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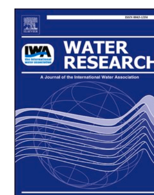

Distribution of toxigenic cyanobacteria in Alpine lakes and rivers as revealed by molecular screening

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ABSTRACT

The increasing frequency of cyanobacteria blooms in waterbodies caused by ecosystem eutrophication could endanger human health. This risk can be mitigated by effective monitoring incorporating molecular methods. To date, most molecular studies on toxigenic cyanobacteria have been limited to microcystins (MCs), disregarding other cyanotoxins, to freshwater planktic habitats while ignoring benthic habitats, and to limited geographic areas (usually one or a few specific waterbodies). In this study, we used PCR-based methods including PCR product sequencing and chemical-analytical methods (LC-MS/MS) to screen many plankton (n = 123) and biofilm samples (n = 113) originating from 29 Alpine lakes and 18 rivers for their cyanotoxin production potential. Both *mcyE* (indicating MC synthesis) and *anaC* (indicating anatoxin (ATX) synthesis) gene fragments were able to qualitatively predict MC or ATX occurrence. The abundance of *mcyE* gene fragments was significantly related to MC concentrations in plankton samples (R^2 = 0.61). *mcyE* gene fragments indicative of MC synthesis were most abundant in planktic samples (65 %) and were assigned to the genera *Planktothrix* and *Microcystis*. However, *mcyE* rarely occurred in biofilms of lakes and rivers, i.e., 4 % and 5 %, respectively, and were assigned to *Microcystis, Planktothrix*, and *Nostoc*. In contrast, *anaC* gene fragments occurred frequently in planktic samples (14 % assigned to *Tychonema, Phormidium (Microcoleus)*, and *Oscillatoria*), but also in biofilms of lakes (49 %) and rivers (18 %) and were assigned to the genera *Phormidium, Oscillatoria*, and Nostocales. The *cyrJ* gene fragment indicating cylindrospermopsin synthesis occurred only once in plankton (assigned to *Dolichospermum*), while saxitoxin synthesis potential was not detected. For plankton samples, monomictic and less eutrophic conditions were positively related to *mcyE*/MC occurrence frequency, while oligomictic conditions were related to *anaC*/ATX frequency. The *anaC*/ATX frequency in biofilm was related to the lake habitats generally showing higher biodiversity as revealed from metabarcoding in a parallel study.

1. Introduction

In aquatic environments, several cyanobacteria genera produce toxins (called cyanotoxins) and can form massive blooms. While mass occurrences of toxic cyanobacteria pose an acute threat to human health, chronic threats could be also caused by long-term exposure (Chorus and Welker, 2021). Although surface blooms are produced mostly by planktic species, benthic species in biofilms are also known to

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cause animal poisonings (Wood et al., 2020; Quiblier et al., 2013). Toxic cyanobacteria occur throughout the world (Du et al., 2019), but there are biogeographic differences in the distributions of producers carrying the respective synthesis pathways (e.g., Pacheco et al., 2016).

From the late nineteenth century onwards, the Alpine region has experienced a temperature increase above the average temperature increase of the northern hemisphere (Auer et al., 2007). The current trend of rising temperatures, intensifying precipitation, increasing frequency

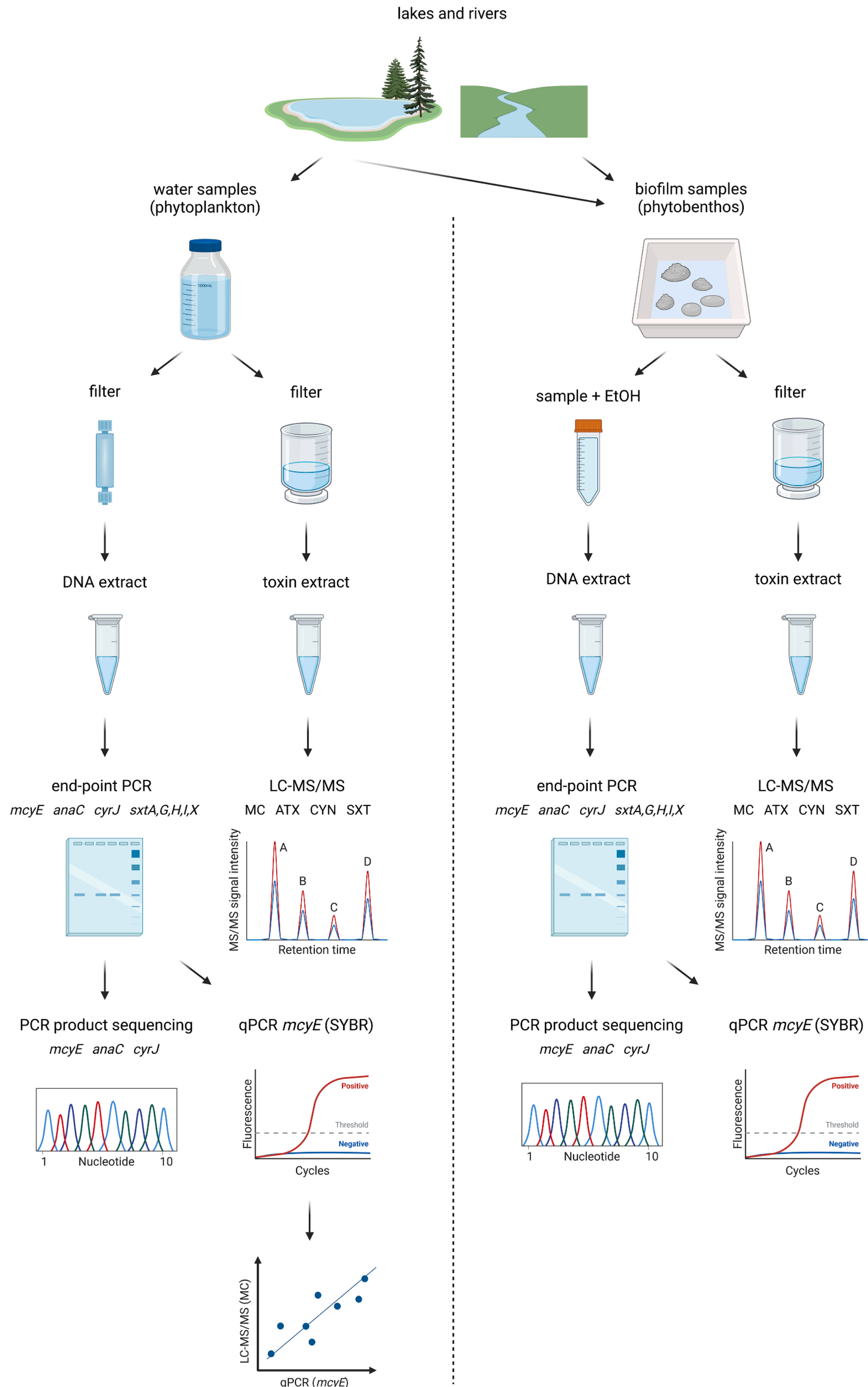

Fig. 1. Workflow for sample analysis in Alpine lakes and rivers. Samples showing PCR products (*mcyE*) were further analyzed with *mcyE*-specific qPCR. MC – microcystins, ATX – anatoxins, CYN – cylindrospermopsins, SXT – saxitoxins.

of drought periods, and melting glaciers is expected to continue (Gobiet et al., 2014). In addition, the intensive tourism in the Alpine region might lead to further degradation of the aquatic environment through increased pollution, nutrient loading, shoreline erosion, and habitat and biodiversity loss (Dokulil, 2014). Together these altered conditions could influence cyanobacteria growth in Alpine water bodies, increasing their biomass (Gallina et al., 2011). Numerous cyanobacteria species achieve maximum growth rates at higher temperatures, often above 25 °C, giving them an advantage over eukaryotic algae (Paerl, 2014). Moreover, high nutrient levels may act synergistically with high temperatures to further reduce species diversity and promote cyanobacteria dominance (Elliott et al., 2006). In addition, other factors associated with climate change, such as changing precipitation patterns, altered hydrological characteristics (e.g., increased vertical stratification and decreased flushing rate), and increasing atmospheric CO_2 concentrations, could all favor cyanobacterial proliferations (Paerl, 2014; Elliot, 2012). During the last three decades expansions from the tropics to subtropical and temperate regions have been documented for two toxigenic cyanobacteria species (of the order Nostocales), most likely due to climate change and nutrient loading (Sukenik et al., 2012).

