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Bloom-forming cyanobacteria, cyanotoxins and significant factors for their dynamics in freshwaters

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List of abbreviations and definitions

AERs – aeruginosins
Alien species – species dispersed outside its native range of distribution
APs – anabaenopeptins
ATX-a – anatoxin-a
bw – body weight
CPs – cyanopeptolins
Cyanotoxins – toxins produced by cyanobacteria
CYN – cylindrospermopsin
i.p. – intraperitoneal
IN – inorganic nitrogen
IP – inorganic phosphorus
LD_{50} –the amount of a material, given all at once, which causes the death of 50% of a group of test animals
MCs – microcystins
N – nitrogen
Native species – species observed naturally occurring during historical times
NRPs – bioactive non-ribosomal peptides
P – phosphorus
qPCR – quantitative real-time polymerase chain reaction
STX – saxitoxin
TN – total nitrogen
TP – total phosphorus

INTRODUCTION

Cyanobacteria are autotrophic gram-negative ancient prokaryotes that form blooms almost in all continents including Europe (Merel et al., 2010; Meriluoto et al., 2017). Cyanobacteria have competitive advantage over other algae in phytoplankton such as buoyancy regulation, colony formation, nitrogen fixation, etc., therefore, they are able to adapt to various environmental conditions, outcompete other microorganisms and consequently determine blooms in inland freshwaters (Carey et al., 2012).

Cyanobacteria species from over the forty genera have ability to produce various cyanotoxins (Carmichael et al., 2001). Worldwide blooms disrupt the aquatic ecosystems functioning and recognized as a major water quality management issue limiting water resource availability for drinking use, recreational purposes (Carmichael et al., 2001), cause economic losses (Ho et al., 2012) and posing serious health problems or even death to humans, wild animal and livestock (Buratti et al., 2017). Thus, the toxicity of bloom depends on cyanobacteria species and their biomass, number of toxic individuals in the population (Kardinaal et al., 2007), and the ability to produce toxic metabolites (Buratti et al., 2017). Seasonal and spatial changes in the composition of dominant cyanobacteria species and their biomass may also cause variation in structure of cyanotoxins and quantity in the bloom (Dolman et al., 2012).

Global challenges such as climate warming and anthropogenic eutrophication will primarily affect more vulnerable ecosystems such as shallow temperate lakes stimulating harmful cyanobacteria proliferation that aggravate management of freshwater aquatic ecosystems (Kosten et al., 2012). Increase of temperature and nutrient concentrations have been suggested as top drivers that shift cyanobacteria composition, amount and intensify bloom events and alien species establishment (Paerl and Huisman, 2008; Carey et al., 2012; Sukenik et al., 2015). Usually few cyanobacteria species dominate during bloom, and the population structure depends on individual species response to interaction of abiotic and biotic variables (Rigosi et al., 2014). Combined field and laboratory experimental studies have revealed more accurate predictions addressing ecological, physiological and molecular mechanisms of cyanotoxin accumulation in freshwaters that are of primary importance for providing a solid background for practical recommendations of bloom management (El-Shehawy et al., 2012; Humbert and Fastner, 2017).

Aim: To investigate diversity, biomass dynamics of potentially toxic bloom-forming cyanobacteria and cyanotoxins profile variations in response to environmental variables under field and laboratory experimental studies.

Objectives:

1. To investigate the diversity and biomass dynamics of potentially toxic bloom-forming cyanobacteria in two shallow eutrophic lakes.
2. To study qualitative and quantitative variations in cyanotoxins and bioactive non-ribosomal peptides during cyanobacteria vegetation period.
3. To identify cyanobacteria species responsible for cyanotoxin production and evaluate the proportion of toxic/non-toxic genotypes in the studied lakes.
4. To determine experimentally, the significance of temperature and nutrient availability on the growth of native and alien cyanobacteria strains, the contents of cyanotoxins and interspecies competition.

Defence statements:

1. Single or a few potentially toxic cyanobacteria species dominate and form intense blooms in summer and early autumn in shallow eutrophic temperate lakes.
2. High diversity of potentially toxic cyanobacteria species gives a broad profile of cyanotoxins and non-ribosomal peptides in eutrophic lakes, however, dominant species reflect prevalent toxic secondary metabolites.
3. Production of cyanotoxins and non-ribosomal peptides in cyanobacteria are species and/or strain specific.
4. Temperature and nutrients affect differently the growth rate, production of cyanotoxins, non-ribosomal peptides and interspecies competition of native and alien cyanobacteria.

Novelty of the work. The strength and novelty of this work is the interrelation of field and laboratory experimental studies on local cyanobacteria at strain level to investigate harmful blooms. Strains isolated just prior to the experiments retain eco-physiological characteristics similar to that in their lakes of origin, therefore, could give a reliable and more accurate answer how a particular ecosystem will be modified under various temperatures and nutrient enrichment conditions.

The study diminishes disproportionality of knowledge on cyanotoxins and non-ribosomal peptides in European freshwaters providing a profile of toxic secondary metabolites. Additionally, the data from the lakes sampled by standardized manner of snapshot survey performed across 28 European

countries allowed to compare profile of cyanotoxins with those in other 135 lakes of the continent. The current study contributes significantly to the data of scarcely examined saxitoxin and non-ribosomal peptides in the lakes and strains of cyanobacteria. It also provides novel data on distribution, productivity and ability of toxin production of recently expanded alien to Europe cyanobacteria species disclosing their competitive abilities with native species and possibility to establish into temperate lakes under changing environmental conditions.

The study substantially contributes to quantitative and qualitative data on insufficiently studied cyanotoxins of STX, ATX-a, CYN and NRPs in Lithuanian freshwaters. The presence of listed cyanotoxins and alien *Sphaerospermopsis aphanizomenoides* species in Lithuanian freshwaters was recorded for the first time. For the analysis of potential producers and cyanotoxins, a multiple methodical background including liquid chromatography with mass spectrometry (LC-MS/MS) as well as determination of *mcy*E synthetase gene copy number in field samples based on qPCR and examination of *mcy*E, *ana*C and *sxt*A synthetase genes in strains were applied. Up to 300 strains of potential toxic bloom-forming native and alien species were isolated and tested for their ability to synthesize cyanotoxins.

Relevance of the study. The study is focused on cyanobacteria blooms and cyanotoxins that pose significant threat to water quality, human health, leading to loss of ecosystem balance and biodiversity, also causing a costly problem in the water management and recreation sectors. Recent global warming aggravates anthropogenic eutrophication effect that subsequently intensifies blooms in freshwater ecosystems making predictions, control and management more complicated. The target of the current work is bloom-forming cyanobacteria and their cyanotoxins in shallow lakes of temperate zone the most vulnerable to climate and environment changes freshwater ecosystems. The combined effect of climate warming and eutrophication promoting blooms can be understood applying combination of field and laboratory experimental investigations that are the key point approach to solve the problem. The study of seasonal data on cyanotoxin composition and concentrations provides the information to the society on periods suitable for safe recreation in the particular lakes.

1. LITERATURE REVIEW

1.1. Cyanobacteria blooms and bloom-forming species in European freshwaters

The shallow eutrophic lakes in Europe are the most susceptible for cyanobacteria proliferation and commonly suffer from blooms in summer. With the reference to WHO (2003), cyanobacterial biomass from 2 to 10 mg L^{-1} in a water body ecosystem indicates low risk level, over 10 mg L^{-1} – medium, whereas beyond 12.5 mg L^{-1} reveals high alert level for bathing waters. Cyanobacteria in eutrophic lakes may comprise biomass of more than 70% of total phytoplankton (Gkelis and Zaoutsos, 2014) at amount reaching high alert level (Dembowska, 2011; Stoyneva-Gärtner et al., 2017a).

Blooms might consist of one dominating or comprise the complex of dominant and prevailing species (Dembowska, 2011; Toporowska et al., 2016). According to Buratti et al. (2017), species from *Microcystis* (blooms recorded in 15 countries), *Dolichospermum* (= *Anabaena*; in 13 countries), *Aphanizomenon* sensu lato (in 12 countries) and *Planktothrix* genera (in 10 countries) are commonly found as bloom-forming cyanobacteria along all the continent. Others have less frequent blooms, for example, *Cylindrospermopsis* are restricted to the warmer regions (Hungary, Italy, Portugal, Spain).

A global survey of the *Microcystis* (Chroococcales) species is characterized by widely recorded blooms in Europe and beyond in at least 108 countries (Harke et al., 2016). *Microcystis* ability to regulate buoyancy and form large colonies that can float fast (Ibelings et al., 1991) gives to species a clear benefit over non-migrating phytoplankton species under prolonged stratification (Paerl and Huisman, 2009; Carey et al., 2012). The most common bloom-forming species in freshwaters is *M. aeruginosa* (Harke et al., 2016), less frequent *M. viridis*, *M. flos-aquae*. Their co-occurence in blooms can reach high alert level for bathing waters (Kobos et al., 2013; Pitois et al., 2018).

Out of Oscillatoriales, the most common bloom-forming cyanobacteria belong to *Planktothrix* genus. *P. agardhii* is well adapted species to eutrophic shallow, mixing lakes in temperate zone particularly in the Northern Hemisphere (Scheffer et al., 1997), reaching biomass peaks during spring and autumn (Kurmayer et al., 2016). During the season, species reach on average 27.6–375.0 mg L^{-1} (Poulíčková et al., 2004; Grabowska et al.,

2014) with maximum biomass 11–605 mg L^{-1} in summer or autumn (Poulíčková et al., 2004; Papadimitriou et al., 2013; Grabowska et al., 2014).

The *Dolichospermum* genus from Nostocales also frequently occurs in eutrophicated freshwaters. The most often bloom-forming species are *Dolichospermum circinale* and *D. flos-aquae* (O'Neil et al., 2012). Other bloom-forming cyanobacterium *D. lemmermannii* has been reported from temperate zone and boreal latitudes (Komárek and Zapomelová, 2008; Lepistö and Holopainen, 2008) and has recently colonized subalpine Italian lakes (Callieri et al., 2014; Buratti et al., 2017). In England, *Dolichospermum* has been detected in 34% (Turner et al., 2018) and in France in 32% of the tested samples and the Nostocales biomass peak at 504 mg L^{-1} with dominant *D. flos-aquae* species (Pitois et al., 2018). Among other Nostocalean, species from the *Aphanizomenon* genus are geographically widespread and able to form blooms (Cirés and Ballot, 2016). *A. flos-aquae, A. gracile* and *Cuspidothrix issatschenkoi* are typical for temperate waters. *A. gracile* is often referred as dominant-prevailing species forming biomass in summer in the range 15–20.5 mg L^{-1} (Rücker et al., 2007; Kokociński et al., 2013).

1.2. Cyanotoxins and non-ribosomal peptides in European freshwater ecosystems

Toxic secondary metabolites (cyanotoxins and non-ribosomal peptides) are the biggest concern related to cyanobacteria blooms. Concentrations of cyanotoxins in the blooms vary considerably in space and time (Kardinaal et al., 2007; Sabart et al., 2010). Alterations of toxins composition and concentrations in the bloom depend on prevailing species and the proportion of toxic and non-toxic individuals in the population (Kardinaal et al., 2007; Sabart et al., 2010) also cyanotoxin cell quota in cyanobacteria and synthetize gene expression (Briand et al., 2008a; Neilan et al., 2013). Blooms can vary in levels of production of toxins and usually 40–70% are reported as toxic ones (WHO, 2003; Sivonen et al., 2009). Thirty years of studies related to the occurrence and production of cyanotoxins has not yet fully disclosed the causality why, when and which species would produce toxins, alone or in combination with other toxins (Pitois et al., 2018).

Cyanotoxins are classified based on toxicological target and chemical structure. According to toxicological target, cyanotoxins are classified into four classes: **1) hepatotoxins** that act on liver (microcystins and nodularin); **2) cytotoxins** that have both hepatotoxic and neurotoxic effects (cylindrospermopsin); **3) neurotoxins** that affect negatively nervous system

(anatoxins, saxitoxins); **4) dermatoxins** that cause irritation of skin, eyes, etc. (lypopolysaccharide, lyngbyatoxins and aplysiatoxin) (Sanseverino et al., 2017). Whereas, cyanotoxins fall into three groups by their chemical structures: **1) cyclic peptides** (microcystins and nodularin), **2) heterocyclic compounds** (alkaloids) (cylindrospermopsin, anatoxins, saxitoxins, lyngbyatoxins, aplysiatoxin) and **3) lipidic compounds** (lypopolysaccharide) (Sanseverino et al., 2017).

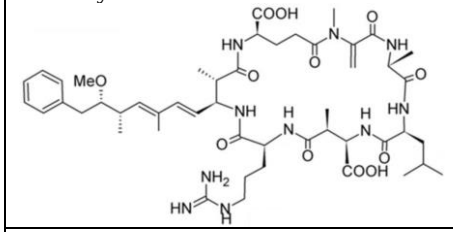
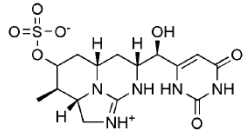
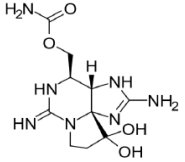
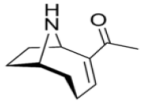
Most common and related to this study cyanotoxins and bioactive non-ribosomal peptides are discussed below.

MICROCYSTINS (hepatotoxins)

Structure and characteristics. Microcystins (MCs) are hydrophilic cyclic heptapeptides that are very stable in the environment due to cyclic structure that gives resistance to heat, hydrolysis and oxidation (De la Cruz et al., 2011) and has more than 246 MCs variants (Meriluoto et al., 2017). Toxicity between the variants differs from 50 μg kg^{-1} to 11 mg kg^{-1} (Puschner, 2018). For example, MC-LR is referred as the most toxic variant (Chorus and Bartram, 1999). WHO set the safe amount for human to ingest the MC-LR to be at 0.04 μg kg^{-1} per day (Kuiper-Goodman et al., 1999). As blooms contain different number of MCs variants, it is difficult to evaluate joint toxic potential effect of the bloom (Puschner, 2018). More detailed information about MCs is presented in Table 1.

Occurrence and concentrations. MCs are very common in all Europe; their effect on aquatic ecosystems and human health is better documented in comparison with other known cyanotoxins (Pantelić et al., 2013; Meriluoto et al., 2017). Concentrations of MCs mainly range from 1 to 10 μg L^{-1} (Greer et al., 2016; Pitois et al., 2018), however, in cyanobacteria scum it could reach even 427 μg L^{-1} (Turner et al., 2018). Total MCs concentration was extremely high (64000 μg L^{-1} or 1300 μg g^{-1} DW) in the pond from the Netherlands, where thick surface scum of *Microcystis aeruginosa* covered one-third of water body for many weeks (Lürling and Faassen, 2012). In Europe, MCs have been detected in 62–91% samples tested (Messineo et al., 2009; Dolman et al., 2012; Pitois et al., 2018). The MC-LR is the most often detected among other known MCs variants (Ho et al., 2012). The frequent variants are MC-RR and MC-YR (Zervou et al., 2017; Turner et al., 2018), while the MC-HilR, MC-WR and MC-LY are the least detected (Zervou et al., 2017).

Table 1. Chemical structure, producers, mode of action and toxic effect of four cyanotoxins (after Sanseverino et al., 2017; Huisman et al., 2018)

Toxin, chemical structure	**Main producing genera**	**Modes of action**	**Toxic effects**
Microcystins 	*Microcystis*, *Dolichospermum*, *Leptolyngbya*, *Nostoc*, *Phormidium*, *Planktothrix*, *Synechococcus*	Inhibition of eukaryotic protein phosphatases	Liver and kidney damage, gastroenteritis, tumour promotion, reduced DNA repair and reproductive toxicity
Cylindrospermopsin 	*Cylindrospermopsis*, *Dolichospermum*, *Aphanizomenon*, *Chrysosporum*, *Raphidiopsis*	Inhibition of protein synthesis, DNA damage and cell death	Damage to multiple organs, gastroenteritis and genotoxicity
Saxitoxins 	*Aphanizomenon*, *Cuspidothrix*, *Cylindrospermopsis*, *Dolichospermum*	Bloch voltage-gated sodium channels of neurons	Paraesthesia, numbness, paralysis and respiratory failure
Anatoxin-a 	*Dolichospermum*, *Aphanizomenon*, *Cuspidothrix*, *Oscillatoria, Phormidium*	Agonist of nicotinic acetylcholine receptors at neuromuscular junctions	Loss of coordination, muscle tremors and respiratory failure

CYLINDROSPERMOPSIN (cytotoxin)

Structure and characteristics. Cylindrospermopsin (CYN) is tricyclic guanidine alkaloid (Ohtani et al., 1992). This toxin is water-soluble and very stable under heat, pH and light (Chiswell et al., 1999). Studies have revealed that CYN toxicity LD_{50} is 200 µg kg^{-1} bw (Chorus and Bartram, 1999). More detailed information about CYN presented in Table 1.

Occurrence. In Europe, CYN was first time detected in German lakes in 2000 (Fastner et al., 2003), then in Hungary, Italy, Spain, Finland, France, the Czech Republic and Poland (Kiss et al., 2002; Manti et al., 2005; Quesada et al., 2006; Spoof et al., 2006; Bláhová et al., 2009; Brient et al., 2009; Kokociński et al., 2009; Pitois et al., 2018). In general, CYN is referred from tropical and subtropical waters (Pearson et al., 2010), but has been reported more frequent in temperate climate. Toxin has been detected mainly at concentrations up to 2–3 µg L^{-1} (Brient et al., 2009; Gkelis and Zaoutsos, 2014; Pitois et al., 2018) with the highest concentrations 9–126 µg L^{-1} (Bogially et al., 2006; Rücker et al., 2007).

SAXITOXINS (neurotoxins)

Structure and characteristics. Saxitoxins (STXs) are heterocyclic guanidines with at least 57 structural variants (Wiese et al., 2010). They are classified to: non-sulphated molecules (STX and neoSTX), mono sulphated (gonyautoxins-GTX), doubly sulphated (C-toxins) and decarbamoyl variants (Pereira et al., 2004). STXs are stable, soluble in water toxins (Jones and Negri, 1997). Under high temperature (e.g. boiling), variants transform into more toxic variants (Calado et al., 2019). STX is the most toxic congener with i.p. LD_{50} of 10 µg kg^{-1} bw of mice (Chorus and Bartram, 1999), following neoSTX with LD_{50} value at 65 µg kg^{-1} bw of mice has been indicated (Wolf and Frank, 2002). More detailed information about STX is presented in Table 1.

Occurrence. STXs have been recorded in Bulgaria, Denmark, Finland, France, Germany, Greece, Ireland, Italy, Norway, Poland, Portugal, Russia, Spain, United Kingdom and the Czech Republic (Kaas and Henriksen, 2000; Pomati et al., 2000; Pereira et al., 2004; Codd et al., 2005; Ballot et al., 2010a; Ledreux et al., 2010; Teneva et al., 2010; Kobos et al., 2013; Cirés et al., 2014; Gkelis and Zaoutsos, 2014; Jančula et al., 2014; Savela et al., 2015; Ballot et al., 2016; Chernova et al., 2017; Stoyneva-Gärtner et al., 2017b). STX has been recorded in samples from 11 to 14% of the tested lakes (Kaas and Henriksen, 2000; Rapala et al., 2005; Wörmer et al., 2011; Jančula et al., 2014; Pitois et al., 2018) with maximum 69% (Dolman et al.,

2012). The concentrations of cyanotoxin have been rather low at 0.01–2.5 µg L^{-1} (Wörmer et al., 2011; Gkelis and Zaoutsos, 2014; Stoyneva-Gärtner et al., 2017b; Pitois et al., 2018) with exceptions of 26.1 µg L^{-1} in Spain (Wörmer et al., 2011) and the maximum values of 1386.0 µg g^{-1} detected in scum in Russia (Chernova et al., 2017).

ANATOXIN-A (neurotoxin)

Structure and characteristics. Anatoxin-a (ATX-a) is a water-soluble neurotoxic alkaloid (Devlin et al., 1977) that is sensitive to environmental conditions and easily decompose or totally degrade to non-toxic compounds (Stevens and Krieger, 1991; James et al., 1998; Duy et al., 2000). The i.p. LD_{50} value for ATX-a in mice has been detected 375 µg kg^{-1} (Chorus and Bartram, 1999). More detailed information about ATX-a is presented in Table 1.

Occurrence. Anatoxin-a has been detected in freshwaters in Denmark, Germany, France, Ireland, Italy, the Netherlands, Poland, Portugal, Spain and the UK (Edwards et al., 1992; Furey et al., 2003; Gugger et al., 2005; Carrasco et al., 2007; Messineo et al., 2009; Osswald et al., 2009; Ballot et al., 2010b; Faassen et al., 2012b; Kobos et al., 2013; Pitois et al., 2018). ATX-a is quite frequent, but not as often detected in waterbodies as hepatotoxins, detected in 29–35% (Carrasco et al., 2007; Pitois et al., 2018) and maximum 57% of the samples (Dolman et al., 2012). The maximum concentration has been up to 6.4 µg L^{-1} (Bumke-Vogt et al., 1999). However, smaller amount of ATX-a is more usual in the European lakes up to 2.19 µg L^{-1} (Carrasco et al., 2007; Dolman et al., 2012; Toporowska et al., 2016; Pitois et al., 2018).

BIOACTIVE NON-RIBOSOMAL PEPTIDES (secondary metabolites of other potentially toxic cyanobacteria)

Structure, characteristics and target. In addition to cyanotoxins, cyanobacteria are able to produce various bioactive compounds, among these more than 60% are peptides (Chlipala et al., 2011). In general, cyanopeptides consist of cyclic and linear non-ribosomal peptides (Janssen, 2019). More than 500 cyanopeptides have been described additionally to already known toxin microcystins (Welker and von Döhren, 2006; Meriluoto et al., 2017). They are classified to five structural groups: cyclic depsipeptides (aeruginopeptins, anabaenopeptilides, cyanopeptolins, micropeptins, oscillapeptilides, oscillapeptins, planktopeptins), depsipeptides (microviridins), linear peptides (aeuginosins, microginins, spumigin), cyclic

peptides possessing an ureido linkage (anabaenopeptins, ferintoic acids, nodulapeptins, oscillamides) or a β-amino acid (nostophycin) (Geada et al., 2017). Cyanopeptolins, anabaenopeptins and aeruginosins are widely known as inhibitors of serine proteases in humans that are involved in blood coagulation (Kodani et al., 1999; Hanessian et al., 2006; Gademann et al., 2010; Kohler et al., 2014; Schreuder et al., 2016). Cyanopeptolins and anabaenopeptins inhibit pancreatic and leucocyte elastases (Bubik et al., 2008; Sedmak et al., 2008), whereas microginins are involved in inhibition of angiotensin-converting enzymes implicated in blood pressure regulation (Okino et al., 1993; Kodani et al., 1999). Cyanopeptolins and aeruginosins are widely known as trypsin and chymotrypsin activity inhibitors (Kodani et al., 1999; Hanessian et al., 2006; Gademann et al., 2010; Kohler et al., 2014). Cyanopeptides have been detected accumulating in fish, frogs, snails and mussel tissues (Gkelis et al., 2006). Aeruginosin 828A has been found to be toxic for crustacean *Thamnocephalus platyurus* with a LC_{50} value of 22.4 μM, which is slightly higher than the toxicity found for MCs (Kohler et al., 2014). Also, aeruginosin 828A inhibits thrombin (IC_{50} = 21.8 nM) and trypsin (IC_{50} = 112 nM).

Producing genera. Planktonic cyanobacteria from the *Microcystis*, *Dolichospermum*, *Nostoc*, *Planktothrix* genera are referred to be producers of wide variety of structurally different linear and cyclic peptides, particularly anabaenopeptins, aeroginosins, microgynins, microviridins (Welker et al., 2004; Sivonen and Börner, 2008).

