm. *Freshwater Biology*, *66*, 1846–1859. https://doi.org/10.1111/fwb.13791

Roy-Lachapelle, A., Solliec, M., Sauvé, S., & Gagnon, C. (2021). Evaluation of ELISA-based method for total anabaenopeptins determination and comparative analysis with on-line SPE-UHPLC-HRMS in freshwater cyanobacterial blooms. *Talanta*, *223*, 121802. https://doi.org/10.1016/j.talanta.2020.121802

Rozwalak, P., Podkowa, P., Buda, J., Niedzielski, P., Kawecki, S., Ambrosini, R., Azzoni, R. S., Baccolo, G., Ceballos, J. L., Cook, J., Di Mauro, B., Ficetola, G. F., Franzetti, A., Ignatiuk, D., Klimaszyk, P., Łokas, E., Ono, M., Parnikoza, I., Pietryka, M., … Zawierucha, K. (2022). Cryoconite – From minerals and organic matter to bioengineered sediments on glacier's surfaces. *Science of the Total Environment*, *807*, 150874. https://doi.org/10.1016/j.scitotenv.2021.150874

Rybak, A. S., Dziuba, M., Pełechata, A., Rybak, M., Akter, S., Czerepska, A., Dulić, T., Gąbka, M., Hindáková, A., Jurczak, T., Kendir, A., Mankiewicz-Boczek, J., Meriluoto, J., & Wejnerowski, Ł. (2024). Characterization of cyanobacterial mats from an artificial hot spring in Uniejów (Poland) and the potential use of their biomass. *Algal Research*, *82*, 103646. https://doi.org/10.1016/j.algal.2024.103646

Sanseverino, I., Niegowska, M., Loos, R., & Lettieri, T. (2023). *Good practice for cyanobacterial and algal bloom detection across Europe*. Publications Office of the European Union. https://data.europa.eu/doi/10.2760/380493

Schembri, M. A., Neilan, B. A., & Saint, C. P. (2001). Identification of genes implicated in toxin production in the cyanobacterium *Cylindrospermosis raciborskii*. *Environmental Toxicology*, *16*, 413–421. https://doi.org/10.1002/tox.1051

Sivonen, K. (1996). Cyanobacterial toxins and toxin production. *Phycologia*, *35*, 12–24. https://doi.org/10.2216/i0031-8884-35-6S-12.1

Sivonen, K., Himberg, K., Luukkainen, R., Niemelä, S. I., Poon, G. K., & Codd, G. A. (1989). Preliminary characterization of neurotoxic cyanobacteria blooms and strains from Finland. *Environmental Toxicology*, *4*, 339–352. https://doi.org/10.1002/tox.2540040310

Spoof, L., Błaszczyk, A., Meriluoto, J., Cegłowska, M., & Mazur-Marzec, H. (2016). Structures and activity of new anabaenopeptins produced by Baltic Sea cyanobacteria. *Marine Drugs*, *14*, 8. https://doi.org/10.3390/md14010008

Svirčev, Z., Obradović, V., Codd, G. A., Marjanović, P., Spoof, L., Drobac, D., Tokodi, N., Petković, A., Nenin, T., Simeunović, J., Važić, T., & Meriluoto, J. (2016). Massive fish mortality and *Cylindrospermopsis raciborskii* bloom in Aleksandrovac Lake. *Ecotoxicology*, *25*, 1353–1363. https://doi.org/10.1007/s10646-016-1687-x

Tokodi, N., Drobac, D., Lazić, G., Petrović, T., Marinović, Z., Lujić, J., Palanački Malešević, T., Meriluoto, J., & Svirčev, Z. (2018). Screening of cyanobacterial cultures originating from different environments for cyanotoxicity and cyanotoxins. *Toxicon*, *154*, 1–6. https://doi.org/10.1016/j.toxicon.2018.09.001

Van de Waal, D. B., Verspagen, J. M. H., Lürling, M., Van Donk, E., Visser, P. M., & Huisman, J. (2009). The ecological stoichiometry of toxins produced by harmful cyanobacteria: An experimental test of the carbon-nutrient balance hypothesis. *Ecology Letters*, *12*, 1326–1335. https://doi.org/10.1111/j.1461-0248.2009.01383.x

Vieira-Lanero, R., Barca, S., Cobo, M. C., & Cobo, F. (2022). Occurrence of freshwater cyanobacteria and bloom records in Spanish reservoirs (1981–2017). *Hydrobiology*, *1*, 122–136. https://doi.org/10.3390/hydrobiology1010009