Thus, addressing the threats posed by cyanobacteria blooms will be beneficial to human health, and yet many water bodies used for recreational purposes are not regularly assessed for cyanotoxins. Making the task of monitoring more practical by reducing cost, time, and effort and increasing its sensitivity would perhaps encourage policymakers to use monitoring for risk surveillance on a regular basis. In recent decades molecular methods have gained attention as an early warning tool (Kurmayer et al., 2017). The advantage of molecular methods includes early detection of toxic genotypes because of the generally lower detection limit (e.g., Zupančič et al., 2021), thus they could complement analytical methods for cyanotoxin measurements. Nevertheless, to date the application of molecular tools in monitoring has been developed slowly. PCR-based methods mostly focus on microcystins (MCs), while other cyanotoxins, such as anatoxins (ATXs), cylindrospermopsins (CYNs), and saxitoxins (SXTs), are monitored less frequently (Pacheco et al., 2016). Moreover, planktic habitats are studied more widely than benthic habitats (Wood et al., 2020). Typically, studies are limited to a relatively small geographic area including only one waterbody (Pacheco et al., 2016).

In this study, we use PCR indicative of cyanotoxin synthesis and subsequent sequencing of PCR products, qPCR, and LC-MS/MS to test both lake plankton and biofilm from littoral and rivers within a larger geographic area of the European Alps ($N = 236$) sampled in the framework of the Alpine Space project Eco-AlpsWater (https://www.alpine-space.eu/project/eco-alpswater/). Our general aim was to assess the distribution of toxigenic cyanobacteria in the Alpine region, both in plankton and in biofilm, with the following objectives: (i) identify the potential for MC, ATX, CYN, and SXT production in Alpine lakes and rivers, (ii) compare different methods for PCR-based detection of cyanotoxin producers, (iii) evaluate spatial and temporal trends of producers, and (iv) identify environmental variables affecting their abundance.

2. Methods

The overall workflow is presented in Fig. 1. For each sample, aliquots were analyzed in parallel for cyanotoxins by LC-MS/MS (i.e., MC, ATX, CYN, SXT) and the presence of gene fragments by PCR indicative of cyanotoxin synthesis, i.e., *mcyE* indicative of MC synthesis, *anaC* indicative of ATX synthesis, *cyrJ* indicative of CYN synthesis, and five *sxt* gene fragments (*sxtA, sxtG, sxtH, sxtI*, and *sxtX*) indicative of SXT synthesis. Currently, no single gene locus is known that could confirm the presence of the *sxt* gene cluster; therefore, a protocol combining several genes is recommended (Kurmayer et al., 2017). For *mcyE* gene fragments all samples yielding distinct or less specific PCR results (i.e., multiple bands on the gel) were further quantified by qPCR. Only this gene was used for qPCR, as *sxt* genes were not detected, *cyrJ* was detected in only one sample, and for *anaC*, no suitable qPCR assay was available. Finally, the results obtained from molecular screening were compared with LC-MS/MS results on cyanotoxin concentrations.

2.1. Sampling of Alpine lakes and rivers

For a general introduction to the water bodies see Salmaso et al. (2022, 2024). This study included a total of 236 samples collected from lakes and rivers sampled in the Alps in five Alpine countries (Austria, Germany, Italy, Slovenia, and Switzerland) between October 2018 and January 2020 (Table 1). All lakes were formed by glacial erosion and can be classified into three categories distinguished by their formation processes and altitude (Dokulil, 2009): high alpine lakes (located at high altitude above tree line), alpine lakes *sensu strictu* (glacial valley lakes) and pre- or subalpine lakes (formed by large glaciers, also known as piedmont lakes). In particular, 123 depth-integrated plankton samples from 29 different lakes and 113 biofilm (phytobenthos) samples from 18 rivers and the littoral of 9 lakes were collected. For five selected lakes (Lake Bled, SI; Lake Garda, IT; Lake Lugano, CH; Lake Mondsee, AT; Lake Starnberg, DE), seasonal plankton sampling was performed for one year resulting in 9–13 samples in monthly intervals (from January to December). For the same five lakes, biofilms were sampled from several sampling points located along the shore between July and October (Suppl. File S1). For the remaining lakes and rivers, sampling was performed either only once in the summer season (from June to October), or a few times between spring and autumn (from March to November).

For plankton sampling, water samples were depth-integrated through the euphotic zone at maximum depth and filtered through Sterivex columns (Millipore Sterivex-GP Pressure Filter Unit, Merck). The filtered volume ranged between 60 and 2000 mL, depending on the turbidity of the sample. Filters were stored at −20 °C until DNA extraction (for details see Domaizon et al., 2019).

Biofilm samples were obtained by brushing biofilm typically from stones collected manually in rivers (Rimet et al., 2020) or in the littoral from defined sampling areas (Rimet et al., 2021), i.e., at 20–50 cm depth from the minimal recorded water level (sampling year) in an area of 100 m^2. Generally, from five stones a total of 100 cm^2 surface area of biofilm was removed by brushing it into 50 mL of Milli-Q water using a tray. An aliquot of 10 mL was fixed immediately by adding 40 mL absolute ethanol and stored at 4 °C until DNA extraction. Sampled stones were either processed directly in the field or transported in a cooling box for processing in the laboratory.

For cyanotoxin analysis, depth-integrated plankton samples (60–4000 mL) or biofilm (0.005–1.224 g dry weight) samples were filtered through GF/C filters (Whatman, Kent, UK, approx. 1.2 µm pore size) and stored at −20 °C until extraction for LC-MS/MS analysis. For biofilm samples, dry weight on the filter (105 °C, overnight) was determined from aliquots.

Table 1
List of plankton and biofilm samples originating from lakes and rivers used in this study. The number of samples analyzed as well as the original number of samples for each category (in brackets) is indicated. In total, 15 samples were excluded due to insufficient DNA quality (i.e., PCR using *cpc*BA-IGS primers was negative).

Country	Lakes – plankton	Lakes littoral – biofilm	Rivers – biofilm	All
Austria	14	2 (13)	6	22 (33)
Switzerland	12	6	1	19
Germany	46	45 (46)	14	105 (106)
Italy	26	10	6	42
Slovenia	25 (28)	12	11	48 (51)
All	123 (126)	75 (87)	38	236 (251)

Various physiographic and environmental variables were recorded, i.e., catchment area, water renewal time, mixing type, water temperature, pH, conductivity, Secchi disk depth (for plankton samples), oxygen concentration, and saturation. In the laboratory, concentration of nitrate, ammonium, total nitrogen, soluble reactive phosphorus, total phosphorus, total alkalinity, and chlorophyll a (for plankton samples) were determined. For details, see Salmaso et al. (2022, 2024).