Occurrence. There is concern that already known toxins very likely represent only some part of the number of bioactive molecules produced by cyanobacteria. For other potentially toxic compounds already identified (e.g. microviridins, microginins, cyanopeptolins) or non-identified cyclic peptides, very limited information is available to date not only for the Europe, but also worldwide (Buratti et al., 2017). High variability in the production of bioactive non-ribosomal peptides is usually recorded in *Microcystis* (Fastner et al., 2001) and *Planktothrix* (Kurmayer et al., 2016). Among the cyanopeptides, excluding MCs, the most structurally known compounds are cyanopeptolins (CPs) – 36%, microginins (MRs) – 14%, aeruginosins (AERs) – 13%, followed by cryptophycins and anabaenopeptins (APs) – 9% (Janssen, 2019). The studies have shown that cyanopeptides are frequently detected in lakes, rivers, estuaries, reservoirs in Finland, Greece, Israel, Italy, Poland, Portugal, and Switzerland (Rohrlack et al., 2009; Lifshits and Carmeli, 2012; Lopes et al., 2012; Ferranti et al., 2013; Grabowska et al., 2014; Gkelis et al., 2015). Five compounds from

anabaenopeptins, aeruginosin and planktocyclin have been presented in all field samples from water reservoir in Poland with pronounced blooms of *Planktothrix agardhii* (Grabowska et al., 2014). Pawlik-Skowrońska et al. (2019) have found that *Planktothrix agardhii* extract rich in anabaenopeptins, oscillamide, aeruginosins and MCs is more toxic to zooplankton than *Microcystis* spp. extracts containing mixture of anabaenopeptins, aeruginosamide, cyanopeptolins and MCs. *Dolichospermum flos-aquae* is referred as producer of anabaenopeptins (Harada et al., 1995). Those data strongly suggest that non-ribosomal oligopeptides, other than MCs, have essential contribution to the toxicity to invertebrates and their effects can vary depending on the cyanobacteria secondary metabolite profile.

1.3. Producers of cyanotoxins

Cyanobacteria bloom toxicity depends on the situation in a lake: variation in the dominant and prevailing potential cyanotoxin producers in space and time during vegetation period, the structure of toxic and non-toxic strains in a population (Kardinaal et al., 2007; Sabart et al., 2010). Species of the same genus differs in the ability to produce cyanotoxins and their spectrum (Bernard et al., 2017). *Microcystis aeruginosa*, *M. botrys*, *M. flos-aquae*, *M. viridis*, *M. wessenbergii* are known as bloom-forming species and except the latter one are worldwide known as MCs producers (Via-Ordorika et al., 2004; Bernard et al., 2017). MCs concentration may reach 15.7 mg g^{-1} in *M. aeruginosa* bloom and the biomass is highly toxic – IC_{50} 245 µg. *Microcystis* spp. is associated with the production of more than one variant of microcystins, e.g. MC-LR, -RR, -YR, -D-Asp3-LR, -D-Asp3-RR (Farkas et al., 2014; Pekar et al., 2016).

Planktothrix agardhii is also widely known as MCs producer (Yéprémian et al., 2007). *P. agardhii* produces various MCs variants such as [Asp, Mdha]-MC-RR, [Asp, Dhb]-MC-RR, [Asp]-MC-LR, [Asp]-MC-HtyR (Kosol et al., 2009). The maximum value 34.5 µg equiv. MC-LR L^{-1} has been detected in *P. agardhii* blooms in October in France, while lower concentrations of 2 µg equiv. MC-LR L^{-1} in summer months (Yéprémian et al., 2007). *Dolichospermum lemmermannii* produce microcystins (Capelli et al., 2017) and anatoxin-a(s) (Onodera et al., 1997). Other potential producers mostly belonging to the Nostocales such as *Chrysosporum bergii*, *C. ovalisporum*, *Dolichospermum planctonica*, *Aphanizomenon flos-aquae*, *A. gracile*, *Cylindrospermopsis raciborskii*, *Raphidiopsis curvata*, *R.*

mediterranea are recognized as CYN producers worldwide (De la Cruz et al., 2013). *Chrysosporum ovalisporum, Cylindrospermopsis raciborskii* have not been confirmed to produce CYN in Europe, however, able to produce analogue 7-epi-CYN (Rzymski and Poniedziałek, 2015). The 7-deocy-CYN has been detected in *Aphanizomenon* sp. isolated from German lakes (Rzymski and Poniedzialek, 2015; Cirés and Ballot, 2016).

Some cyanobacteria are able to produce more than one group of cyanotoxins. For example, based on strain analysis *Aphanizomenon gracile* has been proved as CYN (Kokociński et al., 2013) or STXs (Ballot et al., 2010a; Ledreux et al., 2010) producer. Similarly, *Cuspidothrix issatschenkoi* can synthesize ATX-a (Ballot et al., 2010b) or STXs (Nogueira et al., 2004). Potential producers are also considered species that have present cyanotoxin synthetase genes, but the toxin production has not been detected. For example, only STX synthetase gene has been detected in *Dolichospermum circinale*, *Sphaerospermopsis aphanizomenoides* (Ledreux et al., 2010), *Anabaenopsis elenkinii* and *Aphanizomenon flos-aquae* (Ballot et al., 2010a) isolates from Europe. *Dolichospermum flos-aquae*, *Aphanizomen flos-aquae* (Rapala et al., 1993), *Dolichospermum planctonicum* (Bruno et al., 1994), *Cuspidothrix issatschenkoi* (Wood et al., 2007) and *Raphidiopsis mediterranea* (Namikoshi et al., 2003) species are associated with the production of ATX-a. So, the composition of the species in the population is crucial for prediction of profile of cyanotoxins in the water body.

1.4. Potential risks related to exposure of cyanotoxins

The most severe human intoxications caused by cyanotoxins were recorded in Brazil in 1996, when 76 patients died due to MCs infected water used for dialysis (Carmichael et al., 2001). Moreover, over hundred human deaths attributable to cyanotoxins have been documented for Lake Embu in Kenya (Sanseverino et al., 2017). *Cylindrospermopsis raciborskii* bloom in 1979 containing cytotoxic CYN caused hepatitis like-illness for more than 100 Australian people (Hawkins et al., 1985). Human fatalities due to ingestion of STX-contaminated seafood (PSP poisoning) have been well documented and still occur with about 2000 intoxication cases yearly and a mortality rate of 15% estimated to occur globally in recent past (Testai et al., 2016). Numerous animal death incidences due to cyanotoxins have been recorded worldwide. Cyanotoxins were responsible for more than 100 water bird deaths in the USA (Wilde et al., 2005; Wiley et al., 2007) and sheep mortality in Australia due to STXs (Negri et al., 1995), the death of other

domestic animals (pigs, ducks and calves) has been reported due to ATX-a(s) (Patocka et al., 2011).

In Europe, the massive and lethal human intoxications have not been recorded to date or have not been associated with cyanotoxins. However, Turner et al. (1990) have reported pneumonia and other disease symptoms, which appeared in two army recruits after canoeing in lake during *Microcystis* bloom in Staffordshire (United Kingdom). In 2003–2004, negative effect on human health was reported after mass development of *Dolichospermum lemmermannii* that produced STX (Rapala et al., 2005). People in Sweden and the United Kingdom suffer from rashes, gastroenteritis, fevers, abdominal and muscular pain due to exposure to MCs produced by *Planktothrix* (Bláha et al., 2009). Stoyneva-Gärtner et al. (2017b) analysis has revealed general conformities between Bulgarian regions of cancer distribution and common occurrence of most dangerous cyanotoxins in water bodies of the country. Epidemiological studies in Serbia have shown the elevated occurrence of digestive tract and skin diseases in the population, where dmMC-RR and dmMC-LR contaminated water was supplied for drinking from blooming water reservoirs (Svirčev et al., 2017). Animal poisonings such as dogs due to ATX and/or homoanatoxin exposure have been found in Scotland (Edwards et al., 1992), France (Gugger et al., 2005), Ireland (James et al., 2005), the Netherlands (Faassen et al., 2012), intoxication also for dogs due to MC-LR (Lürling and Faassen, 2013) and cattle in Finland (Sivonen et al., 1990). The major fish kills associated with *C. raciborskii* toxic bloom have been observed in Serbia (Svirčev et al., 2016). STXs are likely to immense fish mortalities in European freshwaters (Moustaka-Gouni et al., 2017). Also, mass bird kills (Ibelings and Havens, 2008) in Danish lakes are known due to ATX-a(s) production by *Dolichospermum lemmermanii* (Onodera et al., 1997).

European Union has not yet well regulated concentration for cyanotoxins in drinking water (Sanseverino et al., 2017), therefore, only suggestions to alert value of 1 μg L^{-1} for CYN (Rodriguez et al., 2007), for STX – 3 μg L^{-1} (Fitzgerald et al., 1999) and for ATX-a 1 μg L^{-1} (Fawell et al., 1999) have been proposed. The World Health Organization has recommended guideline only for frequent MC-LR variant value up to 1 μg L^{-1} in drinking water (WHO 1998) and recreational waters 20 μg L^{-1} (WHO, 2003). Moreover, several cyanotoxins occur simultaneously in the bloom, and the synergistic effect most probably enhances the toxicity of the bloom (Chia et al., 2019). Therefore, it is highly necessary to know the entire profile of toxins in aquatic ecosystems.

1.5. Environmental variables most significant for cyanobacteria proliferation and cyanotoxin production

In recent decades, the frequency and intensity of harmful cyanobacteria blooms have increased in aquatic ecosystems and caused economic losses (Chorus and Bartram, 1999; Carmichael et al., 2001, Carmichael, 2008; Paul, 2008; Paerl and Huisman, 2008). These blooms are complex events and not a single, but various environmental factors contributed to their formation (Heisler et al., 2008). Nowadays, global warming and anthropogenic eutrophication are the biggest challenge and the greatest threats to ecosystems. According to Paul (2008) and Paerl and Huisman (2009), cyanobacteria will thrive under climate change. The response of cyanobacteria under changing temperature and nutrient conditions have been extensively studied, however, experimentally tested response of different species to a particular variable is still very limited (O'Neil et al., 2012).

1.5.1. Effect of temperature on cyanobacteria and cyanotoxins

Due to global warming, the rise of temperature is expected by 4°C close to 2100 (Brown and Caldeira, 2017). Moreover, it is likely that global warming will affect stronger northern hemisphere temperate zone (Stocker et al., 2013), thus, directly or indirectly affecting species development through more intense and longer heat waves (Meehl et al., 2007). Also, due to extended period of stratification in lakes (Wilhelm and Adrian, 2008), the availability of nutrients in surface water is going to be reduced favouring growth of cyanobacteria that have ability to regulate buoyancy to obtain nutrients from deeper layers (Paerl and Huisman, 2008; Paul, 2008; Liu et al., 2011). Therefore, most probably global temperature rise will induce the distribution, duration and intensity of cyanobacterial blooms (Paerl and Huisman, 2009; Paul, 2008) and shift the structure of phytoplankton (Winder et al., 2008). Cyanobacteria growth is favoured by higher temperatures compared to other prevailing algae in ecosystems such as diatoms, chrysophytes and cryptophytes (De Senerpont Domis et al., 2007). An eight-month mesocosm experiment conducted in Austria showed that 4°C temperature rise decreased the diversity of phytoplankton and pushed the community toward cyanobacteria dominance (Rasconi et al., 2017). Temperature can trigger appearance of intensive blooms, e.g. *Dolichospermum lemmermanni* (Callieri et al., 2014). That increase in temperature from 20°C to 27.5°C has shown at least twice higher growth rate

of *Dolichospermum* sp., *Aphanizomenon gracile*, *Cylindrospermopsis raciborskii*, *Microcystis aeruginosa*, *Planktothrix agardhii*, *Synechococcus elongatus* cyanobacteria strains (Lürling et al., 2013). However, response to temperature increase probably is species specific. *P. agardhii* is able to grow under a wide range of temperatures, even at 4°C, therefore, no direct effect of temperature on the species biomass has been determined (Toporowska et al., 2010).

Increased temperature triggers the shift from non-toxic to toxic strains in e.g. *Microcystis* (Davis et al., 2009; Dziallas and Grossart, 2011) and shows that global warming induces toxic potential of blooms. Concerning production of cyanotoxins, some experiments performed with the strains have indicated that mainly toxin-quota is influenced by changing temperature. Temperature experiments performed with *Microcystis* spp. have shown that there is no effect on MCs quota in *M. viridi*s (Song et al., 1998), whereas *M. aeruginosa* is less toxic at higher than at lower temperatures (Gianuzzi et al., 2016). Temperature influences the production of MCs variants by *Dolichospermum* spp. with enhanced concentration of MC-RR at more than 25°C and favours MC-LR synthesis at lower temperatures (De Figueiredo et al., 2004; Katırcıoğlu et al., 2004). CYN has been detected mainly in aquatic ecosystems of warmer regions worldwide. Optimal growth temperature for equatorial strain of *Cylindrospermopsis raciborskii* is 33°C, the highest for strains previously studied, and the maximum net CYN production rate is at the lowest growth rate 0.37 day^{-1} (Mohamed Nor et al., 2019). During the last decade, the toxin has been recorded in Europe, however, understanding of the factors affecting the production is very limited (Meriluoto et al., 2017). The tested *Cylindrospermopsis raciborskii* strains isolated from Australia have shown negative correlation with the temperature (Saker and Griffiths, 2000). The results of other studies have suggested that CYN biosynthesis is constitutive and not directly influenced by abiotic factors (Davis et al., 2014; Pierangelini et al., 2015).

Several studies have suggested that the maximum STX content and increased *sxt*A gene expression in *Aphanizomenon gracile* are at the highest 30°C tested temperature (Cirés et al., 2017), similarly, in other *Aphanizomenon* sp. strain toxin production has tripled from 11.7 to 34.6 fg STX equiv. $cell^{-1}$ with temperature increase from 22°C to 28°C (Dias et al., 2002). In contrast, STX production is stable under wide temperature range (15°C, 20°C and 28°C) in *A. gracile* strain (Casero et al., 2014). According to Harland et al. (2013) and Heath et al. (2014), the effect of physicochemical parameters such as temperature, light, nutrient and metal

concentrations on neurotoxin ATX-a synthesis in *Phormidium autumnale* is strain specific and not strongly supported by statistical analysis. Controlled experiments with the phytoplankton communities from lakes of different trophy at ambient (20°C) and warming (25°C) conditions with or without enrichment have revealed that warming alone induce the increase of cyanobacteria chlorophyll-*a* concentrations, but addition of the nutrients boosts the bloom (Lürling et al., 2018).

1.5.2. Effect of nitrogen and phosphorus availability on cyanobacteria and cyanotoxins

Anthropogenic eutrophication of freshwaters is the other significant driver stimulating the presence of harmful cyanobacteria blooms. Usually, the availability of P is considered as main limiting factor for cyanobacteria growth (Schindler, 2012). A critical TP concentration favouring proliferation of common bloom-forming cyanobacteria from the genera *Aphanizomenon*, *Dolichospermum* and *Microcystis* ranges from 70 to 215 µg L^{-1} (Wagner and Adrian, 2009). Furthermore, Downing et al. (2001) have predicted that cyanobacteria in temperate ecosystems dominate at TP values around 100–1000 µg L^{-1}. However, much less attention is given to nitrogen (Sterner, 2008). N availability is especially highly important in P enriched lakes. At high concentrations of P, the amount of P does not positively affect the total cyanobacteria biomass; however, their biomass still increases continually with rising concentration of N (Dolman et al., 2012). Total nitrogen concentrations exceeded 1.29 mg L^{-1} predetermine high cyanobacteria biomass (Wagner and Adrian, 2009). Many studies have shown that both P and N may control the blooms occurrence and intensity (Paerl et al., 2008; Paerl and Huisman, 2009), however, anthropogenically mediated changes in N:P ratio is the main driver explaining bloom magnitude and composition (Dolman and Wiedner, 2015). According to Klausmeier et al. (2004), the proliferation of cyanobacteria related to N:P ratio is species specific.

Different responses of cyanobacteria species to N and P exist, because diazotrophic cyanobacteria are able to fix gas nitrogen with possible advantage under N limiting conditions (Paerl et al., 2001), whereas non-diazotrophic bloom-forming cyanobacteria prevail under N surplus conditions (Gobler et al., 2007; Davis et al., 2010). Common potentially toxic cyanobacteria able to fix N_2 are species from the genera *Dolichospermum*, *Aphanizomenon*, *Cylindrospermopsis* and non-fixers are *Microcystis* and *Planktothrix* (Paerl and Huisman, 2009). However, the

hypothesis that potentially N_2-fixing Nostocales taxa would be favoured under low N relative to P conditions not always is consistent (Dolman et al., 2012). For example, both diazotrophic species act differently: *Aphanizomenon gracile* reach the highest biomass in lakes with high N:P ratio, however, *Aphanizomenon flos-aquae* prefer low N:P ratios (N-poor lakes) conditions (Dolman et al., 2012). It is known that many cyanobacteria can uptake and store N and P (Donald et al., 2013). For example, *Planktothrix agardhii* store surplus P as polyphosphate (Reynolds, 2006) and N as cyanophycin or phycocyanin (Van de Waal et al., 2010), so it is highly competitive species under limiting conditions.

Dolman et al. (2012) concluded that concentrations of all cyanotoxin groups increase in congruence with higher TP and TN amount and the toxin producers' biomass. Based on stoichiometric theory of phytoplankton toxins regulation (Van der Waal et al., 2014), nitrogen limitation causes a reduction in the cellular quota of N-rich toxins, while phosphorus shortage causes an increase in the most N-rich paralytic shellfish poisoning toxin.

Monchamp et al. (2014) have found that different nitrogen forms possibly influence cyanobacterial community structure leading to corresponding effects on MC concentrations and composition, thus, may influence the overall toxicity of blooms. Field data coupled with experiments show that at lower N concentrations non-toxic strain growth rate was higher than toxic one (Vézie et al., 2002), so blooms of *Microcystis* at lower inorganic N concentrations are less toxic (Welker et al., 2007; Davis et al., 2010). Also, it has been demonstrated that higher P concentrations enhance growth of toxic over non-toxic strain (Davis et al., 2010; Vézie et al., 2002). *Microcystis* as non-diazotroph increase the growth and toxicity under increasing N concentrations, showing positive relationships of MCs production with IN supply (Orr and Jones, 1998). MC concentrations are highest at toxic bloom phase of *Microcystis* that strongly correlates with high C:N ratios and total N:P ratios (Beversdorf et al., 2015). It has been shown that in excess of nitrogen supply there is an increase in MCs cell quotas (Van de Waal et al., 2009; Horst et al., 2014). Environmental factors seem to affect MC variants composition indirectly by determining particular variant dominance. Several studies on anatoxin production in *Aphanizomenon* sp. and *Cuspidothrix isstaschenkoi* strains have revealed that light intensity and nitrate concentration induce toxin concentrations (Cirés and Ballot, 2016).

Despite numerous researches conducted, cyanotoxin production in cyanobacteria and the variables that regulate their biosynthesis remains

unsolved. According to Wells et al. (2015), it is still insufficient evidence that temperature alone affects toxin production in cyanobacteria. Also, Lürling et al. (2017) have found that MC concentrations in *Microcystis aeruginosa* are much higher at the increased temperature and nutrient treatment than under warming alone. Most probably cyanotoxin variations might be regulated by multiple of environmental variables and be specific for the species.

1.5.3. Effect of light intensity on cyanobacteria and cyanotoxins

Light intensity is an important factor for primary producers, including cyanobacteria. The climate projections for high latitudes, particularly higher runoff and low dense clouds, will reduce irradiance availability and thus will favour low-light adapted species (Wells et al., 2015). Cyanobacteria usually dominate in eutrophic turbid ecosystems due to their adaptation to tolerate low light intensity (Paerl and Otten, 2016; Scheffer et al., 1997; Schwaderer et al., 2011). *Planktothrix agardhii* is a shade-tolerant species and usually outcompetes Nostocalean cyanobacteria in temperate freshwaters under low underwater light conditions (Wiedner et al., 2002). On the other hand, some cyanobacteria (e.g. *Microcystis*, *Dolichospermum*, *Aphanizomenon*) have the ability to tolerate high light intensity that let to harmful species thrive throughout the photic zone as well as in the surface (Wells et al., 2015). To acquire optimal light conditions, cyanobacteria species that possess the aerotops can regulate their position in water column and migrate upwards or downwards during the day (Huisman et al., 2005). For example, it has been experimentaly revealed that *Cylindrospermopsis raciborskii* reaches the maximum growth at 75 μmol m^{-2} s^{-1} light intensity (Briand et al., 2004; Dyble et al., 2006), but *Aphanizomenon* species has higher illumination preferences – around 100 μmol $m^{-2}s^{-1}$ (cit. after Mehnert et al., 2010). Sabour et al. (2009) found that sensitivity to light intensity is temperature dependent in *Microcystis ichthyoblabe* and *Sphaerospermopsis aphanizomenoides* strains.

Light intensity is able to shape toxic vs. non-toxic strains in population and production of cyanotoxins. According to Van de Waal et al. (2011), the growth of toxic *Microcystis aeruginosa* strain is greater at higher light intensity compared to non-toxic one. Tested growth of *Planktothrix agardhii* toxic and non-toxic strains does not show direct significant effect of the light (Briand et al., 2008b). However, the results with toxin synthesis are

equivocal as various species or strains act differently. For example, Wiedner et al. (2003) have found increased toxin production in *M. aeruginosa* at light-limited conditions, but Kaebernick et al. (2000) results are contrary as MCs synthesis in the same species is promoted by increasing radiation. Chaffin et al. (2018) results suggest that increase of light intensities from 3 to 300 μmol $m^{-2}s^{-1}$ enhance MC production in *Microcystis* and *Planktothrix* at elevated nitrogen concentrations. The highest STX production in *Cylindrospermopsis raciborskii* strain has been detected at 100 μmol $m^{-2}s^{-1}$ comparing to 50 and 150 μmol $m^{-2}s^{-1}$ (Carneiro et al., 2009). An increased transcription of *mcy* genes under 68 μmol m^2s^{-1} light intensity has been found, while the transcription rates become reduced under low light (16 μmol m^2s^{-1}) and dark conditions (Kaebernick et al., 2000; Kurmayer and Christiansen, 2009). Light intensity determines the production of particular microcystin variants (Tonk et al., 2005). The dmMC-RR variant in *P. agardhii* decreases two-fold, whereas dmMC-LR increases three-fold with more intensive light, but total intracellular microcystin concentration has not changed. Briand et al. (2008b) suggested that environmental factors indirectly affect toxic vs. non-toxic strains *P. agardhii* growth and production of MCs.

1.5.4. Importance of abiotic and biotic variables to interspecies competition

Competition is important regulatory process that affects the phytoplankton assemblages' structure and dynamics in lakes; however, information based on experiments with intermixed species is very limited up to date. Long-term (eight months) mesocosm experiment showed that induced increase of water temperature from ambient level significantly decreased phytoplankton diversity and pushed the community towards the dominance of only a few species, and also a shift towards cyanobacteria dominance (Rasconi et al., 2017). Ryan et al. (2017) have tested temperature (25°C and 30°C) and phosphorus (1 μmol L^{-1} and 25 μmol L^{-1}) effect on the species competitive success and dynamics in mixed phytoplankton communities. *Cylindrospermopsis raciborskii* dominate the communities under low-phosphorus conditions, but remain rare in high-phosphorus conditions, showing that temperature plays a negligible role influencing community composition.

Several experiments have been performed using cyanobacteria strains to test their response to set environmental conditions and/or to test species

competition abilities. Due to lower requirements to phosphorus, *Microcystis aeruginosa* is the best competitor followed by *Planktothrix agardhii* compared to diatom *Cyclotella meneghiniana* at both tested 18°C and 30°C temperatures (Gomes et al., 2015). Similarly, Lürling et al. (2013) have not found significant differences in growth under optimum temperatures for cyanobacteria species (*Microcystis aeruginosa*, *Planktothrix agardhii* and *Cylindrospermopsis raciborskii*) and green algae at tested temperatures in the range of 20–35°C. Monocultures of *C. raciborskii* and *Planktothrix agardhii* grown under different temperatures and light intensity, have revealed that *P. agardhii* growth rates are significantly higher 15°C and 20°C and at low light intensity (60 µmol photons m^2 s^{-1}), but *Cylindrospermopsis raciborskii* grows significantly faster at 25°C and high light intensity (135 µmol photons m^2 s^{-1}) (Bonilla et al., 2012). Experiment testing of co-culture has shown that *C. raciborskii* either dominates or is displaced by *Microcystis aeruginosa* depending on light intensity and phosphorus concentrations (Marinho et al., 2013). The eighteen-day experiments with mixed cultures of *Aphanizomenon flos-aquae* and green algae *Ankistrodesmus falcatus* conducted at four temperatures (14, 19, 24, and 29°C) have indicated that a slightly higher water temperature than the growth threshold value is needed for *A. flos-aquae* to outcompete *A. falcatus* (Yamamoto and Nakahara, 2006).

De Nobel et al. (1997) experiments with continuous cultures have shown that *Dolichospermum* sp. has a higher maximum growth rate, greater affinity for phosphorus and higher N_2 fixation activity than *Aphanizomenon flos-aquae* strain. *Dolichospermum* sp. is the superior competitor for P in competition experiments on the basis of monoculture measurements. Tan et al. (2019) have observed fast growth of *Microcystis aeruginosa* in monoculture with elevated P concentrations (≥ 0.05 mg P L^{-1}); however, the species is strongly inhibited in co-cultures with *Synechococcus* sp.