Vincent, W. V. (2000). Cyanobacterial dominance in the polar regions. In B. A. Whitton & M. Potts (Eds.), *The ecology of cyanobacteria* (pp. 321–340). Springer. https://doi.org/10.1007/0-306-46855-7_12

Visser, P. M., Verspagen, J. M. H., Sandrini, G., Stal, L. J., Matthijs, H. C. P., Davis, T. W., Paerl, H. W., & Huisman, J. (2016). How rising CO_2 and global warming may stimulate harmful cyanobacterial blooms. *Harmful Algae*, *54*, 145–159. https://doi.org/10.1016/j.hal.2015.12.006

Walls, J. T., Wyatt, K. H., Doll, J. C., Rubenstein, E. M., & Rober, A. R. (2018). Hot and toxic: Temperature regulates microcystin release from cyanobacteria. *Science of the Total Environment*, *610–611*, 786–795. https://doi.org/10.1016/j.scitotenv.2017.08.149

Wejnerowski, Ł., Dulić, T., Akter, S., Font-Nájera, A., Rybak, M., Kamiński, O., Czerepska, A., Dziuba, M. K., Jurczak, T., Meriluoto, J., Mankiewicz-Boczek, J., & Kokociński, M. (2024). Community structure and toxicity potential of cyanobacteria during summer and winter in a temperate-zone lake susceptible to phytoplankton blooms. *Toxins*, *16*, 357. https://doi.org/10.3390/toxins16080357

Wejnerowski, Ł., Poniecka, E., Buda, J., Klimaszyk, P., Piasecka, A., Dziuba, M. K., Mugnai, G., Takeuchi, N., & Zawierucha, K. (2023). Empirical testing of cryoconite granulation: Role of cyanobacteria in the formation of key biogenic structure darkening glaciers in polar regions. *Journal of Phycology*, *59*, 939–949. https://doi.org/10.1111/jpy.13372

Wejnerowski, Ł., Rzymski, P., Kokociński, M., & Meriluoto, J. (2018). The structure and toxicity of winter cyanobacterial bloom in a eutrophic lake of the temperate zone. *Ecotoxicology*, *27*, 752–760. https://doi.org/10.1007/s10646-018-1957-x

Wilk-Woźniak, E. (2019). An introduction to the 'micronet' of cyanobacterial harmful algal blooms (CyanoHABs): Cyanobacteria, zooplankton and microorganisms: A review. *Marine and Freshwater Research*, *71*, 636–643. https://doi.org/10.1071/MF18378

World Health Organization. (2024). *Management of cyanobacteria in drinking-water supplies: Information for regulators and water suppliers* (2nd ed.). World Health Organization. https://doi.org/10.2471/B09130

Woszczyk, M., & Schubert, C. J. (2021). Greenhouse gas emissions from Baltic coastal lakes. *Science of the Total Environment*, *755*, 143500. https://doi.org/10.1016/j.scitotenv.2020.143500

Zawierucha, K. (2024). Did bioaggregates on the glacier surface trigger life seeding and pedogenesis in terrestrial environments after the Neoproterozoic snowball earth? *Soil Biology and Biochemistry*, *198*, 109526. https://doi.org/10.1016/j.soilbio.2024.109526

Zong, J. M., Wang, X. X., Zhong, Q. Y., Xiao, X. M., Ma, J., & Zhao, B. (2019). Increasing outbreak of cyanobacterial blooms in large lakes and reservoirs under pressures from climate change and anthropogenic interferences in the middle–lower Yangtze River basin. *Remote Sensing*, *11*, 1754. https://doi.org/10.3390/rs11151754

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Appendix S1. Details about studied strains of cyanobacteria.

Appendix S2. The results of ELISA, TRFIA, HPLC-DAD, and LC-MS for extracts from studied strains of cyanobacteria.

Appendix S3. Data about phaeophytin concentration (a), pH (b), total nitrogen (c), and total phosphorus (d) concentrations in 40-day-old cyanobacterial cultures incubated for *Daphnia* biotests. Strains with detected toxic/bioactive cyanometabolites are marked in red, strains for which no toxic/bioactive cyanometabolites were detected have blue font. Abbreviations: APs—anabaenopeptins, ATX-a—anatoxin-a, CYN—cylindrospermopsin, MCs—microcystins, STXs—saxitoxins.

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