2.2. Cyanotoxin analysis with LC-MS/MS

Intracellular cyanotoxins were extracted from GF/C filters prepared from sample aliquots and quantified by LC-MS/MS as described by Cerasino and Salmaso (2020). The extraction was performed in acetonitrile/water (60/40, v/v) containing 0.1 % formic acid. Extracted toxins were injected into a LC-MS/MS system, composed of a Waters Acquity UPLC system (Waters, Milford, MA, USA) coupled to a SCIEX 4000 QTRAP mass spectrometer (AB Sciex Pte. Ltd., Singapore). The mass detector was operated in scheduled Multiple Reaction Monitoring (MRM) mode, using positive electrospray ionization (ESI+). The 11 most common MC structural variants were quantified following the protocol by Cerasino and Salmaso (2020): MC-RR, MC-[D-Asp$_3$]-RR (RRdm), MC-[D-Asp$_3$]-HtyrR (HtyRdm), MC-YR, MC-LR, MC-[D-Asp$_3$]-LR (LRdm), MC-WR, MC-LA, MC-LY, MC-LW, MC-LF. ATXs, CYNs, and SXTs were analyzed following the LC-MS/MS conditions from Ballot et al. (2020), targeting ATX-a, homoATX-a, CYN, STX, decarbamoylated STX, NeoSTX, gonyautoxins (GTX1, GTX4, GTX5), and C-toxins (C1, C2). For each individual toxin variant, two mass transitions were monitored in the mass spectrometer (the quantifier and the qualifier). The presence of each toxin variant was confirmed only when both transitions were present. Once this condition was fulfilled, quantification was performed by measuring the intensity of the most intense one (quantifier transition) in comparison to a standard curve. Details on compound identification and the monitored product ions in MRM mode are given in Cerasino et al. (2017). Limits of quantification (LOQs) were 0.5–9 ng/g d.w. for MCs, 2.0–4.0 ng/g d.w. for ATXs, 0.4 ng/g d.w. for CYN, and 5.0–27.0 ng/g d.w. for SXTs, depending on the variant. In Germany, samples for cyanotoxin analyses were not collected, and therefore 71 (58 %) of all plankton and 53 (47 %) of all biofilm samples were analyzed, as indicated in Suppl. File S1.

2.3. DNA extraction and quality control

To extract DNA from plankton samples, the DNeasy PowerWater Sterivex kit (Qiagen, Hilden, Germany) was used following manufacturer's instructions. To extract DNA from biofilm samples fixed with absolute ethanol (80 %, v/v, 50 mL) either the NucleoSpin Soil kit (Macherey-Nagel, Germany) (Vasselon et al., 2017) or a combination of precipitation of DNA using sodium acetate and purification of precipitated DNA from cellular debris using the DNeasy Plant Mini kit (Qiagen) was used. DNA quantity and quality were recorded using a microvolume spectrophotometer (NanoDrop) or Qubit fluorometer. DNA extracts were tested by PCR amplifying the phycocyanin gene region *cpc*BA and the intergenic spacer region (IGS), which is indicative of cyanobacteria in general (Neilan et al., 1995), confirming (i) non-inhibiting conditions for PCR and (ii) the presence of cyanobacteria in the samples. The same DNA extracts used in this study have been used for 16S rRNA and 18S rRNA deep-amplicon sequencing (next generation sequencing, NGS) as described (Salmaso et al., 2022, 2024). In this study, absolute and rarefied read numbers of 12 toxigenic cyanobacteria (as identified via PCR product sequencing and listed in Suppl. File S2) have been related to cyanotoxin concentrations (Suppl. File S1).

2.4. PCR-based detection of genes indicative of cyanotoxin synthesis

Reactions were prepared in 0.2 mL 8-strip PCR tubes (Starlab) in 10 μL volume, consisting of 1 μL 10× DreamTaq PCR buffer containing 20 mM $MgCl_2$ (Thermo Scientific), 0.4 μL of 25 mM $MgCl_2$, 0.3 μL of dNTP (10 mM each), 0.3 μL of each primer (10 μM), 0.05 μL of DreamTaq (5 U/μL), 1 μL of undiluted DNA template, and 6.7 μL Milli-Q water. PCR amplification was performed in a MasterCycler Nexus Gradient (Eppendorf) with cycling conditions as follows: 3 min at 95 °C, 35 cycles including 30 s at 94 °C, 30 s at variable annealing temperature (see Table 2), and 1 min at 72 °C, followed by 5 min at 72 °C. PCR products were stained with Midori Green Advance DNA Stain (Biozym), loaded onto an agarose gel (0.8 %), run at 120 V for 60 min, and visualized with Molecular imager ChemiDoc XRS+ (Bio-Rad).

Selected PCR products of expected sizes were either (i) purified directly by QIAquick PCR Purification Kit (Qiagen) or (ii) cut out from 1 % agarose gel after gel electrophoresis in modified TAE buffer and visualization using Ethidium Bromide under UV light (Safe Imager 2.0, Invitrogen), and purified using the Montage DNA Gel Extraction Kit (Merck Millipore, USA). The purified PCR products were sequenced using Sanger sequencing (Eurofins Genomics, Germany) in the forward direction. The sequences were analyzed with BioEdit (v. 7.2.5) using ClustalW (1000 bootstraps) for alignment. Sequences can be found in Suppl. File S2.

2.4.1. mcyE gene quantification via qPCR

DNA extracts showing either distinct or less specific PCR products via the HEPF, HEPR-PCR amplifying the *mcyE* gene indicative of MC synthesis were further analyzed by qPCR to quantify *mcyE* genotypes via the DQ*mcy*F, DQ*mcy*R primers (Al-Tebrineh et al., 2011). In pilot experiments the qPCR assay performance was tested using eight strains representing distantly related cyanobacteria genera, i.e., *Planktothrix, Microcystis, Nostoc,* and *Hapalosiphon* (details in Suppl. File S3). qPCR was performed in 10 μL volume, consisting of 5 μL (2×) Maxima SYBR Green/ROX qPCR Master Mix (Thermo Scientific), 0.8 μL of each primer (10 μM), 2.40 μL Milli-Q water, and 1 μL of DNA template. Routinely undiluted and 10-fold diluted DNA was amplified to test for possible amplification inhibition. qPCR was performed on a Mastercycler® ep realplex (Eppendorf), and cycling conditions followed a standard two step protocol: 10 min at 95 °C, 45 cycles of 15 s at 95 °C and 1 min at 60 °C. At the end of each run, a melting curve analysis was performed with an initial denaturation at 95 °C for 15 s, followed by 15 s at 60 °C, and a gradual increase to 95 °C to compare the PCR product melting temperature (Tm) maximum with the reference Tm (obtained from strains mentioned above). All samples were analyzed in triplicate using heat-sealed optical PCR plates (twin. tec PCR plates 96, Eppendorf). Positive controls (*M. aeruginosa* strain Hofbauer) and negative controls were included.

2.4.2. Statistical analyses to quantify the influence of environmental conditions

Statistical analyses were performed in R (v. 4.0.3 and 4.2.2), SigmaPlot (v. 15.0.0.13), and Canoco 5 (Ter Braak and Smilauer, 2012). R packages ggplot2, ggpubr, scales, vegan, Hmisc, and regclass were used for analysis and visualization, and qGIS (v. 3.22.7) was used for mapping the results. For the multivariate statistical analysis, the response variables (i.e., genotypes and cyanotoxin concentrations) were log transformed, centered, and standardized. Mixing type and habitat were coded as categorical variables.

For redundancy analysis (RDA), environmental variables with > 18 % (plankton) and > 6 % (biofilm) missing data were removed. Significance of progressive inclusion of variables was tested via ordistep (i.e., mixing type, NH_4, chlorophyll a, and catchment area were recognized as the most significant influence variables for plankton, while it was habitat for biofilm). Interdependence of quantitative explanatory variables was tested via Pearson correlations. As a result, total phosphorus, total nitrogen, total alkalinity, and water renewal time were removed from the plankton dataset due to significant correlations with other variables. For plankton, the correlations between variables were as follows ($p < 0.001$): total P vs. chlorophyll a ($r = 0.95$), total P vs. total N

Table 2
List of oligonucleotides (primers) used for PCR and qPCR in this study.