Secondary metabolites of cyanobacteria may be involved in interspecies competition. An allelopathic effect is likely to play a role in driving the seasonal alteration of dominant cyanobacteria species (Ma et al., 2015). Ma et al. (2015) have found that among five tested strains of *Microcystis*, *M. ichthyoblabe* and *M. aeruginosa* exhibit the strongest inhibitory effects on *Aphanizomenon flos-aquae*. Co-culture filtrate has stronger effect than monoculture filtrates suggesting that the allelopathic effect of some *Microcystis* strains is inducible. Similarly, co-culture of *Microcystis aeruginosa* with *Planktothrix agardhii* have resulted in the reduction of the growth of *Planktothrix* and morphological changes in the filaments (Briand

et al., 2019). The production of intracellular compounds by *Microcystis* increases, but by *Planktothrix* decreases if to compare between mono and co-culture conditions, indicating that co-culture induces specific compounds for the interspecies competition. Rzymski and Poniedziałek (2014) have found that CYN concentrations of 1 and 5 μg L^{-1} slightly decrease the growth rates of *Microcystis aeruginosa*, but significantly up-regulate alkaline phosphatase activity; however, the toxin concentrations of 10 and 50 μg L^{-1} induce the toxicity effects demonstrated by significant growth inhibition and *M. aeruginosa* cell necrosis. Also, under both CYN concentrations MC-LR production by *Microcystis* strongly decreases. Co-culturing of species has resulted in an increase of *Cylindrospermopsis raciborskii* contribution at the expense of *Microcystis aeruginosa.*

1.6. Potential risk to aquatic ecosystems due to alien cyanobacteria species spread and establishment in Europe

Climate warming is expected to cause changes in aquatic habitats and species composition (Kovats et al., 2014). It predicts the shift in species distribution on continental-scale that promotes expansion and introduction of invasive or alien species with a high migration rates from outside of Europe. Introduction of new species in the habitats will lead to changes in aquatic communities and biodiversity (Sukenik et al., 2015). As cyanobacteria toxin production is depending on species, so changes in composition, could lead also to changes in toxin concentration and their diversity.

To date, seven alien nostocalean cyanobacteria species to the European freshwaters are known (Kokociński et al., 2017a) and three have already been recorded in Lithuanian lakes: *Cylindrospermopsis raciborskii*, *Raphidiopsis mediterranea*, *Chrysosporum bergii* (referred as *Anabaena minderi* var. *limnetica*) (Kasperovičienė et al., 2005; Koreivienė and Kasperovičienė, 2011; Kokociński et al., 2017b). *Cylindrospermopsis raciborskii* is widespread invasive species originated from tropical and subtropical regions (Sukenik et al., 2012). Warming favours recent spread of species to northern Europe as well (Antunes et al., 2015). Nevertheless, *Cylindrospermopsis raciborskii* was recorded in Lake Jieznas three decades ago (Kavaliauskienė, 1996), the species remains in the same lake at very low biomass 0.02 mg L^{-1} (Kokociński et al., 2017b). Due to difficulties in identification, some authors have suggested that young filaments of *Cylindrospermopsis raciborskii* are misidentified as *Raphidiopsis mediterranea* (Moustaka-Gouni et al., 2009). It is believed that *R.*

mediterranea originated from the Mediterranean region and is referred as invasive alien cyanobacteria species to temperate region (Kaštovský et al., 2010). *Chrysosporum bergii* extended its distribution northwards in Europe and now is considered as an alien species in the Czech Republic, Slovakia, Germany, Poland. Lithuania is known as the northernmost point of distribution so far (Koreivienė and Kasperovičienė, 2011; Kokociński et al., 2017a). *Raphidiopsis mediterranea* and *Chrysosporum bergii* alien species have been detected in Lake Gineitiškės (Lithuania) comprising up to 3.5 mg L^{-1} and 0.26 mg L^{-1} July, respectively (Kasperovičienė et al., 2005; Koreivienė and Kasperovičienė, 2011).

To date, the ecology and production of cyanotoxins of those invasive species have been insufficiently studied. Meriluoto et al. (2017) have highlighted that in further surveys not only native toxic cyanobacteria in Europe should be assessed, but also cyanotoxin producers among alien or invasive species. Distribution and toxin production should not be overlooked of such alien species as *Chrysosporum bergii*, *C. ovalisporum* or *Sphaerospermopsis aphanizomenoides*. Germany and Poland have been reported as the northern occurrence points of *Sphaerospermopsis aphanizomenoides* (Stefaniak and Kokocinski, 2005; Stüken et al., 2006); moreover, recently the species have established there and tend to dominate in phytoplankton (Maileht et al., 2013). According to Budzyńska and Gołdyn (2017), *S. aphanizomenoides* biomass strongly depends on high water temperature and high concentration of phosphates. Also, due to climate change, temperature will favour success of *Cylindrospermopsis raciborskii*; however, it is still not clear, which individuals, toxic or non-toxic, will be most favoured (Funari et al., 2012).

1.7. Cyanobacterial blooms and cyanotoxins in Lithuanian freshwater ecosystems

Freshwater cyanobacteria blooms in Lithuania have been reported since the beginning of the 20th century. Dominant cyanobacteria species, their diversity, density and biomass have been described as a part of total phytoplankton in numerous hydrobiological and floristic studies. The earliest record on *Microcystis flos-aquae*, *Aphanizomenon flos-aquae*, *Oscilatoria princeps* bloom in the River Nemunas observed in 1922 and 1932 was published by Valionis (1936). An overview of prevailing and bloom-forming cyanobacteria species in the country has been provided by Jankavičiūtė (1996). Vitėnaitė (2001) has compiled the checklist of cyanobacteria species

detected in Lithuania for the period 1791–2001. The most comprehensive study of more than hundred Lithuanian lakes has been performed by Kavaliauskienė (1996). The study has noted that cyanobacteria contribution into phytoplankton assemblages in eutrophic and hypertrophic vary from 50.2 to 96.0% and reaches the maximum biomass up to 56.29 mg L^{-1} during summer. Moreover, up to 20 potentially toxic species are listed. Among dominant bloom forming species, *Planktothrix agardhii* (in 35 lakes), *Dolichospermum flos-aquae* (in 33), *Microcystis aeruginosa* (in 23), *Aphanizomenon flos-aquae* (in 17) are mentioned most frequently (Kavaliauskienė, 1996). Other studies also have revealed that species from the genera *Dolichospermum*, *Aphanizomenon*, *Microcystis* and *Planktothrix* mainly form blooms in shallow lakes (Jankavičiūtė, 1962, 1963, 1986; Kasperovičienė, 2001). The shift of phytoplankton structure has been detected in Lake Simnas, cyanobacteria from the *Aphanizomenon*, *Dolichospermum*, *Aphanocapsa* genera (Kasperovičienė, 2007) have replaced diatoms, dinophytes and cryptophytes common in the period 1952–1986 (Markevičienė, 1962; Kavaliauskienė, 1996).

Cyanobacterial blooms have been well documented in the Curonian Lagoon. Ūselytė (1959, 1961,) described intensive development of *Aphanizomenon flos-aquae* and *Microcystis* spp. half a century ago. Pilkaitytė (2003) has found that salty water and increased temperature positively affect filamentous cyanobacteria in the lagoon. Temperature and nitrogen limitation are the major drivers determining the composition and spatial extent of hyper-blooms of cyanobacteria in the Curonian Lagoon (Bartoli et al., 2018). Those blooms occur during high water temperatures, long water residence time and low-wind conditions. Temperature increases resulting from climate warming have likely caused changes in phytoplankton communities during two decades (1990–2008) and cyanobacteria also have become abundant earlier in the season in the Curonian Lagoon (Jaanus et al., 2011). *Planktothrix agardhi* has replaced *Aphanizomenon flos-aquae* as the most abundant cyanobacterium and currently both species frequently build up to 70 mg L^{-1} biomass (Jaanus et al., 2011; Anne et al., 2015). Satellite-based remote sensing has been tested, validated and suggested to complement conventional water quality and bloom monitoring in the Curonian Lagoon (Giardino et al., 2010; Vaičiūtė et al., 2012).

Since 2004, the combined assessment of cyanotoxins and phytoplankton composition has been started in the blooming freshwater ecosystems in Lithuania. Cyanotoxins and microcystins have been determined for the first time in Lake Gineitiškės by using enzyme-linked immunosorbent assay

(ELISA) by Kasperovičienė et al. (2005). According to the studies of Kasperovičienė et al. (2005) and Kasperovičienė (2008), in nine lakes and the Curonian Lagoon, up to 18 potential toxic species mainly prevailed by *Aphanizomenon flos-aquae*, *Microcystis* spp. (*M. flos-aquae*, *M. viridis* and *M. wessenbergii*), *Dolichospermum lemmermanii* have been revealed. The biovolume of potentially toxic species varies from 0.5 mg L^{-1} (Lake Arinas) to 65 mg L^{-1} (the Curonian Lagoon), whereas total MCs concentration assessed using ELISA is from 0.20 μg L^{-1} (Lake Gineitiškės) to 2.62 μg L^{-1} (the Curonian Lagoon).

A comprehensive study on cyanotoxins in the Curonian Lagoon has been provided by Paldavičienė et al. (2009, 2015). Five microcystin variants (MC-LR, MC-RR, MC-LY, MC-YR and dmMC-RR) and nodularin were detected in the lagoon in 2006–2007 using ELISA and liquid chromatography–mass spectrometry (LC-MS/MS). Most common was MC-LR (in 75% of the samples) varying in concentration from 0.1 μg L^{-1} to 134.2 μg L^{-1}. Microcystins MC-RR (up to 30.71 μg L^{-1}) and MC-YR (up to 20.27 μg L^{-1}) were characteristic to the western part of the lagoon. DmMC-RR and the most toxic MC-LY variants were present only in one sample at concentration of 7.5 μg L^{-1} and 0.61 μg L^{-1}, respectively. High concentrations reaching 284.6 μg L^{-1} of hepatotoxin nodularin were found in the Curonian Lagoon. No neurotoxins, cytotoxins and dermatotoxins were found in the study. Šulčius et al. (2015a) have found that MC-LR is present at the highest concentration in *Microcystis aeruginosa* dominated samples, while the dominance of *Planktothrix agardhii* is associated with the occurrence of dmMC-RR as the major microcystin variant. Concentration of total MCs in the scum material was higher (153.60 μg L^{-1}) compared to bloom samples (0.51–4.95 μg L^{-1}), however, STX, ATX-a or CYN were not detected in the analysed cyanobacterial bloom and scum samples.

Paldavičienė et al. (2015a) also have assessed the accumulation of cyanotoxins in hydrobionts of the Curonian Lagoon and showed large zebra mussels (MCs up to 284.06 ng g^{-1} DW) and larger individuals of the roach (MCs up to 196.44 ng g^{-1}). Lesutienė et al. (2018) have found that cyanotoxin concentrations in liver of the pikepeach are significantly higher relative to muscle tissues (means 0.55 ± 0.27μg g^{-1} DW *vs*. 0.19 ± 0.09 μg g^{-1} DW, respectively) and exceed the recommended concentrations for safe long-term human consumption (i.e. 0.28 μg g^{-1} DW). Also, the muddy bottom sediments are the secondary repository of cyanotoxins in the Curonian Lagoon (Paldavičienė et al., 2015b).

In 2010, the collection of pure cultures of algae and cyanobacteria was established in Lithuania. The collection is deposited at the Nature Research Centre (Koreivienė et al., 2016). This led to use the local freshwater cyanobacteria strains for the various purposes such as molecular and chemical analysis, examination of cyanotoxins for experimental purposes. The strains isolated from annually blooming in the Curonian Lagoon non-toxic *Aphanomenon flos-aquae* (2012/KM1/D3 and 2012/KM1/C4) were among the first studied (Šulčius et al., 2015b).

So, in Lithuania, numerous studies related with the assessment of cyanobacteria diversity or blooms have been performed. However, the data on cyanotoxins, especially in the inland aquatic ecosystems, are very scarce and almost have never been tested at the cyanobacteria strain level. To date, STX, ATX-a, CYN and NRPs have not been detected in Lithuanian aquatic ecosystems.

2. MATERIALS AND METHODS

The object of the study was bloom-forming cyanobacteria and production of cyanotoxins and non-ribosomal peptides (NRPs).

Determination of physico-chemical parameters, chl-*a* and phytoplankton analysis, isolation and maintainance of cyanobacteria strains as well as all experiments were carried out at the Nature Research Centre (Lithuania). Analysis of secondary metabolites (structure of cyanotoxins, quantitative analysis and NRPs evaluation in field, experimental material and strain biomass using LC-MS/MS) and molecular analysis (sequencing of 16S rRNA, PC-IGS and *sxt*A in the biomass of *Aphanizomenon* sensu lato strains) were performed at the Division of Marine Biotechnology under supervision of Prof. Dr. Hab. H. Mazur-Marzec (University of Gdańsk, Faculty of Oceanography and Geography, Poland). Cyanotoxin analysis in field samples and molecular analysis (qPCR analysis in the environmental samples, *mcy*E, *anaC* gene detection in cyanobacteria strains) were performed at the Department of Food and Environmental Sciences under supervision of Prof. K. Sivonen (University of Helsinki, Finland).

2.1. Study area

The study was carried out in two shallow eutrophic water bodies: Lake Širvys (54° 59' 16.27", 25° 12' 54.13") situated in eastern Lithuania and Lake Jieznas (54° 35' 33.67", 24° 10' 48.95") – in the southern part of Lithuania (Fig. 2.1.). Worldwide known, that shallow lakes due to low depth and inefficient buffer mechanisms are sensitive ecosystems with limited resilience to climate change and posibilities to maintain lower trophic level. Catchment area of the studied polymictic lakes is dominated by agricultural land (comprise 48%–84%), therefore, inflow of nutrients from the watershed to the lakes strengthen phosphorus loading from the sediments due to anoxia that periodically occur near the bottom (Balevičius, 2009). Meanwhile, Lake Širvys is with the same extent surrounded by natural biotopes for 47%, and possibly, therefore, the lake has lower nutrient concentrations compared to Lake Jieznas. According to Balevičius (2009), Lake Širvys is assigned as problematic and Lake Jieznas is reffered as lake with critical conditions due to the past (intensive farming close to water bodies, agriculture) and present pollution events (farming and urbanization). Lake Širvys is characterized by frequent blooms, while Lake Jieznas experience perrenial blooms. Fish deaths are recorded in both lakes. Lakes are situated in the close proximity to

villages and are the only waterbodies used for recreation and fishing, thus water quality of the lakes is very important for the local communities.

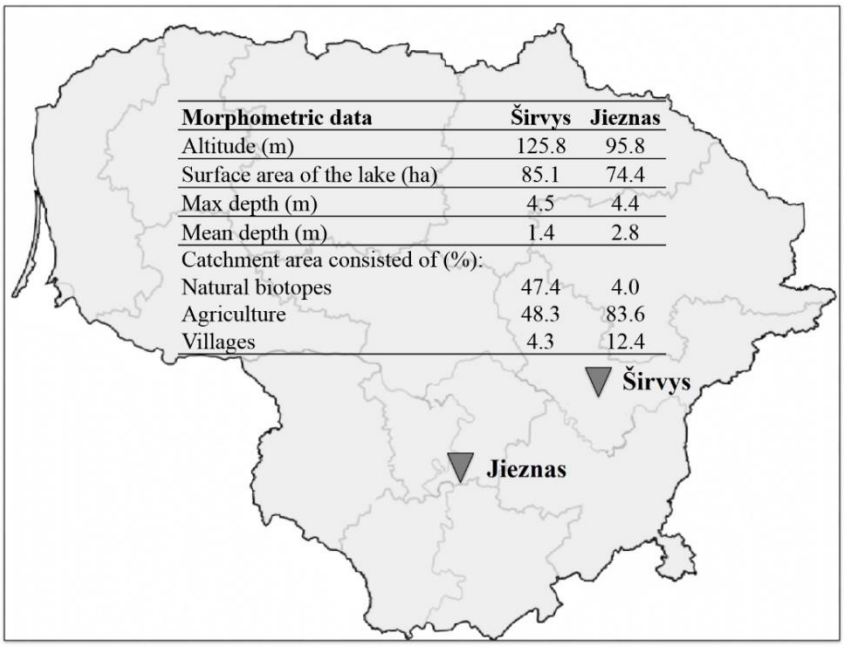


Fig. 2.1. Location and morphometric characteristics of the studied lakes and land use of their catchment area (Balevičius, 2009).

2.2. Sampling and phytoplankton analysis

Surface water sampling and phytoplankton analysis. Samplings were performed during the ice-free period from April to November in Lake Širvys (2014–2015, biweekly) and Lake Jieznas (2015, monthly). Water samples were collected using a Ruttner sampler from the surface (0.1 m) layer in the deepest part of the lakes. Water temperature, pH and conductivity were measured *in situ* using a portable multiline meter F/Set-3 with selective electrodes. Illumination was determined using a light meter LI-250A and water transparency using a Secchi disc. Evaluation of dissolved oxygen was performed according to Winkler titration method (Strickland and Parsons, 1968). Chlorophyll-*a* (chl-*a*) was evaluated using a fluorometer AlgaeLabAnalyser. Phosphorus and nitrogen and their forms were determined in accordance with standard methods (LST ENISO 10304; LST EN ISO 14911; ISO 8245:2003) performed by „Vandens tyrimai“. Air temperature and precipitation data of Vilnius Meteorological Station were

provide by Lithuanian Hydrometeorological Service under the Ministry of Environment.

Surface water samples (1 L of volume) were analysed to assess phytoplankton bloom dynamics and cyanobacteria seasonal succession. Samples for the analysis (n=34) were preserved with formaldehyde solution with the final concentration of 4%. Species identification and counting was performed in Nageotte chamber using light microscope. At least 600 counting units per sample were estimated (Olenina et al., 2006). The biomass was calculated from cell number and mean of cells' volumes of the species estimated using formulae of geometric shapes as described in Olrik et al. (1998) and Olenina et al. (2006). Cyanobacteria species were identified and classified based on morphology after Komárek and Anagnostidis (1998; 2005), Komárek and Komárková (2006), Komárek (2013).

Water column sampling for phytoplankton and cyanotoxins. To reveal the differences of cyanobacteria biomass and cyanotoxin profile in the water column of Lakes Širvys and Jieznas, the one per lake integrated sample (n=2) was added to the surface water sampling in August 2015. Integrated sample of water column from the surface to 0.5 m above the bottom was collected using the constructed long tube-like device of the 43 mm diameter. Lake water was pulled-out from the sampler and evenly mixed in the bucket, then divided to the bottles for further processing and analysis. For phytoplankton analysis, 0.5 L of the collected lake water was fixed with Lugol's solution and investigated by Utermöhl's method (Utermöhl, 1958) using inverted microscope. Chl-*a* was evaluated using a fluorometer AlgaeLabAnalyser. For cyanotoxins analysis, 100–200 ml of lake water was filtered through GF/F filters that were stored at -20°C and later centrally analysed in dedicated laboratories. Analysis of MCs was performed at the University of Wageningen, whereas CYN and ATX analyses at the German Environment Agency. The detailed methodological information is provided in Mantzouki et al. (2018a). The samplings and analysis were performed by fully standardized manner for snapshot survey across the Europe by implementing European Multi Lake Survey (EMLS) project. Integrated samples allowed the comparison of the profile of cyanotoxins from Lake Širvys and Lake Jieznas with the other thirteen lakes in Lithuania and 359 lakes all over the Europe, sampled at the similar time period (Mantzouki et al., 2018b).

2.3. Isolation and maintenance of cyanobacteria strains

Strains were isolated in order to reveal presence of cyanotoxins and/or synthetase genes in cyanobacteria species, and for performing the experiments. Monoculture isolation was performed by micropipette and washing method from the surface water samples collected using a plankton net (20 µm mesh size). The cultures were maintained in modified MWC medium (Lebret et al., 2012) at 20°C, 30 µmol m^{-2} s^{-1} using cool white fluorescent illumination and 12:12 day:night regime. Isolation of strains (15 species, 274 strains) was mainly focused on potential cyanotoxin (MCs, STX, ATX-a and CYN) producers (Table 2.1). Identification of isolates to species level was performed based on morphology according to Komárek and Anagnostidis (1998; 2005), Komárek (2013) and genetic analysis.

Table 2.1. Isolated cyanobacteria strains of potential hepatotoxins, cytotoxin and neurotoxins producers.

Species	Number of strains	Lake
Microcystis aeruginosa	8	Širvys, Jieznas
Microcystis flos-aquae	4	Širvys, Jieznas
Microcystis viridis	5	Jieznas
Microcystis wessenbergii	5	Jieznas
Anabaenopsis cf. *elenkinii*	2	Jieznas
Aphanizomenon flos-aquae	3	Širvys
Aphanizomenon gracile	63	Širvys, Jieznas
Cuspidothrix issatschenkoi	5	Jieznas
Dolichospermum crassum	41	Širvys
Dolichospermum lemmermannii	3	Širvys
Raphidiopsis mediterranea	2	Širvys
Sphaerospermopsis aphanizomenoides	12	Jieznas
Limnothrix planktonica	7	Jieznas
Planktolyngbya limnetica	9	Širvys, Jieznas
Planktothrix agardhii	105	Širvys, Jieznas
Total:	**274**	

2.4. Analysis of cyanotoxins and non-ribosomal peptides

Cyanotoxins and NRPs were analysed in cyanobacteria material collected from lakes' surface water layer (n=34) and in the biomass of isolated strains (n=57). The lakes surface water (150–350 ml, depending on the phytoplankton density) was filtered through GF/F filters and freezed at -20°C prior the analysis. Cyanobacteria cultures were concentrated by centrifugation at 8000 rpm for 6–12 min., supernatant was removed and the biomass was freeze-dried. The extraction of microcystins (MCs) and NRPs, anatoxin-a (ATX-a) and cylindrospermopsin (CYN) was performed using 75% methanol in MiliQ water. The saxitoxin (STX) was extracted with a mixture containing 4 mM ammonium formate buffer (pH 3.5) and acetonitrile (95:5, v/v) at a ratio of 2:3. All samples were vortexed for 5 min. and maintained for 5 min. in a bath sonicator (Sonorex, Bandelin, Berlin, Germany). The filters of the field samples prior to bath sonication were homogenized for 1 min. with ultrasonic disrupter HD 2070 Sonopuls (Bandelin, Berlin, Germany). The extracts were centrifuged at 10 000 rpm for 20 min. and the supernatant was transferred to chromatography vials. The analysis was performed using liquid chromatography tandem with mass spectrometer LC–MS/MS (QTRAP5500, Applied Biosystems, Siex; Concorde, ON, Canada) equipped with a turbo ion spray ionization, operating in positive mode as described in Grabowska and Mazur-Marzec (2011), Grabowska et al. (2014) and Chernova et al. (2017). Data were analysed using Analyst QS® 1.5.1 software.

2.5. Microcystin (*mcy*E) gene copy number evaluation in field samples

The purpose was to assess *Planktothrix agardhii mcy*E synthetase gene copy number in field samples and relate it to dynamics of MCs production and *P. agardhii* biomass.

DNA extraction. For detection of *Planktothrix* genus-specific *mcy*E gene copy number in the phytoplankton biomass from Lake Širvys was collected biweekly during April–October in 2014 on GF/F filters and stored at -70°C (n=13). The DNA was extracted following PowerWater®DNA Isolation Kit according to manufacturer protocol. The filters were inserted into the beat-beating tube with 1 ml of Solution PW1 and heated at 65°C for 10 min., then shaked for 30 s at speed 5 m s^{-1} with FastPrep instrument (Savant Instruments). Samples were centrifuged twice for 1 min. at 4000 rpm and

13000 rpm. The 200 µl of Solution PW2 was added to the sample incubated at 4°C for 5 min. and centrifuged at 13000 rpm for 1 min. Supernatant was transferred to a clean tube with 650 µl of Solution PW3, vortexed briefly and into the tube with Spin Filter that was centrifuged at 13000 rpm for 1 min. The DNA on the Spin Filter was several times washed with PW4 and centrifuged. In the last step, the Spin Filter basket was placed into a clean tube and 50 µl of Solution PW6 was added to the center of the filter, centrifuged at 13000 rpm. The extracted DNA was stored at -20°C.