Assay	Target region	Forward primer (5′–3′)	Reverse primer (5′–3′)	Product length [bp]	Reference	Annealing T [°C]	Application
PCβF, PCαR	*cpc*BA-IGS	GGCTGCTTGTTTACGCGACA	CCAGTACCACCAGCAACTAA	500–740	Neilan et al., 1995	50	PCR
HEPF, HEPR	*mcyE*	TTTGGGGTTAACTTTTTTGGGCATAGTC	AATTCTTGAGGCTGTAAATCGGGTTT	472	Jungblut and Neilan, 2006	50	PCR
DQ*mcy*F, DQ*mcy*R	*mcyE*	TTTAGAACSGGVGATTTAGG	CGRBTVADTTGRTATTCAAT TTCT	128	Al-Tebrineh et al., 2011	60	qPCR
anaC-genF, *anaC*-genR	*anaC*	TCTGGTATTCAGTCCCCTCTAT	CCCAATAGCCTGTCATCAA	366	Rantala-Ylinen et al., 2011	58	PCR
cynsulfF, cylnamR	*cyrJ*	ACTTCTCTCCTTTCCCTATC	GAGTGAAAATGCGTAGAACTTG	586	Mihali et al., 2008	53	PCR
sxtAf, sxtAr	*sxtA*	GCGTACATCCAAGCTGGACTCG	GTAGTCCAGCTAAGGCACTTGC	683	Ballot et al., 2010	55	PCR
sxtGf, sxtGr	*sxtG*	AGGAATTCCCTATCCACCGGAG	CGGCGAACATCTAACGTTGCAC	893	Casero et al., 2014	55	PCR
sxtHf, sxtHf	*sxtH*	AAGACCACTGTCCCCACCGAGG	CTGTGCAGCGATCTGATGGCAC	812	Casero et al., 2014	55	PCR
sxtIf, sxtIr	*sxtI*	AGCGCTGCCGCTATGGTTGTCG	ACGCAATTGAGGGCGACACCAC	910	Casero et al., 2014	55	PCR
sxtXf, sxtXr	*sxtX*	GATGCAACCCATAAACTCGCAC	AAGGTACTCGTTTTCGTGGAGC	656	Casero et al., 2014	55	PCR

($r = 0.75$), total N vs. chlorophyll a ($r = 0.70$), total N vs. Secchi depth ($r = -0.59$), total alkalinity vs. conductivity ($r = 0.89$), total alkalinity vs. water renewal time ($r = -0.51$), and water renewal time vs. catchment area ($r = 0.76$). Analogously for biofilm the variable oxygen percentage was not included: temperature vs. O_2 percentage ($r = 0.68$, $p < 0.001$). This resulted in 11 environmental variables for plankton and four for

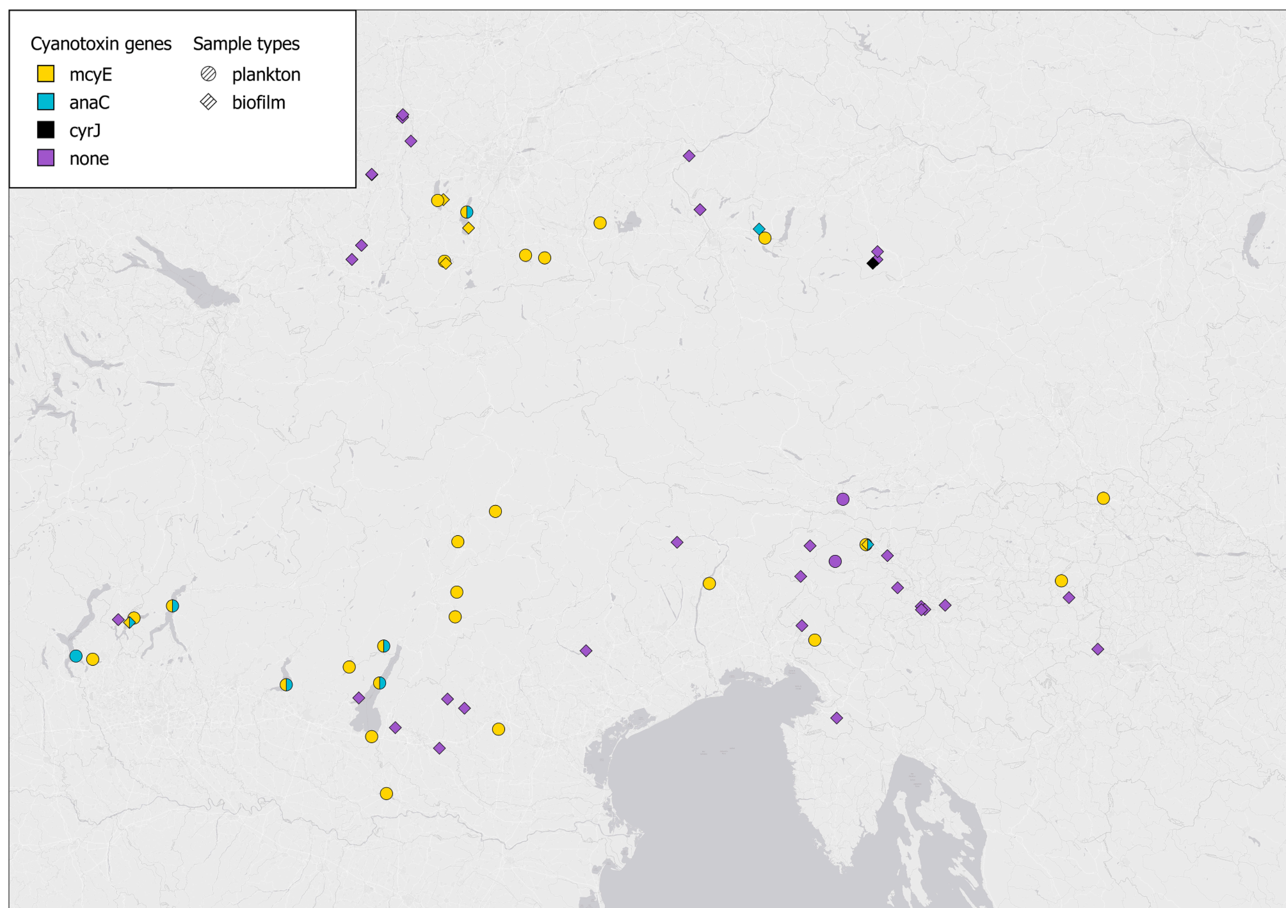

Fig. 2. Distribution of gene fragments indicative of cyanotoxin biosynthesis in the European Alps as determined by (q)PCR. Plankton samples were obtained for several lakes during 2019 while biofilm samples from littoral zones of the same lakes or rivers were collected once during 2018–2019. Note that gene fragments were not necessarily detected at all sampling dates; thus, the symbols indicate occurrence at the respective sample site at least once. For clarity only one littoral sampling point is shown for each lake, though in general multiple sites were sampled for biofilm. Basemap: ESRI Gray (light). For a detailed view, see the interactive map https://zenodo.org/records/11091446 (Suppl. File S9).

biofilm; after removing observations with missing data, 68 plankton and 49 biofilm samples were analyzed.

2.5. Results

2.5.1. DNA quality control and validation of qPCR assay for mcyE

Extracted DNA concentrations ranged from 1.1 to 73.3 ng/μL (median 9.7 ng/μl, mean 12.4 ng/μl, 25 % quartile 6.4, 75 % quartile 14.7, $n = 236$). All sample extracts were tested for the presence of cyanobacterial DNA using *cpc*BA-IGS primers. In general, there was no correlation between PCR positive samples and DNA concentration. Thus, sample extracts with the lowest DNA concentrations (1.1 ng/μL) were also found suitable for PCR amplification. As a result, three plankton and 12 biofilm samples were omitted from further analysis as they repeatedly did not show any PCR product (PC-IGS, HEP or *sxtI*). Thus, in total 236 (123 plankton and 113 biofilm) samples were analyzed by PCR (Table 1).