Detection of *mcy*E gene specific for *Planktothrix* in the field samples. The quantification of *mcy*E gene copy number was performed using quantitative real-time PCR (qPCR) analysis. The PCR one reaction mix amount was in total 25 µl volume, containing: 5 µl DNA, 1.25 µl each primer (300 nM) and 12.5 µl ready-to-use reaction mix prepared according to the manufacturer's instructions SsoAdvanced™ SYBR® Green Supermix (Biorad). One × SsoAdvanced reaction mix contained dNTPS, Sso7d fusion polymerase, MgCl2, SYBR® Green I, stabilizers. The forward primer mcyE-F2 (Vaitomaa et al., 2003) and reverse primer mcyE-plaR3 specifically designed to *Planktothrix* by Rantala et al. (2006) were used. The PCR was performed in 96 Well Skirted PCR Plate (4titude) using device Bio-Rad CFX96. The PCR protocol was performed according to Rantala et al. (2008) with modifications: preincubation step at 95°C, 3 min., denaturation 40 cycles 95°C, 10 s and annealing at 59°C, 45 s, melting curve analysis 95°C, 15 s, 58°C, 30 s, 95°C, 5 s. Each environmental sample was performed in triplicate, negative and positive control, also external standard dilutions.

2.6. Molecular analysis of cyanobacterial strains

The identification of *Aphanizomenon* sensu lato strains using molecular analysis of partial 16S rRNA and PC-IGS was performed due to morphological similarities between species.

***Aphanizomenon* sensu lato strains analysis.** 16S rRNA and PC-IGS analysis was performed for 22 strains belonging to *A. gracile, A. flos-aquae, Sphaerospermopsis aphanizomenoides* (Table 2.2). The amount of 30–40 ml of each strain culture was centrifuged at 8000 × *g* for 6–12 min. and freezed at -80°C. The DNA was extracted using FastDNA™ Spin Kit for Soil (MP Biomedicals, Santa Ana, CA, USA) according to the manufacturer's protocol (modifications: the samples were vortexed for 3 min. instead of 40 s FastPrep instrument) and stored at -20°C. The total amount of PCR was 25

µl contained of 30 ng of genomic DNA, 5 pmol of each forward and reverse primer and 12.5 µl MyTaq™ Red Mix (Bioline Reagents Ltd, London, UK).

Table 2.2. Sequences accession number of the isolated cyanobacteria strains from Lithuanian lakes.

Species	Strain	Lake	Genetic analysis		
			GenBank accesion No.		
			sxtA	PC-IGS	16S rRNA
Aphanizomenon gracile	NRC_JIE/D3-07	J	MG436994	MG553887	MG569712
	NRC_JIE/D5-09	J	MG436995	MG553889	MG569713
	NRC_JIE/F5-09	J	MG436996	MG553890	MG569714
	NRC_JIE/E5-09	J	MG436997	MG553893	MG569715
	NRC_JIE/E3-09	J	–	MG553886	MG569708
	NRC_JIE/C3-08	J	–	MG553888	MG569709
	NRC_JIE/D4-09	J	–	MG553891	MG569710
	NRC_JIE/E3-07	J	–	MG553892	MG569711
	NRC_SIR/E5-09	Š	MG436998	MG553900	MG569721
	NRC_SIR/B41-09	Š	MG436999	MG553903	MG569720
	NRC_SIR/F2-09	Š	MG437000	MG553904	MG569722
	NRC_SIR/G22-09	Š	MG437001	MG553905	MG569723
	NRC_SIR/C31-09	Š	MG437002	MG553902	MG569726
	NRC_SIR/E6-09	Š	MG437003	MG553894	MG569725
	NRC_SIR/D6-09	Š	MG437004	MG553895	MG569724
	NRC_SIR/D5-09	Š	–	MG553897	MG569716
	NRC_SIR/E10-07	Š	–	MG553898	MG569717
	NRC_SIR/C10-07	Š	–	MG553899	MG569718
	NRC_SIR/G5-09	Š	–	MG553901	MG569719
Aphanizomenon flos-aquae	NRC_SIR/G6-07	Š	–	MG553906	MG569728
	NRC_SIR/D6-07	Š	–	MG553907	MG569729
Sphaerospermopsis aphanizomenoides	NRC_JIE/D10-07	J	–	MH379240	MG569730

Abbreaviations: J – Lake Jieznas; Š – Lake Širvys

Partial 16S rRNA. For this present work, 16S–23S rRNA fragment with internal transcribed spacer (ITS) using forward CYA359F (Nübel et al., 1997) and reverse 23S30R (Lepère et al., 2000) primers was amplified. The PCR protocol performed according to Koskenniemi et al. (2007) and

modified: 94°C, 5 min; 35× (94°C, 45 s; 59°C, 45 s; 68°C, 45 s) and 72°C, 7 min.

PC-IGS. Amplification of the phycocyanin *cpc*B-*cpc*A intergenic spacer (PC-IGS) and flanking coding regions were performed using PCβF and PCαR primers and PCR thermal cycling conditions with minor modifications (number of cycles was reduced to 35) (Neilan et al., 1995).

Partial *sxt*A gene. In order to examine the presence of the saxitoxin synthetase gene, the partial *sxt*A gene in 22 strains using sxtaf and stxar primers was amplified. For amplification, PCR protocol with minor modifications (initial denaturation 95°C, 2 min.) was used (Ballot et al., 2010a). Thermal cyclings were performed in a Mastercycler® nexus GSX1 (Eppendorf, Hamburg, Germany).

All PCR products were visualized by 1% 1 × TBE agarose gel electrophoresis, stained with SYBR® Green I nucleic acid gel stain (Sigma-Aldrich). The amplified products of 16S rRNA, PC-IGS and *sxt*A were purified with Extractme® DNA clean-up Kit (Blirt S.A., Gdańsk, Poland) according to manufacturer's protocol and sequenced (Genomed S.A., Warszawa, Poland) using previously described forward primers. Sequences were edited by BioEdit software 7.2.5 (Hall, 1999) and FinchTV version 1.4.0. software (Geospiza). Nucleotide sequences were submitted to the GenBank database under accession no. described in Table 2.2.

***mcy*E or *ana*C gene analysis.** Strains were examined in order to check the presence of *mcy*E or *ana*C genes. The cultures of *Planktothrix agardhii*, *Microcystis* spp. and *Dolichospermum crassum* (141 strains) at exponential phase were centrifuged at 8000 rpm for 6–12 min. and freezed at -70°C. DNA was extracted using E.Z.N.A SP Plant DNA Kit for strains according to the manufacturer's instructions. To verify if the samples contained enough cyanobacterial DNA, the primers CYA359F, CYA781R(a) and CYA781R(b) were used for analysis following PCR protocol described in Nübel et al. (1997) for cyanobacteria rRNA detection with minor modifications: 94°C, 3 min.; 35 × (94°C, 30 s; 56°C, 30 s; 72°C, 1 min.); 72°C, 10 min. The specific primers (Rantala et al., 2006; Vaitomaa et al., 2003) for microcystin *mcy*E gene detection were used in *P. agardhii*, *Microcystis* spp. strains, whereas anatoxin-a synthetase gene *ana*C according to Rantala-Ylinen et al. (2011). The amplified products were separated on a 1% TAE agarose gel, stained with ethidium bromide.

2.7. Phylogenetic analysis

Phylogenetic trees were constructed using MEGA software 7.0 (Kumar et al., 2016) by maximum likelihood (ML) statistical method with 1000 bootstrap replicates. The best-fit of substitution model was assessed for *sxt*A gene T92 (Tamura, 1992) and K2 + G (Kimura, 1980) for concatenated 16S rRNA and PC-IGS sequences. Sequences were concatenated using Mesquite 3.51 software.

2.8. Experimental approach

Three types of experiments were carried out to assess the impact of global warming (temperature experiment) and anthropogenic eutrophication (nutrient experiment) on cyanobacteria growth, cyanotoxins and NRPs production and species competitive abilities (competition experiment). The experiments were performed with four strains of native *Planktothrix agardhii*, *Aphanizomenon gracile* and four strains of alien *Sphaerospermopsis aphanizomenoides*, *Chrysosporum bergii* filamentous cyanobacteria species isolated from four Lithuanian lakes (Table 2.3.). Strains were selected based on previous screening for cyanotoxins (MCs, CYN, ATX-a and STX) and NRPs. Among the selected species, two strains of native species were producers of toxins: the strain NRC_SIR/F5-09 of *P. agardhii* produced MCs and the strain NRC_SIR/B41-09 of *A. gracile* synthesized STX. All experiments were performed at the day cycle (16:8 light:dark) and light intensity (~90 µmol m^{-2} s^{-1}; white fluorescent lamps) in controlled growth chambers. Illumination condition was selected based on light preferences of native *P. agardhii* (Torres et al., 2016) and *A. gracile* (Mehnert et al., 2010) species. Cultures were grown in triplicates in Erlenmeyer flasks of 100 ml volume. The growth rate was evaluated based on chlorophyll-a (chl-*a*) as a proxy of biomass using a fluorometer AlgaeLabAnalyser every other day. The tested strains were re-isolated before the experiments to have pure cultures and to ensure low density of bacteria (< 1%). An initial concentration 10 ± 0.5 µg chl-*a* L^{-1} per strain was used for all treatments which reflected pre-bloom conditions that are according to WHO (2003) < 10 µg chl-*a* L^{-1}. Culturing flasks were manually mixed once a day during the experiment period. At the end of the experiments I and II, each treatment triplicate (n=3) were mixed, centrifuged at 7000 rpm and freeze-dried. The obtained material was used to evaluate cyanotoxins and

NRPs amount according to the above described methodology for strain analysis.

Table 2.3. Cyanobacteria strains selected for the experiments

Species		Strain	Lake	Cyanotoxins and NRPs
Native	*Planktothrix agardhii*	NRC_SIR/F5-09	Širvys	MCs, NRPs
		NRC_JIE/E9-07	Jieznas	–, NRPs
	Aphanizomenon gracile	NRC_SIR/B41-09	Širvys	STX, –
		NRC_SIR/C10-07	Širvys	–
Alien	*Sphaerospermopsis aphanizomenoides*	NRC_JIE/G11-07	Jieznas	–
		NRC_JIE/F11-07	Jieznas	–
	Chrysosporum bergii	NRC_REK/D2-08	Rėkyva	–
		NRC_GIN/B6-08	Gineitiškės	–

2.8.1. Experiment I: Temperature effect

Fourteen day experiment was performed to examine temperature effect on growth of cyanobacteria strains and production of secondary metabolites (toxins, NRPs). Seven different temperatures ranging from 18°C to 30°C with intervals of 2°C were selected for the experiment (Fig. 2.2.). The lower temperatures 18°C and 22°C correspond to the range of average water temperature in temperate lakes during summer. The other temperatures were selected to evaluate global warming impact on native and alien species response. The cultures were maintained in MWC medium and acclimated to each of the 7 tested temperatures for two days prior the experiment.

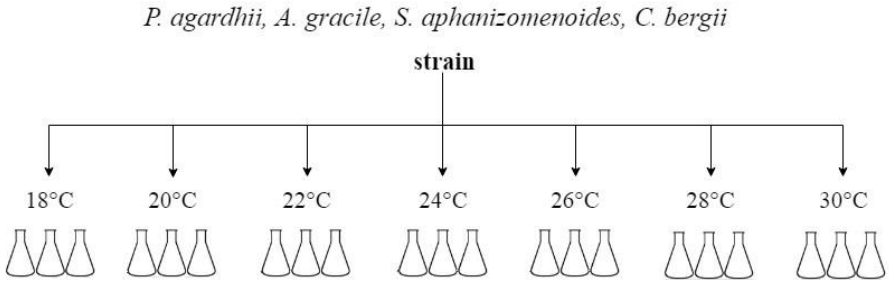


Fig. 2.2. Schematic design of the experiment to test effect of temperature on growth rate and production of cyanotoxins, NRPs of cyanobacteria strains.

2.8.2. Experiment II: Nutrients effect

This experiment was carried out to examine effect of inorganic nitrogen (IN) and phosphorus (IP) concentrations, their atomic ratio (N:P ratio) on growth rate of strains as well as on production of cyanotoxins and NRPs. Phosphorus was added as $KHCO_3$ at five different concentrations characteristic of temperate lakes of various trophy were selected according to Wetzel (1983): concentration 0.035 mg P L^{-1} corresponded to mesotrophic lakes; concentrations 0.071 and 0.140 mg P L^{-1} – eutrophic; 0.255 and 0.51 mg P L^{-1} – hypertrophic (Fig. 2.3.). Nitrogen was added in $NaNO_3$ form to the treatments according to N:P atomic ratio of 7:1; 16:1 and 30:1. Control treatments contained MWC medium (N:P atomic ratio was 20:1 with 1.55 mg P L^{-1}) to ensure cyanobacteria viability at standard conditions. The experiment lasted 12 days. Before the experiment, all cyanobacteria strains were maintained for 3 days in MWC medium free of N and P elements at 24°C temperature in order to starve the cultures.

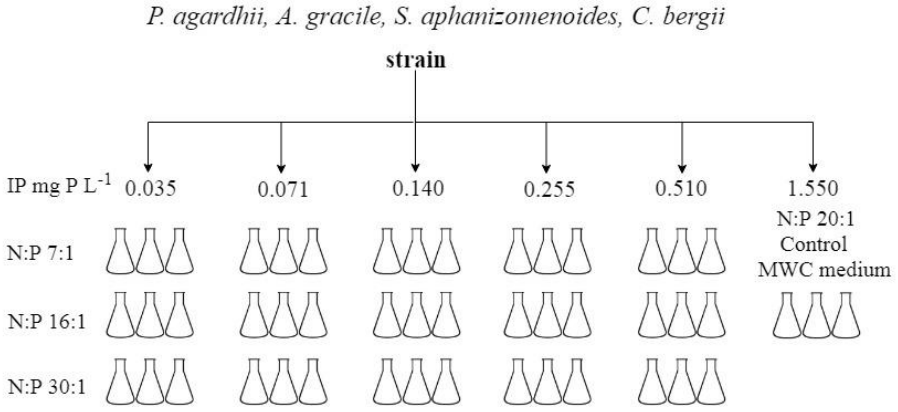


Fig. 2.3. Schematic design of the experiment to test the effect of nutrients concentration on the growth rate and production of cyanotoxins, NRPs of cyanobacteria strains.

2.8.3. Experiment III: Interspecies competition

This type of experiment was carried out to assess native species competitive properties and their ability to cope with alien species establishment under current climate conditions and to predict global warming as well as eutrophication scenarios. Four strains, one of each tested species, were co-cultured in pairs for 12 days for the evaluation of their biomass changes (Fig. 2.4.). Two temperature regimes were chosen, 20°C representing average

summer temperatures and 24°C reflecting possible temperature rise. For the growth medium, two concentrations of P (0.140; 0.51 mg P L^{-1}) at N:P ratio of 30:1 were tested. Cultures of strains at final concentration approximately 10 ± 0.5 µg chl-*a* L^{-1} were mixed as equal parts for the final treatment. For the control treatments, each strain was grown separately, but in the same medium and selected temperatures. Prior the experiment, the strains were maintained for three days in MWC medium free of N and P elements at respective temperatures. The aliquot of 1 ml was removed from each treatment every fourth day and preserved with formaldehyde at the final concentration of 4%. Toxic strains of native species of *P. agardhii* (NRC_SIR/F5-09) and *A. gracile* (NRC_SIR/B41-09) were co-cultured together and separately in one experimental setup with alien species *S. aphanizomenoides* (NRC_JIE/F11-07) and *C. bergii* (NRC_GIN/B6-08) strains. Cyanobacteria biomass was obtained by counting not less than 100 units (1 unit – 100 µm of the filament) using Nageotte chamber with a light microscope.

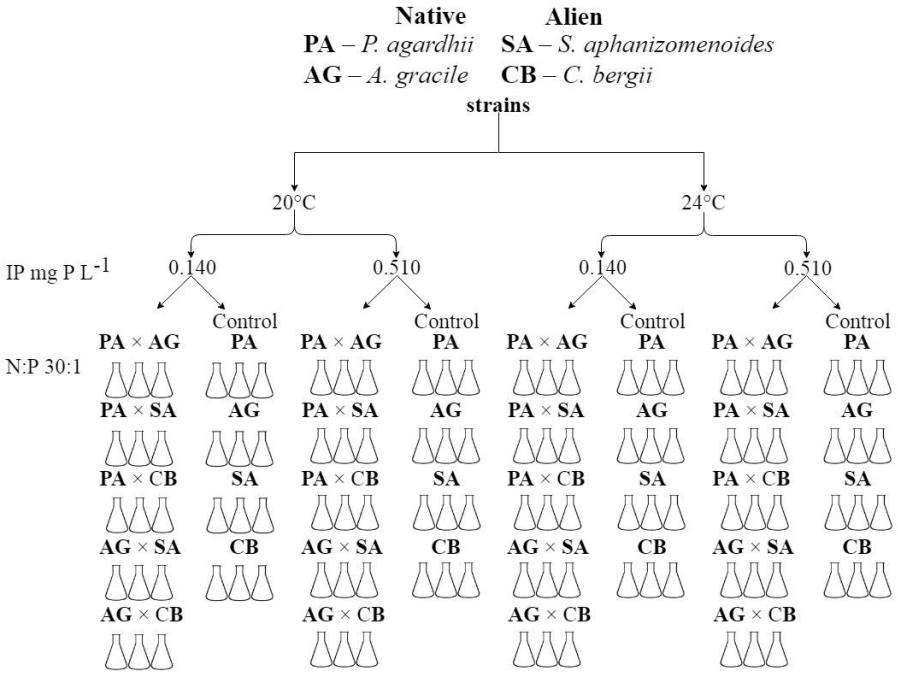


Fig. 2.4. Schematic design of interspecies competition experiment.

2.8.4. Evaluation of growth rate of cyanobacteria strains

The growth rate (μ) was calculated for the culture at the exponential growth phase based on chl-a values measured using a fluorometer AlgaeLabAnalyser. The growth rate was calculated according to equation:

$$\mu = \ln(N\_t - N\_0)/\Delta t,$$

where N_0 and N_t – chl-a values at the beginning and the end of the exponential growth phase, and Δt is the period of the exponential phase expressed in days (Guillard, 1973).

2.9. Statistical analysis

Redundancy analysis (RDA) was applied to assess relationships between the cyanobacteria species biomass, cyanotoxins, NRPs amount and abiotic environmental variables in the studied lakes. The data were square root transformed. General linear model (GLM) analysis was performed in the experiments to reveal significant effect of the tested factors (temperature, nutrients, species origin, alien species, etc.) and their interactions on growth rate or biomass of the tested strains. The linear regression was performed to assess the relationship between abiotic factors and cyanobacteria biomass, the concentration of secondary metabolites. Statistical data analysis was processed using STATISTICA 6.0 and Brodgar 2.7.5. softwares.

3. RESULTS

3.1. Environmental variables in the studied lakes

Cyanobacteria growth and proliferation is dependent on changes in environmental variables in the aquatic ecosystems, especially temperature and nutrients. In general, both studied lakes could be assigned to eutrophic type water bodies with high total phosphorus, nitrogen and chl-*a* concentrations, also low water transparency during the summer (Table 3.1.). Surface water temperature in summer was similar in both lakes – 21.0–21.5°C on average. The highest temperature up to 26°C was detected in August. Secchi depth, TP, TN, atomic ratio of IN:IP (N:P) and nitrates were those variables showing the highest difference between the lakes. Secchi depth in Lake Jieznas was twice (mean 0.55 ± 0.10 m) lower compared to Lake Širvys. Surface water illumination during the study period varied over the wide range in Lake Širvys from 16 to 366 μmol m^2 s^{-1} and Lake Jieznas from 35 to 225 μmol m^2 s^{-1}. The illumination was highly dependent on particular wheather conditions on the day of sampling.

The average nutrient concentrations in Lake Jieznas (TP 0.059 ± 0.026 mg P L^{-1}, TN 1.84 ± 0.3 mg N L^{-1}) were up to twice higher compared to Lake Širvys (TP 0.034 ± 0.012 mg P L^{-1}, TN 1.23 ± 0.3 mg N L^{-1}). In Lake Širvys, NO_3 prevailed (at average 0.672 ± 0.20 mg N L^{-1}) over NH_4^+ (0.129 ± 0.13 mg N L^{-1}) during spring, whereas ammonium comprised higher concentrations in summer–autumn period (0.072 ± 0.07 mg N L^{-1}) than nitrate (0.008 ± 0.02 mg N L^{-1}). In Lake Jieznas, ammonium was dominant among IN forms comprising on average 0.045 ± 0.04 mg N L^{-1} during all the vegetation season. The average N:P ratio in Lake Jieznas (22.8 ± 37.2) was 2–4-fold lower than in Lake Širvys.

3.2. Seasonal variation in diversity and total biomass of cyanobacteria and algae

Cyanobacteria in phytoplankton of the studied lakes were most important during all the studied period and comprised up to 98% in Lake Širvys (2015) and 87% of the total phytoplankton biomass in Lake Jieznas (Fig. 3.1.). Maximum total biomass of cyanobacteria was similar in both lakes (up to 29.2 mg L^{-1} in Lake Širvys and up to 24.7 mg L^{-1} in Lake Jieznas). Cyanobacteria species belonged to the orders Chroococcales, Nostocales and Oscillatoriales, but the community composition differed considerably during

the vegetation period. In Lake Širvys, the dominant cyanobacteria were from the order Oscillatoriales (maximum biomass 28.3 mg L^{-1}), while in Lake Jieznas, species from the Nostocales (maximum 14.0 mg L^{-1}) and the Oscillatoriales (maximum 10.5 mg L^{-1}) prevailed. The highest biomass of cyanobacteria in the studied lakes was observed from July to September/October. In Lake Širvys, cyanobacteria formed two biomass peaks – first in late July and second in September, whereas only one summer peak in August was observed in Lake Jieznas (Fig. 3.1). Species from the other algal taxonomic groups usually form low biomass during the vegetation season. Maximum biomass of green algae (Chlorophyta) was 4.3 mg L^{-1} in September and 7.6 mg L^{-1} in July in Lake Širvys and Lake Jieznas, respectively. Bacillariophyta, Chrysophyta, Cryptophyta together constituted up to 4.1 mg L^{-1} in June and 2.52 mg L^{-1} in September, in Lakes Širvys and Jieznas, accordingly.

Table 3.1. Physicochemical and hydrological variables in Lakes Širvys and Jieznas during the studied period. The data are presented with mean ± SD

Variable	**Širvys**		**Jieznas**
	2014	**2015**	**2015**
Air temperature average, °C*	13.0 ± 5.4	12.7 ± 5.4	13.9 ± 5.3
Air temperature maximum, °C*	18.2 ± 6.2	17.9 ± 6.4	19.4 ± 5.8
Precipitation, mm*	59.3 ± 29.3	48.1 ± 28.9	43.0 ± 28.2
Water temperature, °C	17.6 ± 5.5	16.8 ± 5.2	16.9 ± 5.3
pH	8.3 ± 0.3	8.3 ± 0.3	8.3 ± 0.2
Conductivity, µS cm^{-1}	444.1 ± 7.2	445.4 ± 8.5	441.7 ± 10.6
Secchi disc, m	1.15 ± 0.60	1.30 ± 0.70	0.55 ± 0.10
Dissolved oxygen, mg L^{-1}	10.4 ± 2.0	10.5 ± 2.8	10.9 ± 1.5
Surface illumination, µmol m^{-2} s^{-1}	155.7 ± 115.3	129.9 ± 87.8	119.9 ± 82.3
Total chl-*a*, µg L^{-1}	35.8 ± 17.0	34.5 ± 15.8	61.4 ± 11.2
TP, mg P L^{-1}	0.034 ± 0.012	0.035 ± 0.017	0.059 ± 0.026
IP, mg P L^{-1}	0.011 ± 0.004	0.011 ± 0.003	0.014 ± 0.005
TN, mg N L^{-1}	1.25 ± 0.2	1.23 ± 0.3	1.84 ± 0.3
IN, mg N L^{-1}	0.33 ± 0.4	0.17 ± 0.3	0.12 ± 0.2
NH_4^+, mg N L^{-1}	0.148 ± 0.1	0.043 ± 0.0	0.045
NO_3^-, mg N L^{-1}	0.180 ± 0.4	0.128 ± 0.2	0.003 ± 0.0
N:P, atomic ratio	80.3 ± 103.8	43.8 ± 70.7	22.8 ± 37.2

* Data provided of Vilnius Meteorological Station by Lithuanian Hydrometeorological Service

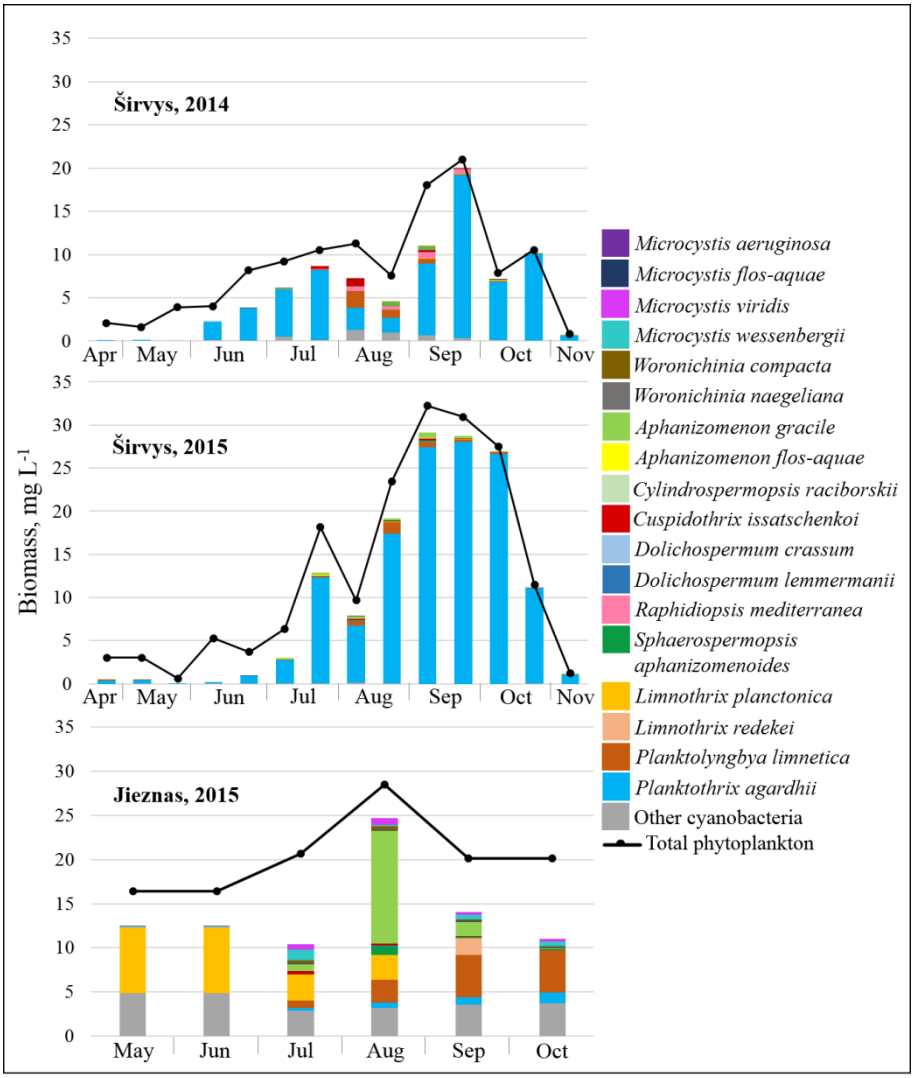


Fig. 3.1. Seasonal variation in potential toxin producing cyanobacteria species biomass in the studied lakes.

Altogether 19 species of potential cyanotoxin (MCs, STXs, ATX-a and CYN) producers belonging to *Anabaenopsis*, *Aphanizomenon* sensu lato, *Cylindrospermopsis*, *Dolichospermum*, *Limnothrix*, *Microcystis*, *Planktolyngbya*, *Planktothrix*, *Raphidiopsis*, *Woronichinia* genera were detected in the studied lakes (Table 3.2.). In general, only four species *Aphanizomenon gracile*, *Limnothrix planctonica*, *Planktolyngbya limnetica* and *Planktothrix agardhii* were dominant or prevailing, whereas other cyanobacteria species comprised negligible part of the phytoplankton (< 5

mg L^{-1}). The studied lakes differed significantly by the structure of dominant cyanobacteria species. Potentially hepatotoxic *P. agardhii* was a single dominant phytoplankton of Lake Širvys comprising up to 28.1 mg L^{-1} biomass with the highest values assessed in autumn (up to 97% to the total cyanobacteria biomass). In Lake Jieznas, potentially neurotoxins producing *Aphanizomenon gracile* dominated and formed maximum 12.7 mg L^{-1} biomass (up to 45% of the total cyanobacteria biomass) in August. The other prevailing species in the lake were *Limnothrix planctonica* with maximum biomass 7.5 mg L^{-1} value that was detected in May. *Planktolyngbya limnetica* comprising up to 5.53 mg L^{-1} prevailed in September. In the studied lakes, three non-native species *Sphaerospermopsis aphanizomenoides*, *Cylindrospermopsis raciborskii* and *Raphidiopsis mediterranea* were found. *Sphaerospermopsis aphanizomenoides* was detected in both lakes. The species comprised the highest biomass in Lake Jieznas up to 1.0 mg L^{-1} (4% of the total cyanobacteria biomass) from August to September, in Lake Širvys, it reached only up to 0.1 mg L^{-1} biomass (0.4% of the total cyanobacteria biomass). *Cylindrospermopsis raciborskii* was recorded only in Lake Jieznas in July and constituted 0.02 mg L^{-1} biomass (0.1% of the total cyanobacteria biomass). *Raphidiopsis mediterranea* up to 0.7 mg L^{-1} (6% of the total cyanobacteria biomass) was detected only in Lake Širvys.

3.3. *Aphanizomenon* sensu lato species identification based on molecular methods

Morphological similarities of potential cyanotoxin producers from *Aphanizomenon* sensu lato group predetermined the necessity to verify species identification based on molecular methods. Therefore, strains of *A. gracile*, *A. flos-aquae* and *Sphaerospermopsis aphanizomenoides* were examined using concatenated sequences of partial 16S rRNA and PC-IGS regions (Fig. 3.2.). Molecular data confirmed *Aphanizomenon gracile* and *A. flos-aquae* identification as a separate species and showed their close similarity in the constructed phylogenetic tree. *Sphaerospermopsis aphanizomenoides* was grouped in separate cluster together with the *S. aphanizomenoides* sequences from the GenBank (NCBI) database.

Table 3.2. Biomass of potential cyanotoxins producing cyanobacteria in the studied lakes

Species	Potential producer*	Širvys, mg L^{-1}		Jieznas, mg L^{-1}
		2014	2015	2015
Chroococcales				
Microcystis aeruginosa (Kützing) Kützing	MCs	0.00–0.02	–	–
Microcystis flos-aquae (Wittrock) Kirchner	MCs	0.00–0.01	–	–
Microcystis viridis (A.Braun in Rabenhorst) Lemmermann	MCs	–	–	0.08–0.77
Microcystis wessenbergii (Komárek) Komárek in Kondrateva	not confirmed	0.00–0.17	–	0.00–1.21
Woronichinia compacta (Lemmermann) Komárek & Hindák	not confirmed	0.00–0.03	single individuals	0.00–0.35
Woronichinia naegeliana (Unger) Elenkin	MCs	0.00–0.06	0.00–0.01	0.00–0.21
Nostocales				
Anabaenopsis cf. *elenkinii* Miller	MCs, STX	–	–	single individuals
Aphanizomenon gracile (Lemmermann) Lemmermann	STXs, CYN	0.00 – 0.50	0.00–0.66	0.00–12.71
Aphanizomenon flos-aquae Ralfs ex Bornet et Flahault	ATX-a, CYN, MCs, STX	–	0.00–0.16	–
Cylindrospermopsis raciborskii (Wołoszyńska) Seenayya & Subba Raju	CYN, ATX, STX	–	–	0.00–0.02
Cuspidothrix issatschenkoi (Usačev) Rajaniemi et al.	ATX-a, STX	0.00–0.91	0.00–0.28	0.00–0.36
Dolichospermum crassum (Lemmermann) Wacklin et al.	ATX-a(S)	0.00–0.02	0.00–0.07	0.00–0.07
Dolichospermum lemmermanii (Richter) Wacklin et al.	ATX-a, STXs	0.00–0.01	–	–
Raphidiopsis mediterranea Skuja	CYN, ATX-a	0.00–0.70	–	–
Sphaerospermopsis aphanizomenoides (Forti) Zapomělová et al.	STX	0.00–0.01	0.00–0.13	0.00–1.03
Oscillatoriales				
Limnothrix planctonica (Wołoszyńska) Meffert	not confirmed	0.00–0.13	0.00–0.07	0.00–7.47
Limnothrix redekei (Van Goor) Meffert	STX	–	single individuals	0.00–1.92
Planktolyngbya limnetica (Lemm.) Komárková-Legnerová et Cronberg	ATX-a	0.00–1.95	0.00–1.33	0.00–5.54
Planktothrix agardhii (Gomont) Anagnostidis et Komárek	MCs	0.01–18.83	0.38–28.06	0.00–1.21

* After Ballot et al. (2010a,b; 2016), Bernard et al. (2017), Ledreux et al. (2010)

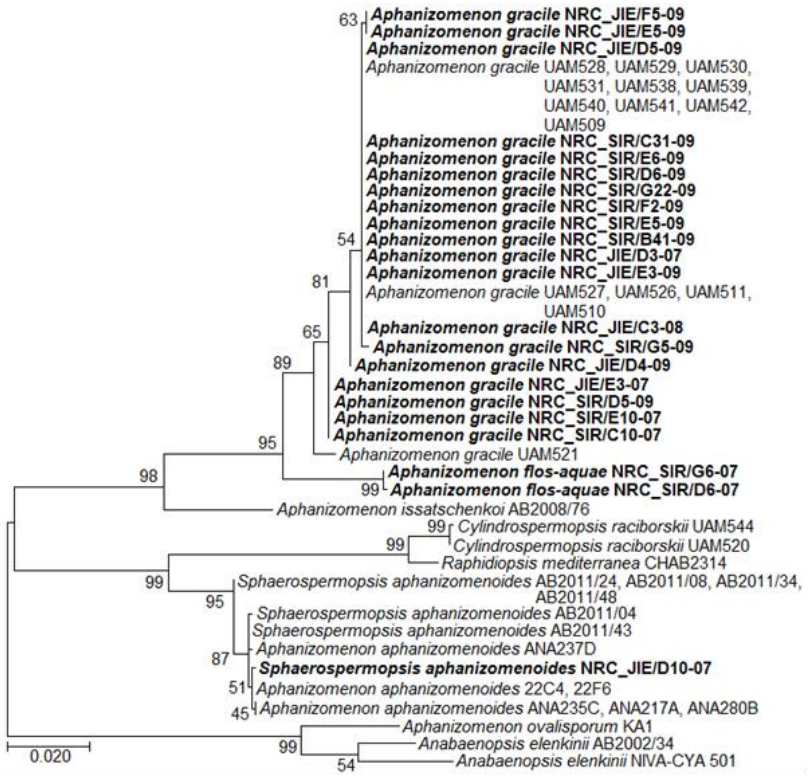


Fig. 3.2. Maximum likelihood tree based on concatenated partial 16S rRNA and PC-IGS sequences. Sequences of the strains from this study are marked in bold (after Karosienė et al., 2019).

3.4. Cyanotoxins and their variation in the environmental samples

High variety and contribution (up to 98% and 76%, respectively in Lakes Širvys and Jieznas) of potentially toxic cyanobacteria in the phytoplankton let us expect various cyanotoxins in the studied water bodies. Among tested cyanotoxins, microcytins, saxitoxin and anatoxin-a were detected, while cylindrospermopsin was absent in both studied lakes. Intracellular cyanotoxin concentrations in cyanobacteria biomass varied considerably during vegetation period (Fig. 3.3.) and were the highest in summer and/or September–October, thus, this is in the line with the increased biomass of cyanobacteria in the lakes.

Hepatotoxins. Five variants of microcystins (MCs) were detected in the environmental samples: dimethylated MC-RR (dmMC-RR), MC-RR, MC-

YR, dimethylated MC-LR (dmMC-LR) and MC-LR. The concentration of intracellular MCs was up to 17-fold higher in Lake Širvys than in Lake Jieznas (up to 0.96 µg L^{-1}, September). In Lake Širvys, the highest values of MCs were found in autumn (14.52 ± 2.4 µg L^{-1}) and coincided with the dominance of potential MCs producer *Planktothrix agardhii* (Fig. 3.3.). Although *P. agardhii* and total biomass of cyanobacteria was higher in 2015, the MCs concentration was slightly lower compared to 2014. The dominant variant was dmMC-RR through the both vegetation seasons and constituted up to 99% of total MCs amount. Other MCs variant, the dmMC-LR contribution to the total MCs amount was up to eight times lower (constituted up to 42%) compared to dmMC-RR and the highest concentration (2.03 µg L^{-1}) reached in August 2014. Concentrations of the other MCs variants (MC-RR, MC-YR, MC-LR) were negligible and each comprised less than 0.20 µg L^{-1}, and MC-YR only was present in a few samples collected in 2014. Contrary, in Lake Jieznas, MC-RR (up to 0.81 µg L^{-1}) prevailed over MC-YR, and MC-LR variants; dmMC-RR and dmMC-LR were absent at all. Thus, different dominant cyanobacteria in the studied lakes (Fig. 3.1) predetermined contrasting values of total concentrations of hepatotoxins and the composition of MCs variants.

Neurotoxins. Neurotoxins were detected in the environmental samples from July to September with the maximum total concentrations in cyanobacteria biomass obtained in August (1.74 µg L^{-1} in Lake Širvys and 1.10 µg L^{-1} in Lake Jieznas) (Fig. 3.3.). Generally, cellular ATX-a prevailed over saxitoxin in Lake Širvys, meanwhile STX was the dominant in Lake Jieznas. In Lake Širvys, the average ATX-a concentrations in 2014 were three times higher than in 2015. The maximum values of the target toxin were detected in late July and early August in 2014 (0.97 and 0.74 µg L^{-1}, respectively) when *Cuspidothrix issatschenkoi* reached the highest biomass (Fig. 3.1). Saxitoxin concentration up to 1.00 µg L^{-1} was detected only in the beginning of August 2014 (Fig. 3.3.) and related with the increase of *Aphanizomenon gracile* biomass (Fig. 3.1). In Lake Jieznas, the highest concentration of STX (1.06 µg L^{-1}) was detected in August, when potential STXs producing *A. gracile* dominated and comprised the highest biomass. The results showed that saxitoxin comprised negligible concentrations even when cyanobacteria formed high biomass. Whereas, the biomass of ATX-a producers was detected at inconsiderable amount in both lakes.

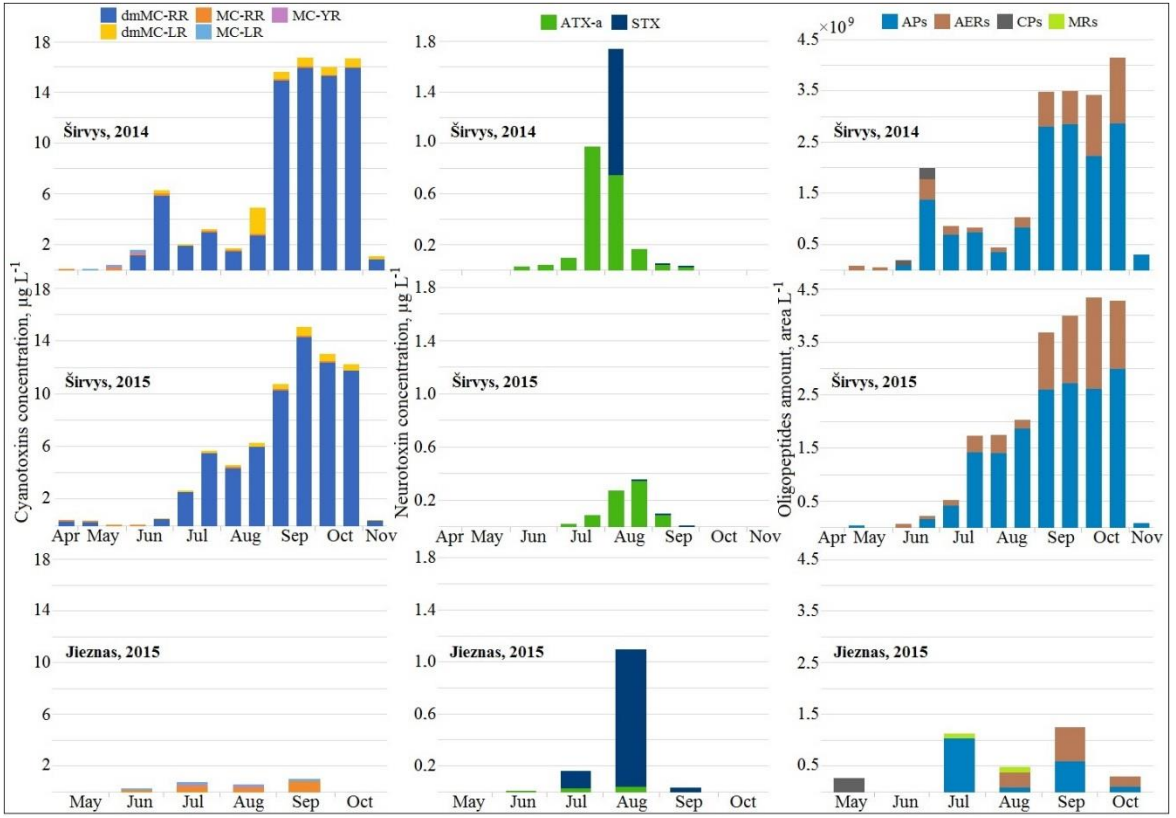


Fig. 3.3. Variation in intracellular cyanotoxins and bioactive NRPs structure in field samples of Lakes Širvys (2014–2015) and Jieznas (2015).

3.5. Variation of non-ribosomal peptides in the environmental samples

Cyanobacteria produce NRPs that may essentialy contribute to the toxicity of blooms enhancing the effect of other cyanotoxins. Among them, oligopeptides belonging to four different classes were detected in the field samples: anabaenopeptins (APs), aeruginosins (AERs), cyanopeptolins (CPs) and microginins (MRs) (Fig. 3.3.). APs and AERs were the dominant during almost all growth season in both lakes. The amount of other peptides was negligible. In Lake Širvys, composition of dominant compounds did not change significantly during the vegetation season or between the study years. APs constituted the major part during vegetation season on average 1.37×10^9 area L^{-1} that constituted 61% of the oligopeptides detected. The AERs relative contribution increased towards autumn with maximum value 1.30×10^9 area L^{-1} and on average constituted 26%. In Lake Jieznas, total amount of oligopeptides was 3.5 times lower than in Lake Širvys. APs were dominant in July comprising 1.03×10^9 area L^{-1} (91%), whereas AERs – constituted 1.96×10^8 area L^{-1} (67%) in September. The MRs were detected only in Lake Jieznas in July-August (up to 24%). The variation in total amount of oligopeptides resembled the changes in *P. agardhii* biomass in Lake Širvys, whereas changes in oligopeptide structure in Lake Jieznas likely depended on the prevailing species composition.

3.6. Comparison of potential toxic cyanobacteria and cyanotoxins in the surface and water column samples from the studied lakes

Cyanobacteria via gas vesicles have the ability to regulate their buoyancy in the water column. Floating to the surface layer, cyanobacteria form dense scums. However, some species prefer shading conditions or wind mixes water layer and distribute cyanobacteria biomass over all column in shallow lakes. So, in this section, cyanobacteria and profile of cyanotoxins of Lakes Širvys and Jieznas from the surface water layer was compared to the integrated water column samples collected in August 2015.

Physico-chemical variables differed significantly over the water column. The concentration of dissolved oxygen varied from 6.8–8.4 mg L^{-1} at the water surface to 1.9–2.5 mg L^{-1} at the bottom of the lake indicating almost anoxic conditions on the sampling day. The illumination below the surface was similar of both studied lakes (187–193 m^{-2} s^{-1}), however, euphotic zone

in Lake Širvys was 1.6 m and 0.9 m in Lake Jieznas. Thus, 1.8-fold lower penetration of the light in Lake Jieznas may determine more pronounced differences in cyanobacteria biomass, dominant species and cyanotoxin profile between the surface and integrated samples. However, the differences of chl-*a* and total cyanobacteria biomass were insignificant comparing surface and water column samples in both lakes (Fig. 3.4.). Both, in the surface and integrated samples, hepatotoxins prevailed among cyanotoxins in the profile of Lake Širvys, while neurotoxins – in Lake Jieznas.

The dominant species in Lake Širvys was *Planktothrix agardhii* constituting 91% and 67% of total cyanobacteria biomass in the surface and water column sample, respectively (Fig. 3.4.). *Planktolyngbya limnetica* was more abundant on the surface, whereas *Aphanizomenon gracile* and *Limnothrix redekei* – in water column. dmMC-RR was the dominant MCs variant with concentrations of 6.63 µg L^{-1} and 4.19 µg L^{-1} at the surface and integrated samples, respectively. The slightly different profile of potentially toxic cyanobacteria possibly predetermined slightly higher dmMC-LR concentration in water column compared to the surface. Cylindrospermopsin was found only in the integrated water in Lakes Širvys and Jieznas.

The dominant *Aphanizomenon gracile* in Lake Jieznas was almost equaly distributed in the water column (52% and 57% at the surface and water column, accordingly). Possibly, due to differences in buoyancy capabilities, *Microcystis viridis* was concentrated at the surface, whereas higher *Planktolyngbya limnetica* biomass was in the water column. Total concentration of MCs (0.53 µg L^{-1}) was almost twice lower at the surface than in the integrated sample (0.98 µg L^{-1}), however, MCs profile was similar to dominant MC-RR and predominant MC-LR variants. Saxitoxins were not tested in the integtated water samples, and concentrations of ATX showed considerable differences among two samples from Lake Jieznas.

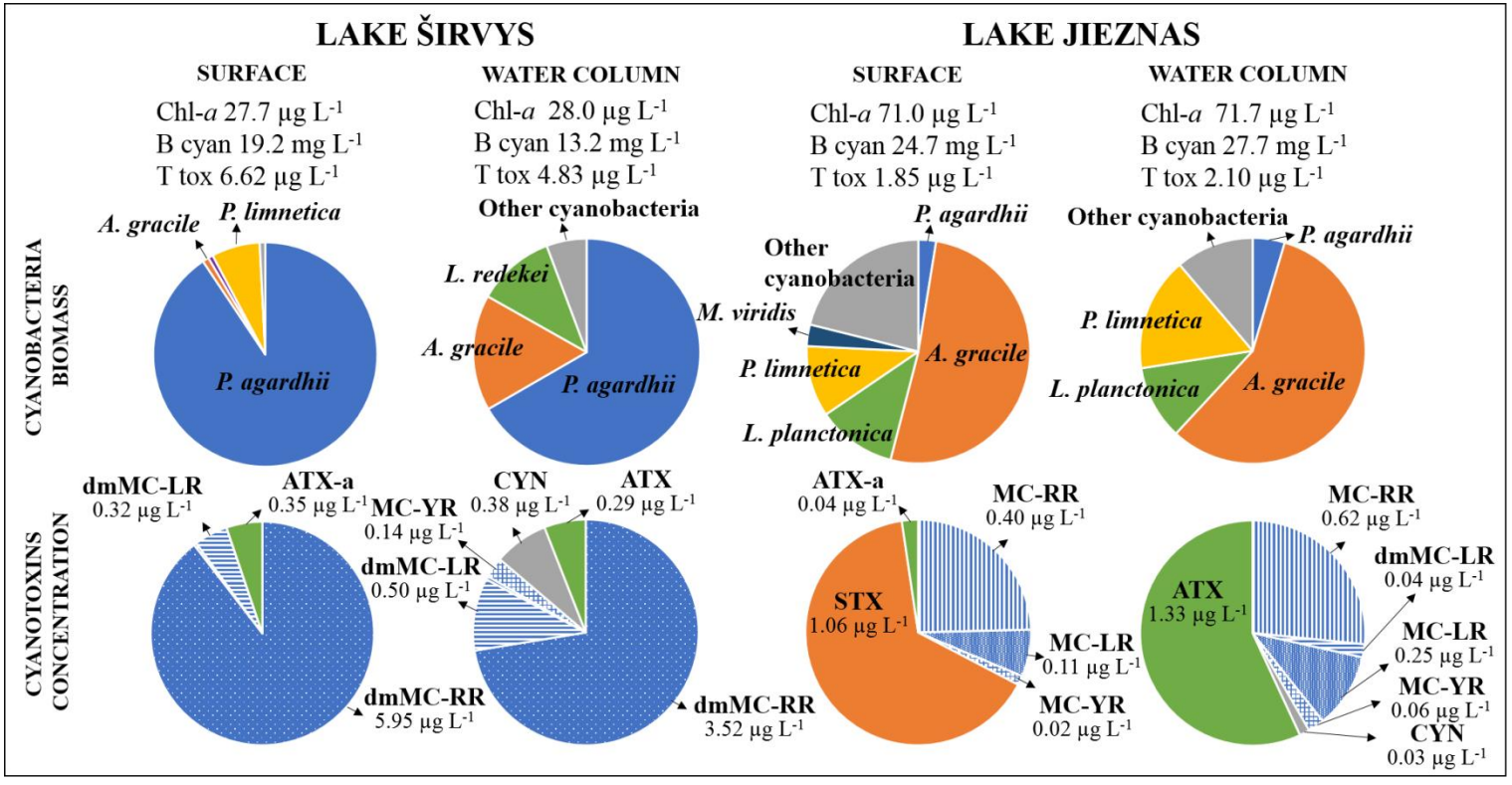


Fig. 3.4. Cyanobacteria biomass and profile of cyanotoxins, and nutrient concentrations in the surface and column samples from the studied lakes.