For *mcyE* quantification via qPCR the specificity, amplification efficiency, assay linearity, and sensitivity were compared using four strains representing distantly related genera: *Microcystis aeruginosa* strain Hofbauer, *P. agardhii* NIVA CYA 126/8, *Nostoc* sp. PCC 9237, and *Hapalosiphon hibernicus* BZ 3–1 (see Suppl. Files S4, S5). For all strains linear curves ($R^2 \geq 0.99$) spanning five orders of magnitude between Cq values and DNA template concentration (expressed as cell equivalents) and amplification efficiencies ranging between 86 % and 107 % were observed (Suppl. File S6). The LOQ was defined as the cell concentration for which all three replicates were found positive and ΔCq between two subsequent 10-fold dilutions was 3.6 ± 0.7 (mean ± SD). Accordingly, the LOQ was set to 14 cell equivalents (*M. aeruginosa*) per template.

2.5.2. Frequency of occurrence and distribution of cyanotoxin genes

The gene fragments indicative of cyanotoxin synthesis were distributed across the five Alpine countries (Fig. 2, Suppl. File S1). In plankton, the *mcyE* (q)PCR product occurred most frequently (107 of 123 samples), followed by the *anaC* PCR product (17 of 123 samples). In biofilm samples, the frequency of *mcyE* was much lower (7 of 113 samples), while the *anaC* PCR product was still found frequently (44 of 113 samples). The *cyrJ* PCR product only occurred in two plankton and in two biofilm samples. In contrast, *sxtA-I* PCR products were never observed co-occurring in one sample, rendering SXT synthesis unlikely. In most samples, one gene locus indicative of either MC or ATX synthesis was recorded. However, for 16 plankton and four biofilm samples gene fragments indicative of both MC and ATX synthesis co-occurred, and in two plankton samples both the *mcyE* and *cyrJ gene* fragments were observed (detailed results are available in Suppl. File S1). The seasonal trend in abundance of MC producers in lakes was also assessed and is described in Suppl. File S7.

For *mcyE*, the obtained PCR products (Suppl. File S2, S8) were confirmed by sequencing (i.e., in 54 PCR products out of 60 products attempted to be sequenced) and assigned to *P. rubescens* (De Candolle ex Gomont) Anagnostidis & Komárek, 1988/*P. agardhii* (Gomont) Anagnostidis & Komárek, 1988 or less frequently to *Microcystis aeruginosa* (Kützing) Kützing, 1846 or the genus *Nostoc* Vaucher ex Bornet & Flahault, 1888 (92–100 % identity; Suppl. File S2). The *anaC* PCR product was confirmed by sequencing of 21 PCR products (out of 31 attempted to be sequenced) and assigned to *Tychonema bourrellyi* (Lund) Anagnostidis & Komárek 1988, or the genus *Oscillatoria* sp. Vaucher ex Gomont 1892, or *Microcoleus autumnale* (Gomont) Strunecky, Komárek & Johansen 2013 (former *Phormidium autumnale* Gomont 1892), or unknown Nostocales Borzì 1914 (sequence identity 87–100 %; Suppl. File S2). For *cyrJ*, the target PCR product was confirmed by sequencing in one sample (out of four PCR products attempted to be sequenced), and the sequence was the most similar to genus *Dolichospermum* (Ralfs ex Bornet et Flahault) Wacklin, Hoffmann et Komárek, 2009, 92 % identity). In summary, while the *mcyE* and *anaC* PCR products were typically confirmed by sequencing, for *cyrJ*, sequencing of PCR product was needed to exclude unspecific PCR amplification.

2.6. Cyanotoxin occurrence and structural variants

In total, MCs were detected in 61 (86 %) out of 71 plankton samples (up to 660.5 ng/L, intracellular concentrations) and from four (8 %) out of 53 biofilm samples (three [4 %] lake samples and one [3 %] river sample; up to 1900 ng/g dry weight) (Suppl. File S1). MC cell quotas (ratio between MC and *mcyE* cell equivalent concentration) ranged from 0.02 to 32.7 pg/cell equivalent (median 0.23 pg/cell equivalent, $N = 61$). In addition, we found ATXs occurring in 20 (28 %) out of 71 plankton samples (up to 669.1 ng/L) and in 21 (40 %) out of 53 biofilm samples (19 [25 %] lake samples and two [5 %] river samples; up to 16,922 ng/g dry weight). We did not detect CYNs or SXTs in any sample. Within biofilm samples, ATXs mostly occurred as the only cyanotoxin (i. e., 18 [86 %] out of 21 ATX-positive samples). In contrast, within plankton samples ATXs always co-occurred with MCs ($n = 20$).

In plankton, the most common MC structural variants included MC-RRdm (84 % out of 61 MC-positive samples), MC-LRdm (59 %), MC-HtyRdm (44 %), MC-RR (31 %), and MC-LR (15 %). Frequently, multiple (up to five) variants of MCs co-occurred in a single sample. From biofilm samples, only two MC variants were recorded (RRdm and MC-LR), co-occurring in two out of four MC-positive samples (Suppl. File S1). Within ATXs, ATX-a was the only recorded variant in plankton samples. In biofilm samples, ATX-a was the most frequently recorded (81 % of 21 ATX-positive samples), while homoATX-a occurred in the six samples of lake Lugano only (in two cases co-occurring with ATX-a).

2.7. Relationship between (q)PCR results and cyanotoxin occurrence

We qualitatively compared PCR and LC-MS results for all samples analyzed for both *mcyE* and MC or *anaC* and ATX (i.e., 71 plankton, 30 littoral biofilm, and 23 river biofilm samples). In general, for both plankton and biofilm samples *mcyE* PCR products indicative of MC synthesis were found related to MC as detected from sample aliquots. For one plankton sample and three biofilm samples MC was recorded but no *mcyE* PCR product was obtained, i.e., PCR for *mcyE* indicated a false negative. Nevertheless, for most samples the recorded *mcyE* PCR product indicated MC occurrence (Table 3). The false negative plankton sample originated from Lake Bohinj (SI), December (1.0 ng MC/L, and false negative biofilm samples originated from Lake Bled (SI), Lake Lugano (CH), and Corbiolo River (IT), with measured MC concentrations of 11.0, 1.9, and 334.5 ng/g d.w., respectively.

Analogously, most samples containing ATX were indicated through the *anaC* PCR product. However, a small percentage of ATX-containing

Table 3
Cross-table showing the frequency of *mcyE* and *anaC* gene fragment occurrence as recorded via (q)PCR and respective MC or ATX occurrence as recorded via LC-MS/MS from plankton and biofilm sample aliquots. Top: microcystins (MC), bottom: anatoxins (ATX). (+/- indicates positive/negative).

Plankton				Biofilm			
		LC-MS/MS (MC)				LC-MS/MS (MC)	
		+	–			+	–
qPCR (*mcyE*)	+	60 (85 %)	4 (6 %)	qPCR (*mcyE*)	+	1 (2 %)	4 (8 %)
	–	1 (1 %)	6 (8 %)		–	3[1] (6 %)	45 (85 %)
		LC-MS/MS (ATX)				LC-MS/MS (ATX)	
		+	–			+	–
PCR (*anaC*)	+	15 (21 %)	0	PCR (*anaC*)	+	16 (31 %)	6 (12 %)
	–	5[2] (7 %)	51 (72 %)		–	5[3] (10 %)	25 (48 %)

[1] one sample found *mcyE* positive had very low MC signal (very close to LOQ).
[2,3] four (plankton) or three (biofilm) samples found *ana*C positive had very low ATX signal (very close to LOQ).

samples (7 % in plankton and 10 % in biofilm) did not show a corresponding *anaC* product, i.e., PCR indicated a false negative. False negative plankton samples originated from lakes Bohinj and Pernica (SI), and Lake Mondsee (AT), with ATX concentrations between 1.1 and 56.3 ng/L. False negative biofilm samples originated from Lake Bled and Koseze pond (SI), and Salzach river (AT), with ATX concentrations between 12.4 and 80.7 ng/g d.w.