Abbreviations: B cyan – total biomass of cyanobacteria; T tox – total concentration of cyanotoxins.

3.7. *Planktothrix agardhii mcy*E gene in the strains and in the environmental samples from Lake Širvys

Only those individuals that have genes responsible for cyanotoxin synthesis could produce toxin. The number of such toxic individuals in the population determine the toxicity of bloom. In order to assess *Planktothrix agardhii* population toxigenicity, quantitative real-time polymerase chain reaction (qPCR)-based molecular techniques were applied to detect *mcy*E gene of *Planktothrix* in the environmental samples collected during vegetation period in Lake Širvys (2014). The qPCR analysis determined the highest copy numbers up to 26×10^9 L^{-1} of *mcy*E gene specific to *Planktothrix* in the field samples collected from Lake Širvys in September-October (Fig. 3.5., A) and coincided with the period of the greatest biomass of *P. agardhii* and MCs concentration. The strong correlation assessed between *P. agardhii* specific *mcy*E gene copy numbers and total MCs concentrations in the field samples ($r = 0.85$, $p < 0.05$) confirmed the species as the main MCs producer in the lake. The lowest *mcy*E gene copy number and MCs concentration were detected in summer, especially end of June and beginning of July, compared to the *P. agardhii* biomass.

To assess the proportion of toxigenic and non-toxigenic individuals in the population over the vegetation season, *mcy*E gene of *P. agardhii* was also examined using conventional PCR in strains isolated each month throughout the vegetation period from Lake Širvys in 2014 (Table 3.3.). Toxic genotypes constituted 80–100% of *P. agardhii* population during vegetation season, and *mcy*E gene was present in 93.6% of all strains tested (Fig. 3.5., B). The study showed that percentage of toxic strains in the population slightly increased in summer-autumn period.

Both the qPCR analysis of the environmental samples and *P. agardhii* strains isolated from that period showed that most of the *P. agardhii* individuals in the populations had potential to produce MCs. High cyanobacterium biomass and percentage of toxic genotypes in the population indicated that *P. agardhii* had high risk to the lake ecosystem as formed bloom to a large extent toxic.

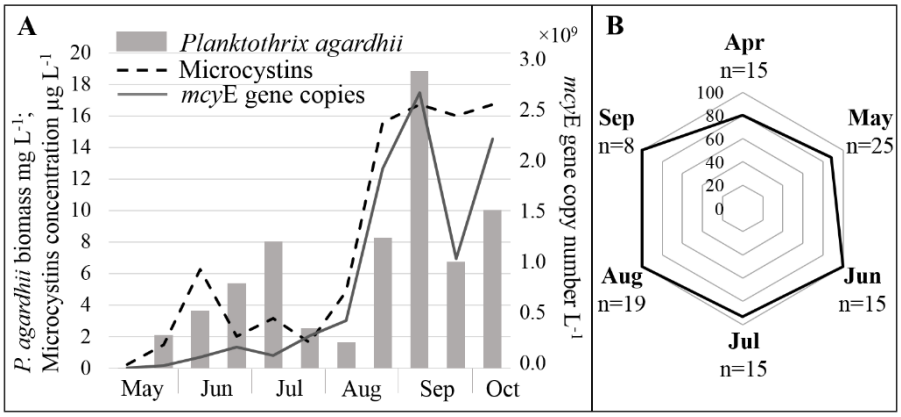


Fig. 3.5. A – *Planktothrix agardhii* biomass, concentration of microcystins and *mcy*E gene copy numbers in the environmental samples of Lake Širvys in 2014. B – Relative number (%) of toxigenic strains of *P. agardhii* (n – number of strains tested).

3.8. Impact of the environmental factors on the variability of cyanobacteria biomass, cyanotoxins and non-ribosomal peptides amount

Biomass of potentially toxic cyanobacteria and profile of their secondary metabolites depend on the environmental variables, therefore, there is essential to identify the most important factors in order to select conditions for the experimental study with the cyanobacteria strains. Redundancy analysis (RDA) presented in Figures 3.6 and 3.7 showed the relationship of biomass of the potentially toxic cyanobacteria and concentration of metabolites to the environmental variables in the field samples from two studied lakes. Various abiotic variables were tested: surface water temperature, pH, conductivity, illumination below water surface, IP, IN, ammonium, nitrate N:P. Some environmental variables such as IN, NH_4^+, NO_3^- were eliminated from the analysis due to high collinearity with N:P.

The analysis revealed that the selected environmental variables explained 86% of the variation in the presence of potentially toxic cyanobacteria (Fig. 3.6.). Only IP was suggested to be statistically significant factor related to cyanobacteria biomass ($F = 4.31$, $p < 0.001$). The IP had the strongest relationship to biomass of *Aphanizomenon gracile* ($r = 0.52$, $p < 0.05$), *Woronichinia compacta* and *Sphaerospermopsis aphanizomenoides* ($r = 0.50$, $p < 0.05$), *Microcystis aeruginosa* and *M. viridis* ($r = 0.40$ and $r = 0.44$, $p < 0.05$, respectively), also *Limnothrix planctonica* ($r = 0.37$, $p < 0.05$)

cyanobacteria species. Temperature values correlated with *Cuspidothrix issatschenkoi* and *Dolichospermum crassum* biomass (r = 0.53 and r = 0.36, p < 0.05), whereas the N:P atomic ratio negatively correlated with *Planktothrix agardhii* (r = -0.48, p < 0.05).

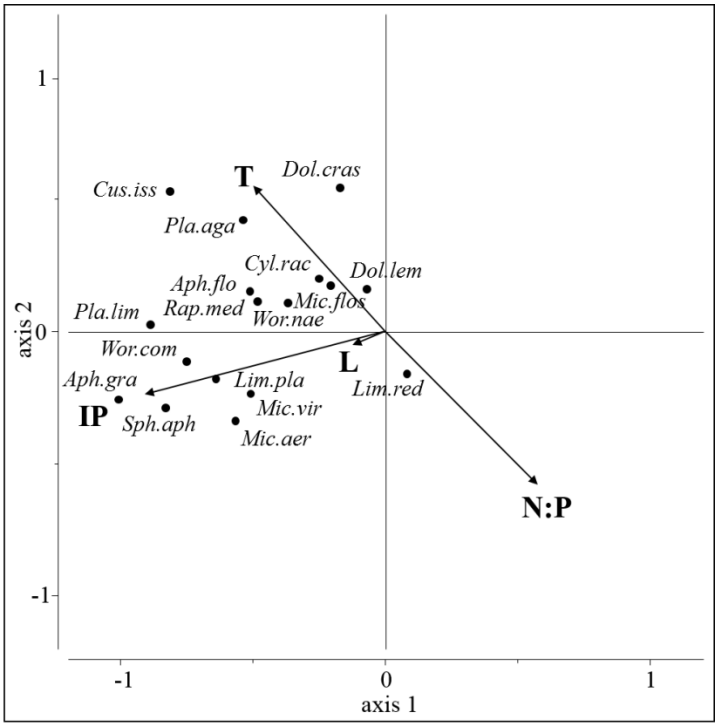


Fig. 3.6. Redundancy analysis (RDA) biplot of the environmental factors as explanatory variables and potential toxin producing cyanobacterial species biomass.

Environmental variables: T – surface water temperature; N:P – atomic ratio of inorganic nitrogen and phosphorus; IP – inorganic phosphorus; L – illumination.

Species: *Mic. aer – Microcystis aeruginosa*; *Mic. flos – Microcystis flos-aquae*; *Mic. vir – Microcystis viridis*; *Wor. com – Woronichinia compacta*; *Wor. nae – Woronichinia naegeliana*; *Aph. gra – Aphanizomenon gracile*; *Sph. aph – Sphaerospermopsis aphanizomenoides*; *Dol. cra – Dolichospermum crassum*; *Dol. lem – Dolichospermum lemmermanii*; *Aph. flos – Aphanizomenon flos-aquae*; *Cus. iss – Cusspidothrix issatschenkoi*; *Raph. med – Raphidiopsis mediterranea*; *Cyl. rac – Cylindrospermopsis raciborskii*; *Pla. lim – Planktolyngbya limnetica*; *Lim. pla – Limnothrix planctonica*; *Lim. red – Limnothrix redekei*; *Pla. aga – Planktothrix agardhii*.

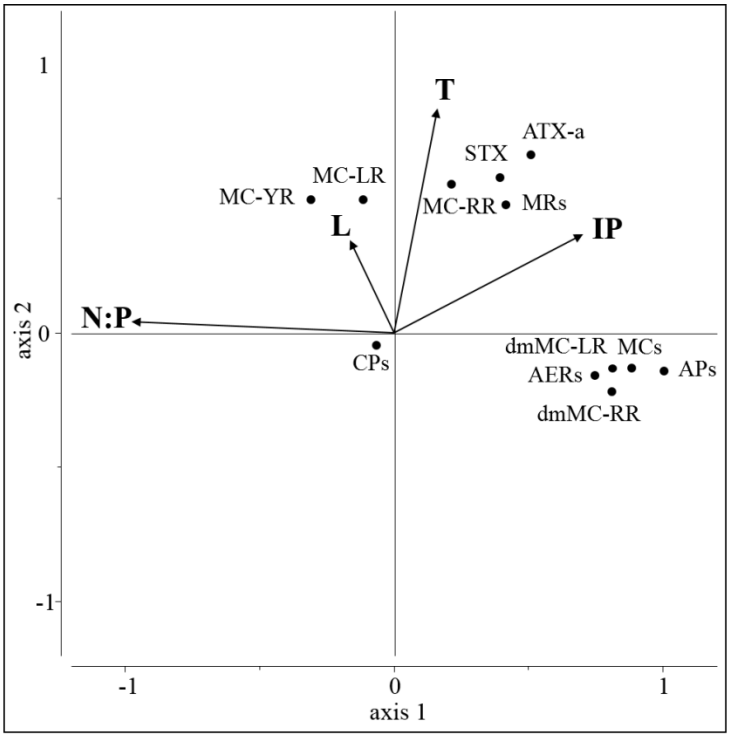


Fig. 3.7. Redundancy analysis (RDA) biplot of the environmental factors as explanatory variables and cyanotoxins also bioactive NRPs amount.
Environmental variables: T – surface water temperature; N:P – atomic ratio of inorganic nitrogen and phosphorus; IP – inorganic phosphorus; L – illumination.
Secondary metabolites: STX – saxitoxin; ATX-a – anatoxin-a; MCs – total microcystins concentration; APs – anabaenopeptins; AERs – aeruginosins; CPs – cyanopeptolins, MRs – microginins.

Variables included into the analysis explained 81% of the total variance of cyanotoxins and other metabolites (Fig. 3.7.). The RDA analysis showed that N:P ratio was the most significant environmental variable for the presence and amount of cyanotoxins and oligopeptides ($F = 5.31$, $p < 0.001$). The negative correlation was detected between N:P ratio and the amount of total microcystins ($r = -0.47$, $p < 0.05$), their variants: dmMC-RR and dmMC-LR ($r = -0.46$ and $r = -0.36$, $p < 0.05$), also APs ($r = -0.54$, $p < 0.05$) and AERs ($r = -0.40$, $p < 0.05$) amount. Inorganic phosphorus positively correlated to STX, APs and MRs ($r = 0.49$, $r = 0.38$ and $r = 0.45$, $p < 0.05$, respectively). Surface water temperature positively correlated to the concentration of ATX-a ($r = 0.53$, $p < 0.05$).

3.9. Determination of toxic strains using LC-MS/MS and genetic analysis

Assessment of the potential toxin producers by applying statistics on phytoplankton and cyanotoxins data from the environmental samples can not unequivocally disclose the real producers. Therefore, a total of 274 isolated strains from the studied lakes were tested using LC-MS/MS and genetic analysis to confirm the toxin producing species (Table 3.3.).

3.9.1. Cyanobacteria strains responsible for production of cyanotoxins

Planktothrix agardhii was detected as MCs producer, because the analysis of *P. agardhii* strains showed that 91 strains contained *mcy*E synthetase gene and the tested six strains of these produced MCs, preferably dmMC-RR, MC-RR, dmMC-LR and MC-YR. The *Microcystis* genera were assessed also as MCs producer as two strains of *M. flos-aquae*, five strains of *M. viridis* and one *M. aeruginosa* strain synthesized MCs, whereas other strains had *mcy*E gene. *M. aeruginosa* strain synthesized MC-RR, MC-LR and MC-YR, *M. viridis* strains – dmMC-RR, MC-RR, MC-YR and MC-LR, whereas *M. flos-aquae* toxic strains were responsible only for MC-RR production. *M. wessenbergii* did not produce MCs.

Among potential neurotoxin producers, only *Aphanizomenon gracile* was responsible for the target toxin production. STX production confirmed by LC-MS/MS analysis and *sxt*A gene presence was detected in the 11 *A. gracile* strains out of 63 tested. Partial *sxt*A gene sequences (544 bp) of *A. gracile* strains were highly homogenous (> 99% sequence identity) among the strains (Fig. 3.8.). *A. gracile* strains *sxt*A gene was closely related to other potentially STX producing cyanobacteria *Aphanizomenon* spp., *Anabaenopsis elenkinii*, *Dolichospermum planctonica*, *D. circinalis*, but clearly separated from more taxonomically distant cyanobacteria species such as *Cylindrospermopsis raciborskii* and *Lyngbya wollei*.

Sixty strains of species *Cuspidothrix issatschenkoi*, *Dolichospermum crassum*, *D. lemmermannii*, *Raphidiopsis mediterranea*, *Planktolyngbya limnetica*, potentially producing cyanotoxins, were tested for neurotoxin ATX-a by LC-MS/MS and/or molecular methods, however, none of these were supported as producers at strain level. The cytotoxin CYN was not detected in any tested strain of the potential producers of *Aphanizomenon gracile*, *A. flos-aquae* and *Raphidiopsis mediterranea*.

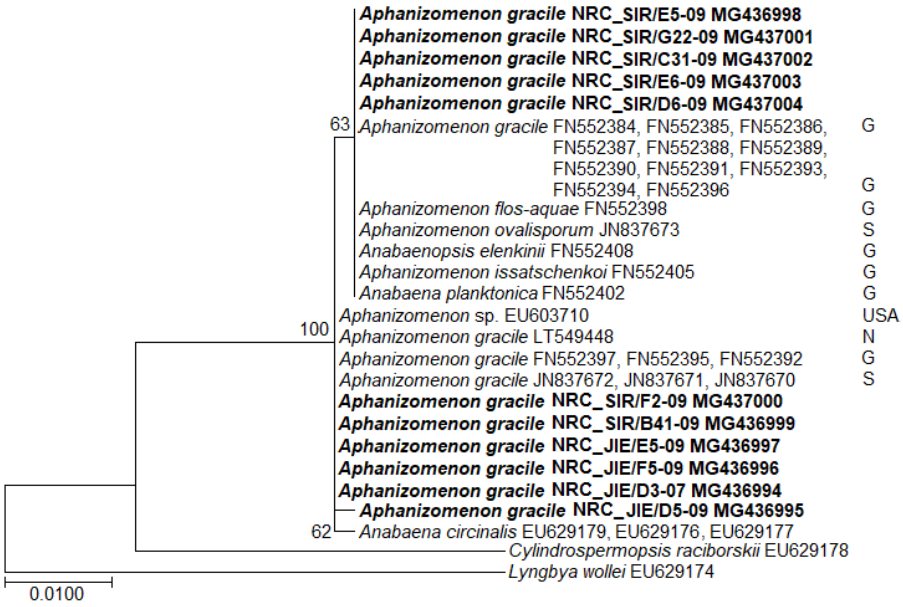


Fig. 3.8. Maximum likelihood tree based on partial *sxt*A gene sequences. Sequences of the strains from this study are marked in bold (after Karosienė et al., 2019). Abbreviations from GenBank (NCBI) strains: G – Germany; N – Norway; S – Spain.

3.9.2. Cyanobacteria strains producing non-ribosomal peptides

LC-MS/MS analysis revealed *Microcystis* spp. (11 strains), *Aphanizomenon gracile* (3 strains), *Dolichospermum lemmermannii* (2 strains) and *Planktothrix agardhii* (7 strains) as NRPs producers, whereas oligopeptides were not detected in *Microcystis wessenbergii*, *Anabaenopsis* cf. *elenkinii*, *Cuspidothrix issatschenkoi*, *Limnothrix planctonica* and *Planktolyngbya limnetica*. NRPs were detected in 39 strains of various cyanoabcteria, and the anabaenopepins (13 strains) and cyanopeptolins (11 strains) were found most often (Table 3.3.). Out of 19 *Microcystis* strains, only three were able to synthesize two classes of NRPs at the same time, whereas eight strains produced peptides belonging to single class. CPs were the most often recorded class in *Microcystis* genus (6 strains). *Dolichopermum lemmermannii* and three strains of *Aphanizomenon gracile* produced APs, also the CPs and MRs were detected in the latter cyanobacterium. Seven strains of *Planktothrix agardhii* tested showed that the species was able to produce three NRPs classes and APs was the most common. The same

cyanobacteria species similarly to the production of cyanotoxins (except *Dolichospermum lemmermannii* and *Microcystis aeruginosa*) produced different NRPs, and a particular oligopeptide could be produced by the same species strains. Thus, production of oligopeptide is species and/or strain specific.

Table 3.3. Presence of cyanotoxins and non-ribosomal peptides in the tested cyanobacteria strains (LC-MS/MS and/or genetic analysis)

Species	Lake	Strain	Cyanotoxins			NRPs			
			MCs	ATX-a	STX	APs	AERs	CPs	MRs
Chroococcales									
Microcystis aeruginosa	Širvys	D7-09	+	-	-	-	-	-	-
		G2-08	+	-	-	n.a.	n.a.	n.a.	n.a.
		1 strain	-	-	-	n.a.	n.a.	n.a.	n.a.
	Jieznas	B6-07	-	-	-	-	-	+	-
		C6-07	-	-	-	-	-	+	-
		B11-05	-	-	-	+	-	-	+
		D7-07	-	-	-	-	+	+	-
		1 strain	-	-	-	-	-	-	-
Microcystis flos-aquae	Širvys	G5-04	-	-	-	n.a.	n.a.	n.a.	n.a.
		D11-09	+	-	-	-	+	-	-
		E10-09	+	-	-	-	+	-	-
	Jieznas	E11-05	-	-	-	+	-	-	+
Microcystis viridis	Jieznas	D8-06	+	-	-	-	-	+	-
		G4-08	+	-	-	-	-	+	-
		F10-05	+	-	-	-	-	-	-
		G10-05	+	-	-	-	-	+	-
		C4-08	+	-	-	-	+	-	-
Microcystis wessenbergii	Jieznas	5 strains	-	-	-	-	-	-	-
Total:		**17 strains**							
Nostocales									
Anabaenopsis cf. *elenkinii*	Jieznas	2 strains	-	-	-	-	-	-	-
Aphanizomenon gracile	Širvys	E5-09	-	-	+	-	-	-	-
		E6-09	-	-	+	-	-	-	-
		D6-09	-	-	+	-	-	-	-
		B41-09	-	-	+	n.a.	n.a.	n.a.	n.a.
		F2-09	-	-	+	n.a.	n.a.	n.a.	n.a.
		G22-09	-	-	+	n.a.	n.a.	n.a.	n.a.
		C31-09	-	-	+	n.a.	n.a.	n.a.	n.a.
		E9-08	-	-	-	+	-	+	+
		G5-09	-	-	-	+	-	+	-

Species	Lake	Strain	Cyanotoxins			NRPs			
			MCs	ATX-a	STX	APs	AERs	CPs	MRs
Aphanizomenon gracile	Širvys	D5-09	-	-	-	+	-	-	-
		4 strains	-	-	-	-	-	-	-
		23 strains	-	-	-	n.a.	n.a.	n.a.	n.a.
	Jieznas	D3-07	-	-	+	n.a.	n.a.	n.a.	n.a.
		D5-09	-	-	+	n.a.	n.a.	n.a.	n.a.
		F5-09	-	-	+	n.a.	n.a.	n.a.	n.a.
		E5-09	-	-	+	n.a.	n.a.	n.a.	n.a.
		E3-07	-	-	-	n.a.	n.a.	n.a.	n.a.
		21 strains	-	-	-	n.a.	n.a.	n.a.	n.a.
Dolichospermum crassum	Širvys	39 strains	n.a.	-	n.a.	n.a.	n.a.	n.a.	n.a.
		2 strains	-	-	-	-	-	-	-
Dolichospermum lemmermannii	Širvys	D7-07	-	-	-	+	-	-	-
		C7-07	-	-	-	+	-	-	-
		1 strain	n.a.	-	n.a.	n.a.	n.a.	n.a.	n.a.
Aphanizomenon flos-aquae	Širvys	3 strains	-	-	-	n.a.	n.a.	n.a.	n.a.
Cuspidothrix issatschenkoi	Jieznas	5 strains	-	-	-	-	-	-	-
Raphidiopsis mediterranea	Širvys	2 strains	-	-	-	n.a.	n.a.	n.a.	n.a.
Sphaerospermopsis aphanizomenoides	Jieznas	12 strains	-	-	-	n.a.	n.a.	n.a.	n.a.
Total:		**131 strains**							
Oscillatoriales									
Limnothrix planctonica	Jieznas	7 strains	-	-	-	-	-	-	-
Planktolyngbya limnetica	Širvys	6 strains	-	-	-	-	-	-	-
	Jieznas	3 strains	-	-	-	-	-	-	-
Planktothrix agardhii	Širvys	G6-05	+	-	-	+	+	+	-
		G5-05	+	-	-	+	+	-	-
		E8-07	+	-	-	+	-	-	-
		F5-07	+	-	-	+	+	+	-
		C12-09	+	-	-	+	-	-	-
		C11-09	+	-	-	+	-	-	-
Planktothrix agardhii	Širvys	85 strains	+	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
		7 strains	-	-	-	n.a.	n.a.	n.a.	n.a.
	Jieznas	C9-07	+	-	-	-	-	+	-
		E9-07	-	-	-	-	-	-	-
Total:		**121 strains**							
Total number of cyanobacteria strains:		**274 strains**							

n.a. – not analysed; - – not detected

3.10. Experiment I: Temperature effect on native and alien cyanobacteria

The experiment was performed to test climate warming impact on two native and two alien species under wide range of temperatures.

3.10.1. Temperature effect on growth rate of cyanobacteria strains

Results from the General linear model (GLM) analysis showed that temperature had a significant effect on cyanobacteria growth rate ($F_{(6,161)}$ = 15.83, $p < 0.001$). Also, the significant impact interaction of temperature with taxa ($F_{(18,140)}$ = 7.79, $p < 0.001$) and species origin (native/alien) ($F_{(6,154)}$ = 5.89, $p < 0.001$) on strains was assessed, but not in case with toxic/non-toxic strains ($p > 0.05$). All strains showed positive growth rates under all tested temperatures (18–30°C) used in the experiments except the strains of *Chrysosporum bergii* which growth was inhibited at 18°C (Table 3.4.). In the case of native species, similar average growth rate of *Aphanizomenon gracile* and *Planktothrix agardhii* strains under all the tested temperatures was assessed. The strains of *P. agardhii* compared to other species showed considerable potential for growing at the lowest 18°C temperature. The General linear model analysis revealed significant toxic and non-toxic strains effect regardless of temperature ($F_{(1,82)}$ = 41.0, $p < 0.001$) on the growth rate of *P. agardhii* and *A. gracile*.

Alien species demonstrated different growth characteristics (Table 3.4.). *Sphaerospermopsis aphanizomenoides* strains tolerated a wide range (20–30°C) of temperatures well and the highest growth rate was at 30°C (0.70 day^{-1} by NRC_JIE/F11-07 strain). However, the growth rate of *C. bergii* increased considerably up to 2–9-fold with rising temperature, especially at 30°C with the highest growth rate of 0.56 day^{-1}.