In a second step, *mcyE* genotype abundance (expressed as *mcyE* cell equivalents/mL) in plankton samples ($n = 71$) were related to total MC concentrations. Data were log(x + 1) transformed and passed the Kolmogorov-Smirnov test for normality ($p \geq 0.09$, alpha = 0.05) and Brown-Forsythe ANOVA test for homogeneity of variances ($p = 0.09$). A significant linear relationship spanning four orders of magnitude (1–1000 cell equivalents/mL vs 1–1000 ng MC/L) was observed (Pearson $R^2 = 0.61$, $p < 0.001$, $n = 71$; Fig. 3). Four samples contained *mcyE* (> 13 cell equivalents/mL) but did not contain MC. The confidence interval was the narrowest at *mcyE* concentrations between 10 and 100 cell equivalents/μL. Thus, the likelihood of prediction potential was considered at maximum in this range implying that 10–100 *mcyE* cell equivalents/mL resulted in 4.5–19.9 (± 1.3) ng/L of total MC concentration. For biofilm samples, linear regression analysis was not possible (49 negative samples out of $n = 53$).

2.8. Relationship between toxigenicity and environmental variables

When comparing the rarefied sequence read numbers of identified toxigenic cyanobacteria (Salmaso et al., 2022, 2024) with the total MC concentrations in plankton samples, *Planktothrix* strain NIVA-CYA 115 (but no other toxigenic taxa) showed a significant linear relationship ($R^2 = 0.38$, $n = 71$). Similarly, ATX concentrations showed a significant linear relationship only with rarefied read numbers from *Tychonema* strain CCAP 1459/11B ($R^2 = 0.84$, $n = 71$). Thus, in plankton habitats *P. rubescens/P. agardhii* were considered the main MC producers, while *T. bourrellyi* was considered the main ATX-producer. Within biofilm samples, higher correlations were observed between ATX concentrations and rarefied read numbers of *Kamptonema* strain PCC 6407 ($R^2 = 0.78$, $n = 53$) and of *Apanizomenon* strain NIES 81 (R2 = 0.32, $n = 53$). ATX synthesis has been documented for *Kamptonema* PCC 6407 (Mejean et al., 2014), while *Aphanizomenon* strain NIES 81 has been phylogenetically related to ATX producers (Österholm et al., 2020). Thus, the observed ecological relationships between *mcy*E and MC concentrations or *ana*C frequency and ATX concentrations were primarily caused by ecological diversification among the various toxigenic species.

In plankton, the RDA (adjusted $R^2 = 0.55$, $p < 0.001$) accounted for 64 % of variance using constrained variables (catchment area, mixing type, water temperature, pH, conductivity, Secchi, NO_3, NH_4, soluble reactive phosphorus, and chlorophyll a), while 36 % of total variation was explained by unconstrained factors. The first two RDA axes explained 97 % of the constrained variance, which represented 62 % of the total variance (Fig. 4A). The first RDA axis ($p < 0.001$) was mostly determined by oligomictic mixing type (centroid 1.00) and catchment area (biplot score 0.61; also positively correlated with water renewal time), and was positively correlated with *anaC* presence (species score 1.81) and ATX concentration (1.66), and negatively correlated with *mcyE* concentration (−0.98) and MC concentration (−0.86). The second RDA axis ($p < 0.01$) was mostly defined by polymictic mixing type (centroid 0.60) and Secchi depth (biplot score −0.47) and was negatively correlated with *mcyE* concentration (−1.12), MC concentration (−0.80), *anaC* presence (−0.54), and ATX concentration (−0.49). In summary, *mcyE/* MC concentrations were negatively related to oligomictic and polymictic conditions, and to habitats with larger catchment areas. In contrast, *anaC* frequency of occurrence and ATX concentration increased under oligomictic conditions and in lakes with larger catchment areas (and longer water renewal time). Both *mcyE*/MC and *anaC/* ATX were associated with increased Secchi disk depth. Generally, the samples formed three subgroups (Fig. 4A): (i) Lake Garda with high *anaC*/ATX frequency and oligomictic conditions, (ii) lakes Bled, Mondsee, and Lugano with high *mcyE*/MC frequency and monomictic/ meromictic conditions, and (iii) other waterbodies associated with dimictic/polymictic conditions as well as higher temperature and pH, which relates to higher primary production. It should be noted that lakes Garda, Bled, Mondsee, and Lugano were sampled monthly for one year, while other waterbodies were sampled only in the spring, summer, or autumn.

In biofilm, the RDA (adjusted $R^2 = 0.14$, $p < 0.01$) accounted for 21 % of variance using constrained variables (temperature, pH,

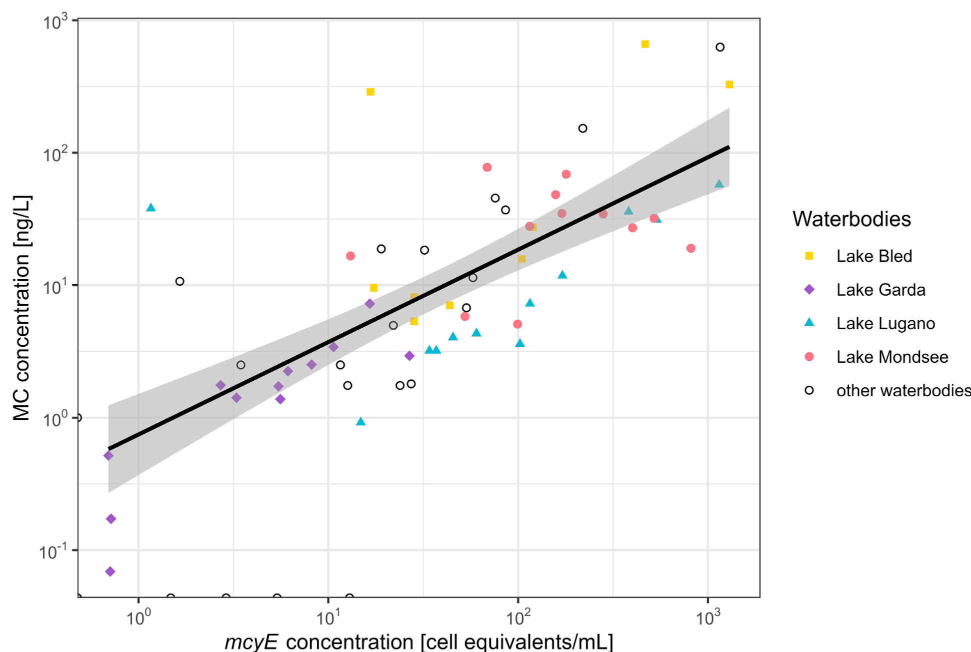

Fig. 3. Linear regression between *mcyE* cell equivalent concentration and microcystins (MC) concentration in plankton samples. The grey area represents the 95 % confidence interval. Linear regression equation (log [x + 1] transformed data): $y = 0.01 + 0.64x$, $R^2 = 0.61$, $p < 0.001$, $n = 71$. Accordingly, 100 cell equivalents/mL correspond to 14 ng MC/L.