Table 3.4. Growth rates (day^{-1}) of the tested native and alien cyanobacteria species cultured under different temperatures. Data presented with mean ± SD

Species	Strain	Temperature, °C						
		18	20	22	24	26	28	30
NATIVE								
PA	F5	**0.38** ± 0.02	**0.41** ± 0.01	**0.42** ± 0.01	**0.42** ± 0.02	**0.43** ± 0.01	**0.42** ± 0.00	**0.45** ± 0.03
	E9	**0.37** ± 0.01	**0.43** ± 0.01	**0.45** ± 0.03	**0.48** ± 0.01	**0.51** ± 0.03	**0.51** ± 0.04	**0.46** ± 0.01
AG	B41	**0.25** ± 0.02	**0.38** ± 0.01	**0.41** ± 0.01	**0.41** ± 0.01	**0.41** ± 0.01	**0.48** ± 0.03	**0.41** ± 0.03
	C10	**0.41** ± 0.01	**0.48** ± 0.02	**0.51** ± 0.00	**0.49** ± 0.01	**0.52** ± 0.01	**0.51** ± 0.01	**0.49** ± 0.02
ALIEN IN EUROPE								
SA	G11	**0.22** ± 0.01	**0.52** ± 0.01	**0.55** ± 0.01	**0.63** ± 0.02	**0.59** ± 0.02	**0.54** ± 0.01	**0.32** ± 0.01
	F11	**0.26** ± 0.01	**0.48** ± 0.01	**0.68** ± 0.02	**0.55** ± 0.04	**0.68** ± 0.01	**0.63** ± 0.02	**0.70** ± 0.02
CB	D2	**-0.13** ± 0.11	**0.05** ± 0.02	**0.28** ± 0.01	**0.22** ± 0.06	**0.33** ± 0.02	**0.39** ± 0.03	**0.46** ± 0.00
	B6	**-0.05** ± 0.03	**0.29** ± 0.02	**0.46** ± 0.00	**0.46** ± 0.01	**0.55** ± 0.01	**0.54** ± 0.01	**0.56** ± 0.01

Abbreaviations: *PA – Planktothrix agardhii* strains: F5 – NRC_SIR/F5-09 (toxic); E9 – NRC_JIE/E9-07; *AG – Aphanizomenon gracile* strains: B41 – NRC_SIR/B41-09 (toxic); C11 – NRC_SIR/C10-07; *SA – Sphaeropermopsis aphanizomenoides* strains: G11 – NRC_JIE/G11-07; F11 – NRC_JIE/F11-07; *CB – Chrysosporum bergii* strains: D2 – NRC_REK/D2; B6 – NRC_GIN/B6

3.10.2. Temperature effect on production of cyanotoxins and non-ribosomal peptides

Two toxic strains of the native species *Aphanizomenon gracile* and *Planktothrix agardhii* were tested for temperature effect on cyanotoxins and NRPs production. Saxitoxin concentrations in the toxic strain of *A. gracile* (NRC_SIR/B41-09) varied from 29.4 to 102.0 ng g^{-1} freeze-dried weight under all experimental treatment (Fig.3.9.). The maximum toxin concentration was at 26°C, whereas STX values at other tested temperatures were up to 5 times lower. However, the correlation analysis did not indicate the relation separately between temperature and *A. gracile* growth rate with STX concentrations ($p>0.05$).

The toxic *P. agardhii* (NRC_SIR/F5-09) strain produced three MCs variants, among these dmMC-RR, dmMC-LR concentrations considerably

prevailed over MC-YR (Figure 3.9.). The total MCs concentration was higher at temperatures 18–24°C with the concentrations ranged from 9.2 × 10^5 to 9.6 × 10^5 ng g^{-1} freeze-dried weight. The MCs concentration was reduced up to 2.2 times at 30°C (4.1 × 10^5 ng g^{-1} freeze-dried weight). The linear regression revealed significant relationship between temperature and total MCs concentration ($r = -0.80$, $p < 0.05$), also with dmMC-RR ($r = -0.90$, $p < 0.05$). The MCs variant proportion under different temperatures remained the same.

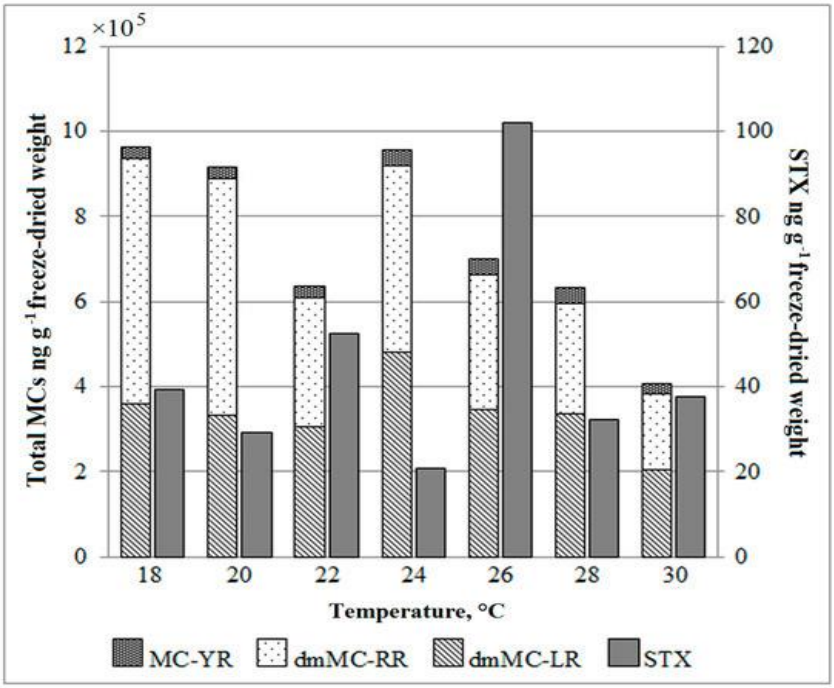


Fig. 3.9. Variation of microcystins concentration in *Planktothrix agardhii* and saxitoxin in *Aphanizomenon gracile* biomass grown under different temperatures (after Savadova et al., 2018).

Only two *P. agardhii* strains among the other used in the experiments were rich in bioactive NRPs and were selected for further analysis to test temperature impact. Three groups of NRPs were identified in both strains: anabaenopeptins (APs), aeruginosins (AERs) and cyanopeptolins (CPs). The highest total relative amounts of NRPs were detected at the lowest tested temperatures (18–20°C) in toxic and non-toxic strains in the range from 5.85 × 10^{11} to 7.40 × 10^{11} area g^{-1} freeze-dried weight and from 4.85 × 10^{11} to 5.80

$\times 10^{11}$ area g^{-1} freeze-dried weight, respectively (Fig. 3.10.). Oligopeptides structure differs among toxic and non-toxic strains. In toxic strain, composition of peptides was similar at the temperature range from 22°C to 30°C. At lower temperatures 18–20°C, APs prevailed. In non-toxic strain, peptides from AERs class were dominant under all tested temperatures. Linear regression analysis performed for toxic *P. agardhii* strain determined significant negative relationship between temperature and three detected oligopeptide classes: r = -0.84 for APs, r = -0.88 for AERs and r = -0.86 for CPs, $p < 0.05$. In non-toxic strain, out of produced oligopeptides only APs negatively correlated with temperature (r = -0.87, $p < 0.05$).

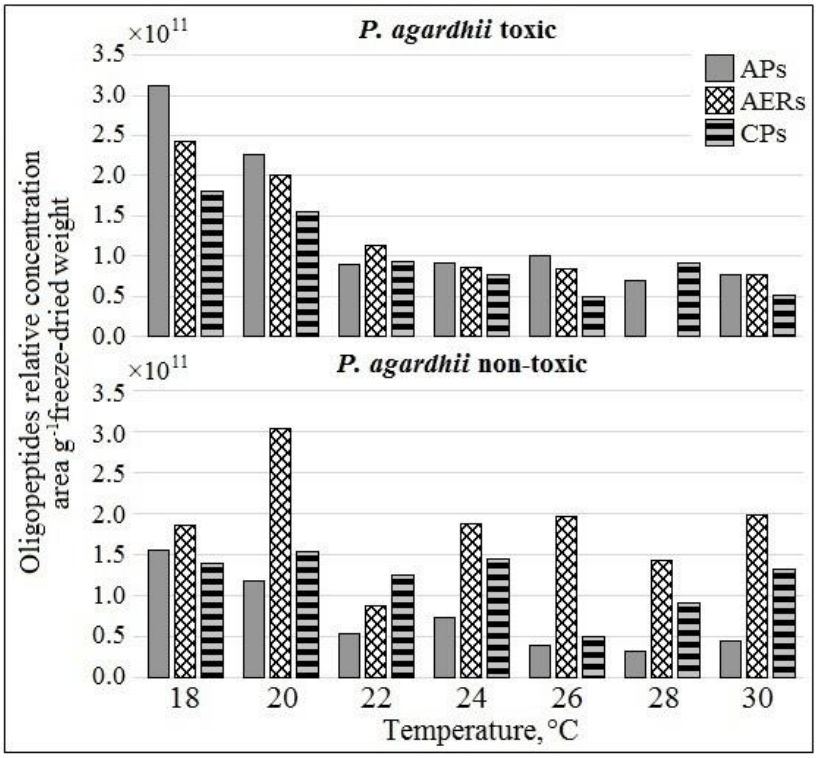


Fig. 3.10. Bioactive NRPs composition in toxic and non-toxic *Planktothrix agardhii* strains grown at different temperatures. Oligopeptide classes: APs – anabaenopeptins, AERs – aeruginosins, CPs – cyanopeptolins.

3.11. Experiment II: Effect of nutrients concentration on native and alien cyanobacteria

The experiment was performed to test eutrophication impact on two native and two alien species under wide range of nutrients at 24°C temperature.

3.11.1. Nutrients effect on the growth rate of cyanobacteria strains

The General linear model (GLM) showed that IP concentrations, N:P atomic ratio, their interaction and IN significantly affected the growth of all tested cyanobacteria strains (Table 3.5.). Also, GLM analysis revealed that growth rate was significantly affected by strain, species and species origin. The highest growth rates were found for strains of *Aphanizomenon gracile* (0.43 ± 0.08 day^{-1}) and *Sphaerospermopsis aphanizomenoides* (0.40 ± 0.12 day^{-1}) at all the tested nutrient concentrations, and especially at two highest IP concentrations (Fig. 3.11.). IP and IN concentrations had impact on both species growth rate, whereas for *A. gracile* N:P atomic ratio was also significant. The lowest growth rate among the tested cyanobacteria was characteristic to *Planktothrix agardhii* strains (0.14 ± 0.25 day^{-1}) and showed the biggest differences in growth under treatments. The maximum growth rate of *P. agardhii* reached at the highest IP concentrations (0.51 mg P L^{-1}) and at N:P atomic ratio 30:1, whereas low IP concentrations and N:P atomic ratio inhibited the cyanobacterium growth. The GML analysis revealed that the IP and IN concentrations, N:P atomic ratio was important for the growth of species of both strains. The growth rate (0.18 ± 0.11 day^{-1}) of *Chrysosporum bergii* was strain specific and considerably differed under all tested nutrient concentrations. The tested variables showed no significant impact on *C. bergii* growth rate (Table 3.5.).

Table 3.5. General linear model (GLM) results for factors impact on the growth rate of cyanobacteria strains

Response variable	Factor	F value, p
Growth rate	**Tested for all cyanobacteria:**	
	IP	$F_{(32, 75)} = 126.56$, $p < 0.01$
	N:P atomic ratio	$F_{(16, 40)} = 142.79$, $p < 0.01$
	IP × N:P atomic ratio	$F_{(64, 121)} = 11.10$, $p < 0.01$
	IN	$F_{(112, 151)} = 35.23$, $p < 0.01$
	Strain (tested under different IP; N:P atomic ratio, IN, respectively)	$F_{(35, 242)} = 34.11$, $p = 0.00$ $F_{(21, 307)} = 24.19$, $p = 0.00$ $F_{(91, 14)} = 38.13$, $p = 0.001$
	Species (tested under different IP; N:P atomic ratio, IN, respectively)	$F_{(15, 168)} = 54.31$, $p < 0.01$ $F_{(9, 270)} = 554.91$, $p < 0.01$ $F_{(45, 9)} = 35.94$, $p < 0.001$
	Native/Alien (tested under different IP; N:P atomic ratio, IN, respectively)	$F_{(5, 63)} = 4.01$, $p < 0.001$ $F_{(3, 113)} = 19.41$, $p < 0.001$ $F_{(15, 5)} = 8.67$, $p < 0.01$
	Tested for *P. agardhii*:	
	IP	$F_{(4, 74)} = 660.16$, $p < 0.001$
	N:P atomic ratio	$F_{(2, 74)} = 800.00$, $p < 0.001$
	IP × N:P atomic ratio	$F_{(8, 74)} = 28.06$, $p < 0.001$
	IN	$F_{(14, 74)} = 321.83$, $p < 0.01$
	Tested for *A. gracile*:	
	IP	$F_{(4, 75)} = 183.78$, $p < 0.001$
	N:P atomic ratio	$F_{(2, 75)} = 6.03$, $p < 0.001$
	IP × N:P atomic ratio	$p > 0.05$
	IN	$F_{(14, 75)} = 54.44$, $p < 0.01$
	Tested for *S. aphanizomenoides*:	
	IP	$F_{(4, 73)} = 226.29$, $p < 0.001$
	N:P atomic ratio	$p > 0.05$
	IP × N:P atomic ratio	$p > 0.05$
	IN	$F_{(14, 73)} = 66.14$, $p < 0.01$
	Tested for *C. bergii*:	
	IP	$p > 0.05$
	N:P atomic ratio	$p > 0.05$
	IN	$p > 0.05$
	IP × N:P atomic ratio	$p > 0.05$

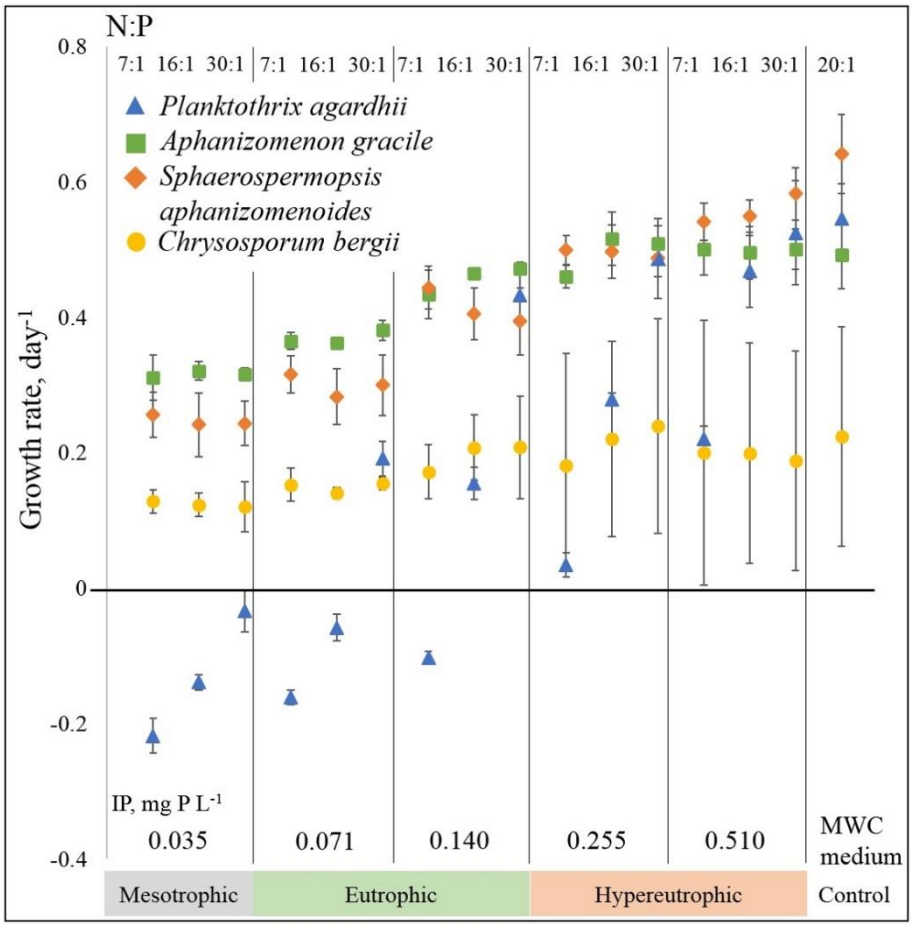


Fig. 3.11. Growth rates (day^{-1}) of native and alien cyanobacteria species cultured under different nutrient concentrations and N:P ratio at 24°C.

3.11.2. Nutrients effect on production of cyanotoxins and non-ribosomal peptides

The toxic *Aphanizomenon gracile* strain (NRC_SIR/B41-09) grew positively under all experimental treatments and the STX concentration considerably ranged from 17.29 to 481.03 ng g^{-1} of freeze-dried weight in the experiment samples (Fig. 3.12.). The highest toxin concentration was detected at the lowest IP concentration (0.035 mg P L^{-1}) and at N:P atomic ratio 7:1 and 30:1. Generally, the STX concentrations were higher at N:P atomic ratio 7:1. However, the regression analysis did not reveal a relationship between *A. gracile* growth rate, IP concentrations, N:P atomic ratio to saxitoxin concentrations ($p > 0.05$).

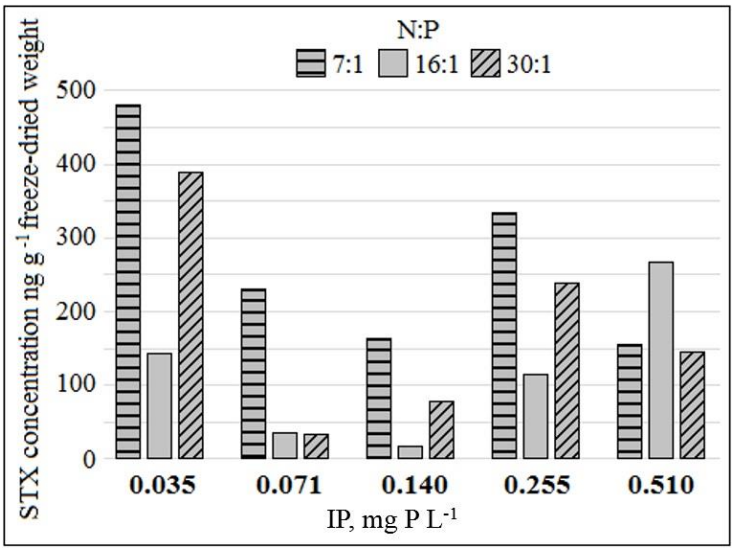


Fig. 3.12. Saxitoxin amount in *Aphanizomenon gracile* strain NRC_SIR/B41-09 grown under various IP concentrations and N:P ratio.

Total MCs concentration in the toxic *Planktothrix agardhii* strain NRC_SIR/F5-09 ranged from 9.83×10^4 to 1.16×10^6 ng g^{-1} freeze-dried weight (Fig. 3.13.). The proportion of MCs variants slightly varied in all tested treatments. Two MCs variants, dmMC-RR and dmMC-LR, prevailed and the highest amount was detected at the lowest IP concentration (0.140 mg P L^{-1}). The MC-YR formed negligible part in all treatments. The dmMC-RR was higher up to 1.8-fold than dmMC-LR. The tested *P. agardhii* strains formed insufficient biomass at lower IP concentrations required for the analysis of secondary metabolites and that hampered to draw clear conclusions on nutrient effect on MCs and NRPs variation.

Three oligopeptides classes (APs, AERs and CPs) were detected in *P. agardhii* toxic (MCs producing) and non-toxic strains used in the experiment (Fig. 3.14.). The relative concentration of total oligopeptides in the non-toxic *P. agardhii* strain were up to 3.07-fold higher than in the toxic strain under all examined treatments. APs and AERs were predominant and shared approximately an equal part in the toxic strain. Contrary, AERs dominated over APs in the non-toxic strain.

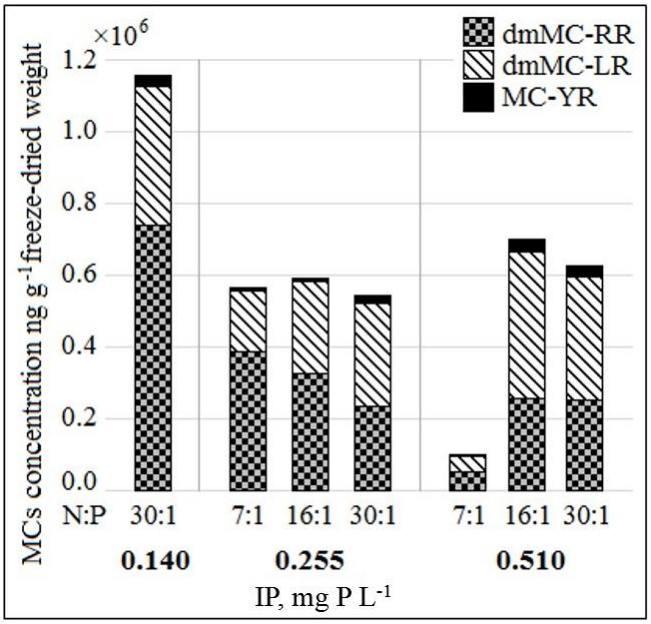


Fig. 3.13. The concentrations of microcystin in toxic *Planktothrix agardhii* strain NRC_SIR/F5-09 under the tested IP concentrations and N:P ratio.

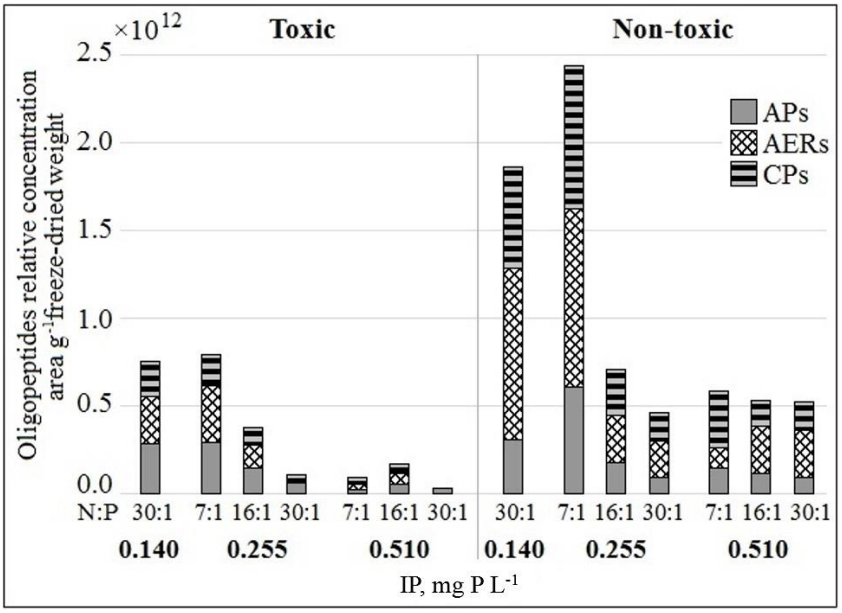


Fig. 3.14. Relative concentration of oligopeptides in the biomass of toxic and non-toxic *Planktothrix agardhii* strains under the tested IP concentrations and N:P ratio. Classes of oligopeptides: APs – anabaenopeptins, AERs – aeruginosins, CPs – cyanopeptolins.

The correlation analysis revealed strong negative relationship between IP concentration and concentration of dmMC-RR, APs and CPs in toxic *P. agardhii* strain ($r = -0.86$, $r = -0.81$ and $r = -0.85$, $p < 0.05$, respectively), while positive relationship was found between toxic *P. agardhii* strain growth rate and the amount of dmMC-LR, MC-YR ($r = 0.78$, $r = 0.82$, $p < 0.05$, respectively). The growth rate of non-toxic *P. agardhii* strain correlated with relative amount of APs and CPs ($r = -0.82$, $r = -0.78$, $p < 0.05$, respectively).

3.12. Experiment III: Interspecies competition

The results based on the analysis of biomass values on the final day of the experiment using the General linear model (GLM) showed that nutrient (IP and IN concentrations had the greatest effect on cyanobacteria species competition, also the temperature effect, temperature/nurient interaction and species taxa had also strong significant effect (Table 3.6.). For native *Planktothrix agardhii* the most important factors were nutrients (IP and IN $F_{(1, 16)} = 37.33$, $p < 0.001$) and alien species ($F_{(1, 16)} = 9.05$, $p < 0.01$), whereas for native *Aphanizomenon gracile* – nutrients ($F_{(1, 16)} = 16.44$, $p < 0.001$) and temperature ($F_{(1, 16)} = 6.86$, $p < 0.05$). For alien *Sphaerospermopsis aphanizomenoides*, most of the tested factors showed high significance (Table 3.6), while for *Chrysosporum bergii* – combination of nutriens and temperature ($F_{(1, 16)} = 14.60$, $p < 0.01$) and native species ($F_{(1, 16)} = 6.16$, $p < 0.05$).