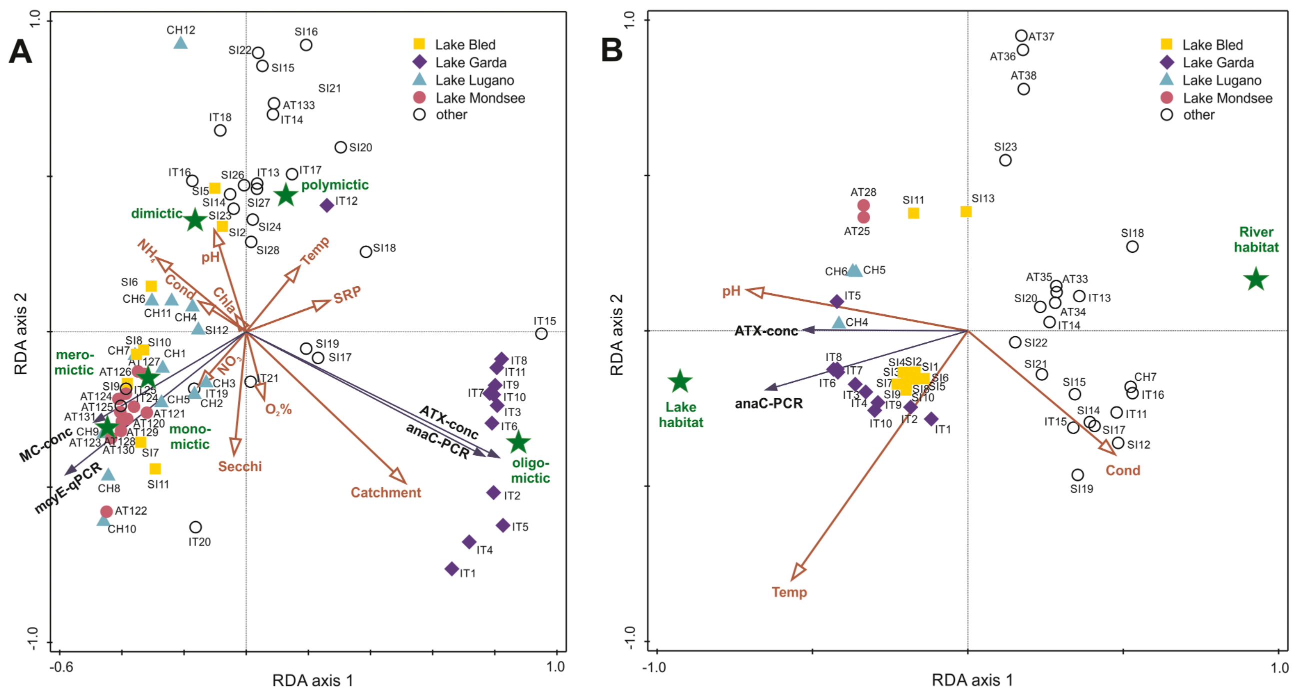

Fig. 4. Ordination of *mcyE* concentration, *anaC* gene frequency, and MC or ATX concentration (black arrows) in relation to selected environmental variables (orange arrows, green stars) in (A) plankton and (B) biofilm samples by means of redundancy analysis (RDA). Strength and direction of relationships are indicated by the length and direction of vectors. Temp - temperature, SRP - soluble reactive phosphorus, Cond - conductivity, Chla - chlorophyll a. The various lake mixing types were included as nominal variables, i.e., as centroids of the samples in which they occurred.

conductivity, habitat), while 79 % of total variation was explained by unconstrained factors (Fig. 4B). The first two RDA components explained 99 % of constrained variance, representing 21 % of total variance. Lake habitat (centroid 0.56), temperature (biplot score −0.69), and pH (−0.67) were the most important factors in the dispersion of sites along the first RDA axis, which was negatively correlated to *anaC* presence (species score −1.25), and ATX concentration (−0.97). Due to the low sample size (only four positive samples in biofilm), *mcyE* and MC concentrations were not included. In summary, *anaC* and ATX were more commonly present and showed higher concentrations in biofilm in the lake habitat and in water bodies with higher temperatures (and higher oxygen percentage) and higher pH.

3. Discussion

3.1. Potential cyanotoxin producing genera

This study demonstrates the high frequency of *mcyE* genes in plankton samples, and of *anaC* genes in plankton and in biofilm samples. In plankton samples, the main producers of MCs as revealed by *mcyE* PCR product sequencing were assigned to *P. rubescens/P. agardhii* which were commonly found in all seasonally studied lakes (Bled, Mondsee, Garda, Lugano, Starnberg; Suppl. File S6, S7). Thus *P. rubescens/P. agardhii* is most likely to constitute the major MC producer in these lakes. In particular, the red-pigmented species *P. rubescens* is best adapted to deep and physically stratified lakes in the Alps (Ostermaier and Kurmayer, 2010), and therefore its prevalence is not surprising. Moreover, *M. aeruginosa*, known as the most frequent MC producer globally, was detected in numerous other (more eutrophic) lakes (i.e., Pernica, Frassino, Ragogna, Mantova Superiore, Staffelsee, Vogrscek, Slivnica, Fimon, Varese, Caldaro, Serraira). In many of these lakes, which were identified as eutrophic, *Microcystis* was also recorded through NGS by Salmaso et al. (2024). In general, the toxigenic taxa identified via PCR and subsequent PCR product sequencing were also observed through 16S rDNA metabarcoding from Salmaso et al. (2024) in plankton. In contrast, in biofilm new toxigenic taxa not recorded via the same 16S rDNA metabarcoding were found (Suppl. File S2). For example, we identified *Nostoc* as potential MC-producer in four biofilm samples (AT-B-33, IT-B-02, IT-B-08, IT-B-12), while *Nostoc* was not detected via 16S metabarcoding in any biofilm sample. Since the *mcyE* sequence identity with *Nostoc* was only 92–96 %, it cannot be excluded that *Nostoc* was incorrectly identified. Similarly, the genus *Planktothrix*, which was identified in four biofilm samples in this study (IT-B-03, DE-B-15, DE-B-22, DE-B-27), was not always detected by NGS; rather, *Microcystis* or *Oscillatoria* were the most frequent toxigenic cyanobacteria identified by NGS in biofilm samples. The results of this study agree with earlier surveys reporting on the high frequency of MC-producers in lake plankton in general (e.g., Mantzouki et al., 2018; Christophoridis et al., 2018; Jančula et al., 2014).

ATX-producing genera detected using *anaC* sequences in biofilm samples included *Tychonema, Phormidium, Oscillatoria*, and unknown genotypes from Nostocales. In most plankton samples, the *anaC* sequence was assigned to *Tychonema/Oscillatoria/Phormidium* (samples IT-P-004, IT-P-007, IT-P-015, IT-P-020), as they share the exact same sequence (Suppl. File S2). Accordingly, Salmaso et al. (2024) showed that the genera *Tychonema, Phormidium*, and *Microcoleus* shared very similar 16S rRNA gene sequences in the V3–V4 hypervariable region within phylogenetic clade 1, implying a rather close systematic relationship, also suggested through ongoing systematic revision (Strunecký et al., 2023). In this study it is most likely that *anaC* genes originated from *T. bourrellyi*, as it has been detected in these samples by NGS (Salmaso et al., 2024). The genus *Tychonema* has been described as an important ATX producer in Alpine lakes (Cerasino and Salmaso, 2020; Salmaso et al., 2023), and has been observed repeatedly in Italian lakes included in this study (i.e., lakes Garda, Como, and Iseo) (Salmaso et al., 2016; Shams et al., 2015). For some *anaC* PCR products from biofilm samples (obtained from Bled, Starnberg, Lugano, and Staffelsee), the highest identity with NCBI GenBank was 92 % (Nostocales HT-58-2; Suppl. File S2). Identification is probably influenced by the lack of sequences from benthic cyanobacterial strains in the reference databases

as shown by Salmaso et al. (2022) for 16S rDNA. In the only sample where potential CYN-producers were detected in this study (AT-B-36), no potential CYN-producing taxon was detected by NGS by Salmaso et al. (2024).

In a few planktic samples, *anaC* and *mcyE* genotypes (and the resulting ATX and MC) were found co-occurring (Fig. 2, Suppl. File S1). This notable co-occurrence of two cyanotoxins has been described previously and explained by the fact that the cyanobacteria *T. bourrellyi* and *P. rubescens* share several ecological traits and can co-occur in nature (Cerasino and Salmaso, 2020; Nava et al., 2017; Salmaso et al., 2016).

3.2. Environmental variables linked to cyanotoxin occurrence

The relationships between environmental variables and cyanotoxin synthesis gene occurrence/cyanotoxin abundance (Fig. 4) can be interpreted best in association with the presence of the respective cyanotoxin-producing species. In planktic samples, *mcyE* genes and MCs were associated with monomictic lakes, which are generally considered typical habitats for *P. rubescens*, which likely constitutes the main MC producer in the Alpine lakes (Ostermaier and Kurmayer, 2010). *P. rubescens* typically grow under more oligotrophic conditions due to their effective light harvesting and nutrition strategy (Kurmayer et al., 2016). On the other hand, *anaC* genes and ATXs were associated with oligomictic conditions, larger catchment areas, and longer water renewal time. Correspondingly, a positive association between water temperature, possibly influencing stability of the water column, and *T. bourrellyi* biovolume has been reported (Nava et al., 2017). Previously, a strong link between ATX concentration and water temperature was reported for nine lakes in Italy (Cerasino and Salmaso, 2012). In this study, *anaC*/ATX were also found to be correlated to higher transparency (Fig. 4). Nava et al. (2017) suggested that solar irradiation in spring might favor *T. bourrellyi* growth more than the growth of *P. rubescens*. It has been argued that *T. bourrellyi* has less floating capability than *P. rubescens*, which is known to carry gas vesicles adapted to overcome hydrostatic pressure resulting from deep mixing events (D'Alelio et al., 2011).

Notably, 70 % of the biofilm samples from littoral zones of lakes revealed *anaC*, while *anaC* appeared much less frequently among river biofilms (5 %). Since biodiversity of cyanobacterial ASVs, as observed within biofilms among lakes (Salmaso et al., 2024), was observed to be much higher when compared to the rivers, it might be speculated that the chance of ATX production is increasing with lentic conditions. It might be argued that lentic lake habitats as sampled during this study allow for the development of richer benthic communities as opposed to lotic river habitats (Salmaso et al., 2024). This study adds to the pool of recent knowledge indicating that ATXs in benthic communities in Europe can be frequent which are sometimes associated with animal mortality (Bauer et al., 2022; Fastner et al., 2018; Cantoral Uriza et al., 2017; Faassen et al., 2012; Gugger et al., 2005). Overall, the concentrations of cyanotoxins observed in both plankton and biofilm samples were low (see 3.3 Cyanotoxin occurrence and structural variants). However, it is known that relatively low cyanotoxin concentrations in the water column can increase by several orders of magnitude due to accumulation at the surface and direct transport by wind towards the shoreline (Chorus and Welker, 2021).

As toxic cyanobacteria have been previously studied in some of the lakes included in this study, these results may also provide insight into longer-term trends in these lakes. For example, in 2005–2007, the *mcyB* gene fragments indicative of MC synthesis in *P. rubescens* have been quantified for 12 alpine lakes, i.e. Lake Mondsee and other alpine lakes in Austria, Germany and Switzerland, using depth-integrated sampling (Ostermaier and Kurmayer, 2010). According to this earlier survey, the average *mcyB* cell equivalent numbers ($n = 6$) ranged from 7 to 100 *mcyB* cell equivalents/mL (Wolfgangsee, Attersee, Schwarzensee, AT) to $1.4–2 \times 10^4$ *mcyB* cell equivalents/mL (Afritzersee, Wörthersee, AT; Hallwilersee, Zürichsee, CH). The lakes Mondsee (AT) and Ammersee (DE), which were also included in our study, together with some other lakes (Offensee, Irrsee, Fuschlsee, AT) reached 200–1500 *mcy*B cell equivalents/mL. In this study, between 13 and 800 *mcyE* cell equivalents/mL were observed in Lake Mondsee ($n = 13$), while the maximum of 1300 *mcyE* cell equivalents/mL occurred in Lake Bled (SI) (Fig. 4). Thus, the *mcyE* cell equivalents/mL observed in this study (2019–2020) are in the lower third of the range observed in 2005–2007. As the abundance of the *mcyB*/*mcyE* genes is mainly determined by the population increase or decrease of *P. rubescens* (Ostermaier and Kurmayer, 2010), continued measures to suppress the growth of *P. rubescens* will be crucial for the management of cyanobacterial blooms in the future.

3.3. Relationship between PCR and cyanotoxin abundance

The positive correlation between *mcyE* cell equivalent abundance and MC concentration (Fig. 4) is consistent with previous studies (e.g., Otten et al., 2015; Panksep et al., 2020) and implies that qPCR may be suitable for a quantitative risk estimation, as previously suggested (e.g. Gaget et al., 2017). For samples with inconsistency (Table 3), two explanations are proposed. First, false positives (genes were present and toxins were absent; more common in *mcyE*/MC), could be explained through an inactivated MC synthesis (Kurmayer et al., 2004). A previous study demonstrated that such mutants occur naturally and persist in Alpine lakes (Ostermaier and Kurmayer, 2009). Second, false negatives (genes were not detected but resulting toxins were detected; more common in *anaC*/ATX) might indicate a too high specificity of the PCR assay detecting only certain ATX-producing taxa, as suggested earlier (Sabart et al., 2015). For example, the PCR for *anaC* was designed from a conserved region inferred from comparing the *anaC* sequences from strains *Anabaena* sp. 37 and *Oscillatoria* sp. PCC 6506 (Rantala-Ylinen et al., 2011) and tested on different *Anabaena* and *Oscillatoria* strains, but not on other genera, such as *Phormidium* or *Aphanizomenon*, which are also common in the Alpine region (Salmaso et al., 2024).

According to the linear regression curve (Fig. 3) 10–100 cells of *mcyE* cell equivalents/mL result in 4.5–19.9 (± 1.3) ng/L of total MC concentration. If we assume that one toxigenic cyanobacteria cell has a biovolume of 45 μm^3 (i.e., *Planktothrix*), we can estimate that for 1 mm^3/L biovolume (or biomass) there is a MC concentration of 4.4–10 μg/L. These values are similar or slightly higher than estimates by Ostermaier & Kurmayer (2010) and WHO guidelines (Chorus and Welker, 2021; Table 5.2), which reported approximately 3.1–3.3 μg/L of MC-LR equivalent per 1 mm^3/L biomass. Thus, the observed quantitative *mcyE*/MC relationship from plankton samples of 29 Alpine lakes is comparable with published cyanobacteria biovolume/MC relationships and would constitute a first quantitative risk estimate based on qPCR methodology. Accordingly, 1 mm^3/L of *mcyE* cell biovolume will result in 4.4–10 μg/L of total MC, which might be considered complementary or an early warning for indicating cyanotoxin concentrations. In summary, this study shows that PCR results can be unspecific due to the presence of various amplification bands (Suppl. File S8), and it is important to note that even unclear PCR results (e.g., a ladder of products of similar intensity) do not exclude the presence of the target gene. Non-specific amplification has been observed before (e.g., Legrand et al., 2016). For clarification in such cases, effective follow-up methods could be qPCR (offering quantification) and PCR product sequencing (confirming the presence of target genes).

4. Conclusions

This study aimed to assess the distribution of potentially toxic cyanobacteria in Alpine lakes and rivers in both plankton and biofilm samples. It is concluded that:

- Within the study area *mcyE* genes and resulting MC occur most frequently in plankton, while ATXs and *anaC* genes are prevalent in both plankton and biofilm.

- End-point PCR results can be unspecific and in such cases the presence of the target fragment should be confirmed by PCR product sequencing.
- Both *mcyE* and *anaC* gene fragments are able to qualitatively predict MC or ATX occurrence.
- The *mcyE* cell equivalent concentrations can be applied to quantitatively predict MC concentrations in accordance with published biovolume/MC relationships.

We conclude that molecular methods provide an efficient approach for rapid screening of waterbodies for the presence of toxigenic cyanobacteria in both planktic and benthic habitats.

CRediT authorship contribution statement

Maša Jablonska: Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation, Conceptualization. **Leonardo Cerasino:** Writing – review & editing, Methodology, Investigation, Data curation. **Adriano Boscaini:** Writing – review & editing, Investigation, Data curation. **Camilla Capelli:** Writing – review & editing, Investigation, Data curation. **Claudia Greco:** Writing – review & editing, Project administration, Investigation. **Aleksandra Krivograd Klemenčič:** Writing – review & editing, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation. **Ute Mischke:** Writing – review & editing, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation. **Nico Salmaso:** Writing – review & editing, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation. **Rainer Kurmayer:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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

All PCR, and cyanotoxin data along with environmental metadata have been included in Suppl. Table 1. Other data will be made 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.2024.121783.

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