Native *P. agardhii* and *A. gracile* species competed for nutrients, especially in the treatments with higher IP and IN concentration (Fig. 3.15). According to statistical analysis, nutrients were the most important factors for native species ($F_{(1, 16)} = 37.33$, $p < 0.001$ for *P. agardhii*; $F_{(1, 16)} = 16.44$, $p < 0.001$ for *A. gracile*). At the elevated nutient concentrations and 24°C temperature, co-cultured *A. gracile* biomass was 2.5 times higher (59.7 mg L^{-1}) compared to *P. agardhii* (23.7 mg L^{-1}). Compared to controls, *A. gracile* biomass was aproximately similar; however, the biomas of *P. agardhii* in co-cultured was 3-6.5 times lower in the treatments with higher IP and IN concentrations. This indicated that *P. agardhii* growth was suppressed by *A. gracile*.

Table 3.6. Results of the General linear model (GLM) factors on the biovolume of cyanobacteria strains

Response variable	Factor	F value, p
Final day biovolume	**Tested for all cyanobacteria biovolumes including controls:**	
	IP and IN	$F_{(1, 112)} = 29.50$, $p < 0.001$
	Temperature	$F_{(1, 112)} = 23.10$, $p < 0.001$
	Temperature × IP and IN	$F_{(1, 112)} = 15.44$, $p < 0.001$
	Species	$F_{(3, 112)} = 14.02$, $p < 0.001$
	Temperature × IP and IN × Species	$F_{(3, 112)} = 5.80$, $p < 0.001$
	Temperature × Species	$F_{(3, 112)} = 5.62$, $p < 0.001$
	IP and IN × Species	$F_{(3, 112)} = 5.48$, $p < 0.001$
	Tested for *P. agardhii*:	
	IP and IN	$F_{(1, 16)} = 37.33$, $p < 0.001$
	Temperature	$p > 0.05$
	N:P atomic ratio	$p > 0.05$
	Alien	$F_{(1, 16)} = 9.05$, $p < 0.01$
	Alien × Temperature	$F_{(1, 16)} = 5.65$, $p < 0.05$
	Tested for *A. gracile*:	
	IP and IN	$F_{(1, 16)} = 16.44$, $p < 0.001$
	Temperature	$F_{(1, 16)} = 6.86$, $p < 0.05$
	N:P atomic ratio	$p > 0.05$
	Alien	$p > 0.05$
	Temperature × IP and IN	$F_{(1, 16)} = 5.23$, $p < 0.05$
	Tested for *S. aphanizomenoides*:	
	IP and IN	$F_{(1, 16)} = 74.46$, $p < 0.001$
	Temperature	$F_{(1, 16)} = 70.46$, $p < 0.001$
	N:P atomic ratio	$p > 0.05$
	Native	$F_{(1, 16)} = 61.18$, $p < 0.001$
	Native × Temperature	$F_{(1, 16)} = 47.88$, $p < 0.001$
	Temperature × IP and IN	$F_{(1, 16)} = 45.31$, $p < 0.001$
	Native × Temperature × IP and IN	$F_{(1, 16)} = 12.86$, $p < 0.01$
	Native × IP and IN	$F_{(1, 16)} = 12.49$, $p < 0.01$
	Tested for *C. bergii*:	
	IP and IN	$F_{(1, 16)} = 7.11$, $p < 0.05$
	Temperature	$p > 0.05$
	N:P atomic ratio	$p > 0.05$
	Native	$F_{(1, 16)} = 6.16$, $p < 0.05$
	Temperature × IP and IN	$F_{(1, 16)} = 14.60$, $p < 0.01$
	Native × IP and IN	$F_{(1, 16)} = 5.49$, $p < 0.05$

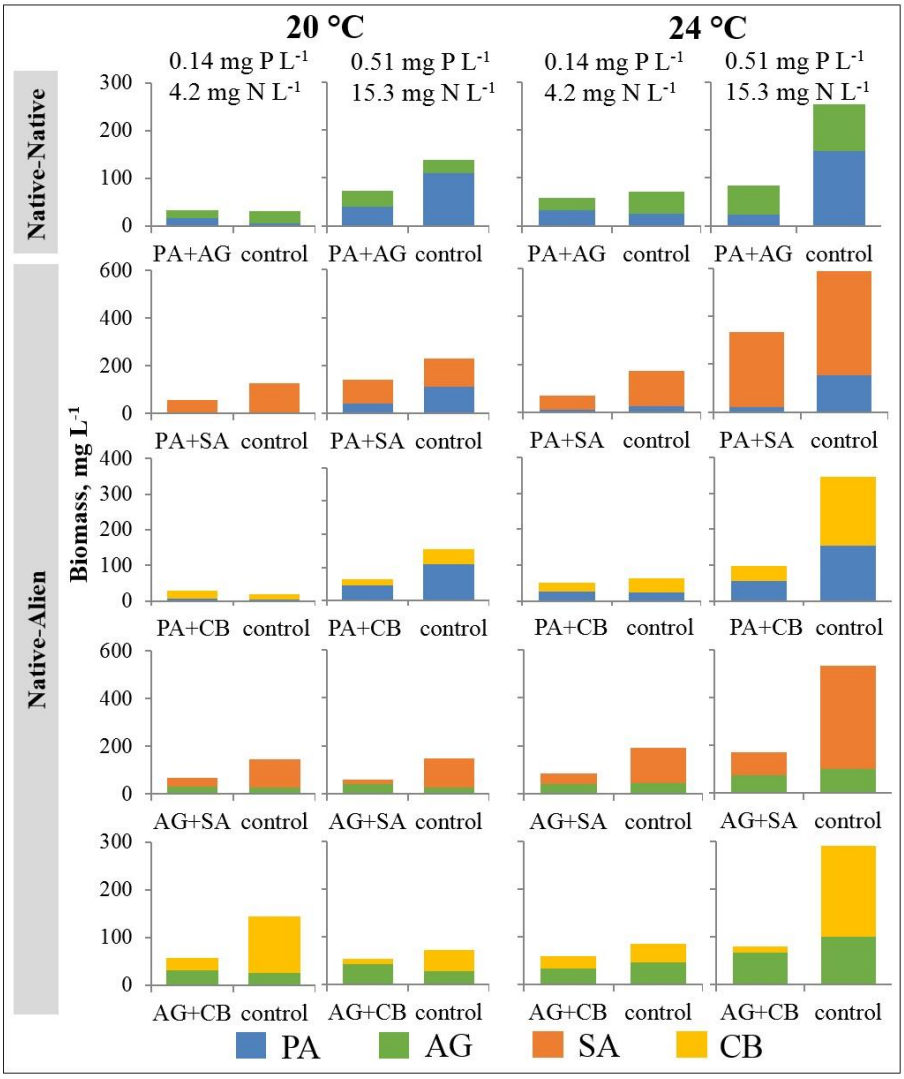


Fig. 3.15. Biomass of co-cultured native *P. agardhii*, *A. gracile* and alien *S. aphanizomenoides*, *C. bergii* species under different temperatures and IP concentrations (N:P ratio 30:1) at the end of the experiment. Cyanobacteria species: PA – *P. agardhii*, AG – *A. gracile*, SA – *S. aphanizomenoides*, CB – *C. bergii*.

Interactions among native and alien species were species specific. The statistic analysis showed that alien species significantly affected *P. agardhii* ($F_{(1,\ 16)}$=9.05, p<0.01) growth, however, the effect of alien *S. aphanizomenoides* was more obvious compared to *C. bergii*. The biomass of co-cultured *P. agardhii* was 2.3–14 times lower compared to *S.*

aphanizomenoides, especially in the treatment with elevated nutrient concentrations at 24°C (21.8 mg L^{-1} *vs.* 312 mg L^{-1}) (Fig. 3.15). Co-cultured *P. agardhii* biomass was up to seven times lower compared to controls, while only slight decrease of the biomass was seen for *S. aphanizomenoides.*

Availability of nutrients and temperature rather than interspecies competition had effect on *P. agardhii* and *C. bergii* growth. *P. agardhii* biomass at elevated IP and IN concentrations was 2–7.4 times higher (e.g. 47.1 mg L^{-1} *vs.* 6.3 mg L^{-1} at 20°C), whereas *C. bergii* build up the highest biomass in the treatments with high nutrients at 20°C (47.1 mg L^{-1}) (Fig. 3.15). Interspecies competition was more obvious in co-culture treatments at high nutrient concentrations and temperature, where species biomass was lower 2.8 and 4.6 times for *P. agardhii* and *C. bergii*, respectively.

A. gracile biomass in co-cultures was similar to *S. aphanizomenoides* and about two times higher than *C. bergii* (on average, 42.4 mg L^{-1} vs. 19.5 mg L^{-1}) in most of the treatments. Higher nutrients predetermined slight increase of *A. gracile* biomass and this was supported by GLM ($F_{(1, 16)} = 16.44$, $p < 0.001$ for IP and IN). At 20°C temperature, *A. gracile* biomass in co-cultures with both alien cyanobacteria was up 1.5 times higher than in control treatments. Compared to controles, *S. aphanizomenoides* biomass was by 3.3–6.7 times lower, whereas *C. bergii* biomass – up to 15 times lower in co-cultures with native *A. gracile*. The assessment revealed that *A. gracile* did not suffer from alien (Table 3.6.), but had negative effect on both alien species ($F_{(1, 16)} = 61.18$, $p < 0.001$ for *S. aphanizomenoides*; $F_{(1, 16)} = 6.16$, $p < 0.05$ for *C. bergii*).

4. DISCUSSION

4.1. Cyanobacteria blooms and bloom-forming species

Since early 1980s toxic cyanobacteria proliferation in Europe has already been recognized as a growing problem due to heavy blooms followed by intoxications reported in 11 out of 26 countries (Skulberg et al., 1984). Recently, harmful cyanobacteria blooms is a global problem, therefore, the diversity and dynamics of cyanobacteria in phytoplankton of freshwater aquatic ecosystems have been extensively investigated. Nevertheless, scientific knowledge about toxic cyanobacteria in Europe has extended rapidly, some key questions are still open and the recent cyanotoxin poisonings in continent clearly illustrate serious health hazard to the human population (Meriluoto et al., 2017).

Chl-*a* concentrations corresponding to phytoplankton blooms may reach 30–50 μg L^{-1} in meso-eutrophic and 300–400 μg L^{-1} in hypereutrophic ecosystems (Humbert and Fastner, 2017). The average chl-*a* concentrations in Lake Širvys (34.5 ± 15.8 μg L^{-1}) and Lake Jieznas (61.4 ± 11.2 μg L^{-1}) indicated intense blooms in the lakes. Maximum total biomass of cyanobacteria in both lakes (up to 29.2 mg L^{-1} in Lake Širvys and up to 24.7 mg L^{-1} in Lake Jieznas; Fig. 3.1) was two times greater than high alert level threshold (12 mg L^{-1}) assessed to bathing waters (WHO, 2003). Similar biomass of cyanobacteria up to 30 mg L^{-1} has been found in other Lithuanian blooming lakes (Kasperovičienė, 2007). In European lakes, cyanobacteria can form substantial biomass reaching up to 200 mg L^{-1} (Dembowska, 2011; Humbert and Fastner, 2017; Stoyneva-Gärtner et al., 2017a). Cyanobacteria comprised up to 98% of total phytoplankton biomass during the blooms that appeared in summer and/or autumn in the studied lakes. It is in the same line with the studies in Lithuanian freshwaters, where cyanobacteria biomass has constituted 50–96% (Kavaliauskienė, 1996; Kasperovičienė et al., 2005) and more than 90% of total phytoplankton in other European water bodies (Yéprémian et al., 2007; Grabowska et al., 2014). The potentially toxic 19 species belonging to *Microcystis*, *Dolichospermum*, *Woronichinia*, *Limnothrix*, *Aphanizomenon*, *Planktothrix*, *Anabaenopsis*, *Cylindrospermopsis*, *Cuspidothrix*, *Raphidiopsis*, *Sphaerospermopsis*, *Planktolyngbya* were found in the lakes (Table 3.2.). *Planktothrix agardhii* and *Aphanizomenon gracile* species dominated in the shallow lakes selected for the current study. Those species are related to frequently mixed shallow turbid eutrophic freshwaters (Häggqvist et al., 2017). Bloom-forming

cyanobacteria species are well known from Lithuanian shallow lakes (Jankavičiūtė, 1962, 1963, 1986; Kasperovičienė, 2001) and worldwide (Buratti et al., 2017). The results obtained in this study and from other research (Kasperovičienė, 2007; Grabowska et al., 2014) illustrate that *Planktothrix agardhii* and *Aphanizomenon gracile* usually co-occur with *Limnothrix*, *Planktolyngbya* and *Pseudanabaena*.

Planktothrix agardhii was recognized as one of the most common bloom forming species in shallow temperate lakes (Scheffer et al., 1997). In Lake Širvys, the biomass of *P. agardhii* reached up to 28 mg L^{-1} (max. 97% of total phytoplankton biomass in September). In European eutrophicated freshwaters, the species forms the highest biomass in autumn up to 15 mg L^{-1} in Germany (max. 78% of total phytoplankton biomass; Rücker et al., 1997) or even up to 54–70 mg L^{-1} in Poland (45–100% of total phytoplankton biomass; Pełechata et al., 2006; Grabowska et al., 2014). Recurrent dominance of *P. agardhii* in shallow eutrophic and hypertrophic lakes is a common phenomenon (Mur, 1983; Rücker et al., 1997) and also is observed in Lake Širvys, where *P. agardhii* blooms have been known since 2009 (Koreivienė, unpublished).

Lake Jieznas is described as hypertrophic lake, where cyanobacteria species *Microcystis aeruginosa*, *Aphanizomenon flos-aquae* and *Dolichospermum macrospora* dominate (Kavaliauskienė, 1996). These species have been replaced recently by dominant *Aphanizomenon gracile* and prevailing *Limnothrix planctonica*, *Planktolygbya limnetica*. Similar prevailing species composition (*Aphanizomenon gracile*, *Planktolyngbya limnetica*, *Limnothrix redekei*) is also characteristic of shallow eutrophic Lake Jeziorak Mały from Poland (Zębek, 2006). Dominant *Aphanizomenon gracile* in Lake Jieznas comprised maximum biomass of 12.7 mg L^{-1} in August (max. 45% of total phytoplankton biomass) (Fig. 3.1.). In other European lakes, *A. gracile* is frequent and dominant species that form similar biomass up to 15 mg L^{-1} (Rücker et al., 2007; Dolman et al., 2012) and the highest value recorded 33 mg L^{-1} (max. 80% of total phytoplankton biomass; Mischke and Nixdorf, 2003) in lakes in Germany, also up to 20.5 mg L^{-1} in freshwaters of Poland (max. 65.8% of total phytoplankton biomass; Kokociński et al., 2013; Toporowska et al., 2016). The prevailing species in Lake Jieznas overlapped, but distinct niches as *Limnothrix planctonica* comprised higher biomass from May to August and *Planktolyngbya limnetica* increased biomass from July to October (Fig. 3.1.). Similarly, Zębek (2006) has found the highest biomass of *P. limnetica* in July.

4.2. The profile of cyanotoxins and non-ribosomal peptides

Although the data on cyanotoxins in the European continent have been relatively well-covered, Meriluoto et al. (2017) have highlighted disproportionalities in the knowledge about MCs versus other cyanotoxins. The occurrence of STX produced by freshwater cyanobacteria and bioactive NRPs is still very limited (Testai et al., 2016; Buratti et al., 2017; Janssen, 2019). The current study substantially filled the gap not only on lacking data particularly to Lithuanian inland waters, but also provided the profile of secondary metabolites of toxic cyanobacteria and the data on scarcely found cyanotoxins. Potentially toxic cyanobacteria species that dominated in the studied lakes (Table 3.2.) determined different profiles of cyanotoxins and their seasonal variation. In general, concentrations of MCs, ATX-a and NRPs were significantly higher in Lake Širvys along cyanobacteria growth season, whereas STX occurred mainly in August and the concentrations were similar in both lakes (Fig 3.3.). ATX-a, STX and NRPs were detected in inland water bodies in Lithuania for the first time. The previous few studies on cyanotoxins have been mainly focused on MCs assessment (Kasperovičienė et al., 2005; Kasperovičienė, 2008).

MCs producing cyanobacteria blooms have been described in eighty countries worldwide (Catherine et al., 2017). In Europe, they occur in all countries and a substantial number of data have been published in the past decades (Descy et al., 2009; Turner et al., 2018). Hepatotoxic MCs are the most common among the cyanotoxins in freshwaters across Europe (Meriluoto et al., 2017) and concentrations usually found in the range of 0.1–10 μg L^{-1} (Greer et al., 2016; Pitois et al., 2018) and in the scums can reach the amount as high as 2800 μg L^{-1} (Faassen and Lürling, 2013).

MCs were detected in the period from April to November in Lake Širvys and constituted up to 16.72 μg L^{-1} (average 5.63 ± 6.14 μg L^{-1}), whereas hepatotoxins were detected in Lake Jieznas from June to September with the maximum concentration 0.96 μg L^{-1} (average 0.59 ± 0.34 μg L^{-1}). In the studied Lithuanian lakes, MCs concentrations in summer and autumn vary from 0.25 to 1.71 μg L^{-1} as has been determined by ELISA (Kasperovičienė et al., 2005; Kasperovičienė, 2008).

The acute hepatotoxicity of different MCs variants ranges from 50 to 1200 μg kg^{-1} bw (LD_{50} i.p. in mice) (Catherine et al., 2017). Therefore, it is crucial to determine not only the total MCs concentration, but also to reveal the profile as contribution of a particular MCs variant to bloom toxicity is not similar (Faassen and Lürling, 2013). Five different variants of MCs were

detected in the studied lakes: MC-YR, dmMC-RR, MC-RR, dmMC-LR and MC-LR (Fig 3.3.). The most toxic MC-LR variant was detected at low concentrations up to 0.15 μg L^{-1} in the studied lakes and did not exceed the recommended guideline 20 μg L^{-1} for bathing waters (WHO, 2003). In Lake Širvys, less toxic dmMC-RR was the dominant variant with maximum value reached up to 16.00 μg L^{-1} that comprised 96% of total MCs concentration. In Lake Jieznas, the prevailing variant was MC-RR with maximum value 0.81 μg L^{-1} that comprised 84% of total MCs concentration. All other variants comprised negligible part of total MCs concentration.

Several researches have shown that MCs typically co-occur with other bioactive NRPs and never occur alone (Janssen, 2019). This coincided with the data of the current study, where MCs production coupled with higher relative amount of NRPs with most common APs and AERs in the studied Lake Širvys. Similarly, according to Grabowska et al. (2014), field samples from the water reservoir with dominant *Planktothrix agardhii* were rich in MCs, APs, AER and planktocyclin. Moreover, high amount of NRPs detected in Lake Širvys could also contribute to the bloom toxicity. *P. agardhii* extract rich in MCs, APs and AERs is toxic to zooplankton (Pawlik-Skowrońska et al., 2019). Thus, non-ribosomal oligopeptides, other than MCs, has an essential contribution to the toxicity to invertebrates and their toxic effect can vary depending on the cyanobacteria secondary metabolite profile.

Neurotoxins are much less common compared to MCs, but are still found in a large variety of European freshwater ecosystems. Over 65 cyanobacterial neurotoxins have been described to date of which the most studied and diverse are anatoxins and saxitoxins (Kellmann et al., 2013). ATX-a and/or homo-ATX-a are more often recorded than ATX-a(s) and STX (Meriluoto et al., 2017). Despite low quantities of neurotoxins (ATX-a up to 0.97 μg L^{-1} and STX up to 1.06 μg L^{-1}), they contributed to the toxicity of blooms in the studied lakes mainly in the summer months (Fig. 3.3.). In Europe, the ATX-a has been detected mainly at low concentrations up to 2.19 μg L^{-1} (Carrasco et al., 2007; Dolman et al., 2012; Toporowska et al., 2016; Pitois et al., 2018). Much higher amounts of STX have been recorded in the continental freshwaters (usually up to 2.5 μg L^{-1}; max. 26.1 μg L^{-1} in Spain) (Wörmer et al., 2011; Gkelis and Zaoutsos, 2014; Stoyneva-Gärtner et al., 2017b; Pitois et al., 2018) than was detected in two Lithuanian lakes. There are no guidelines for neurotoxins in Europe; however, neurotoxin concentration in Lithuanian lakes did not exceed recommended alert values

for STX – 3 µg L^{-1} (Fitzgerald et al., 1999) and for ATX-a 1 µg L^{-1} (Fawell et al., 1999).

Unfortunately, standardized methods studies on cyanotoxins are lacking as major part of investigations usually focused on one type of cyanotoxins (Meriluoto et al., 2017). The pioneering large-scale research on profile of various cyanotoxins (MCs, CYN and ATX) in integrated water column samples across Europe have revealed that total concentration of toxins is below 1 µg L^{-1} in 75% out of 137 lakes (Mantzouki et al., 2018b). The integrated water samples from Lakes Širvys and Jieznas were taken and analysed by standard method as well. Profile of cyanotoxins in the integrated water samples compared to the surface samples was similar in Lake Širvys (6.62 µg L^{-1} and 4.83 µg L^{-1}, respectively) and Lake Jieznas (1.85 µg L^{-1} and 2.10 µg L^{-1}, respectively) (Fig. 3.4.). Cyanotoxin profile in Lake Širvys was similar to other Lithuanian lakes, where MCs dominated (Fig. 4.1). The dmMC-RR was the most often detected and abundant among five MCs variants recorded; the amounts of other variants differed from lake to lake. The highest part of dmMC-RR was detected in Lake Širvys and other Lithuanian lakes. It is in agreement with the data from other European lakes (Mantzouki et al., 2018b), where dmMC-RR is rare, but shows the highest concentrations in the profile of tested cyanotoxins. According to Mantzouki et al. (2018b), the most abundant are MC-YR and dmMC-LR, followed by MC-LR which suggests that risk assessment should be broaden and addressed not only to MC-LR, but also to other variants of cyanotoxin. Cytotoxin CYN (0.38 µg L^{-1}) was also detected in the integrated samples from Lakes Širvys and Jieznas. Due to prevailing ATX (1.33 µg L^{-1}) in Lake Jieznas, the profile differed significantly from the other Lithuanian lakes studied (Fig. 4.1). Although ATX have also been detected in 52 other European lakes (Mantzouki et al. 2018b), neurotoxin concentration in Lake Jieznas was the highest.

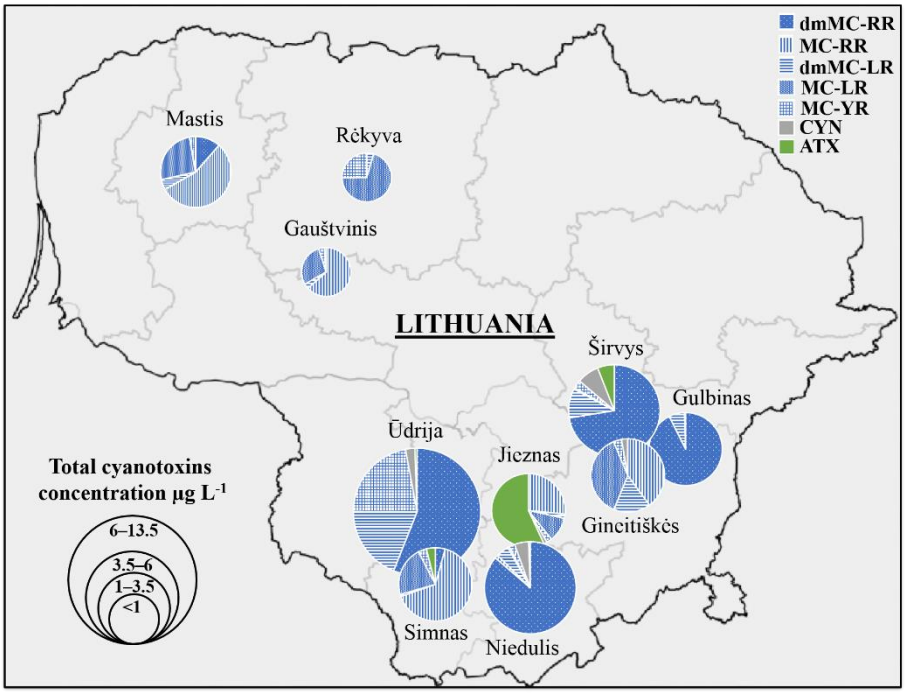


Fig. 4.1. The profiles of cyanotoxins (MCs, CYN and ATX) in Lithuanian lakes included in snap-shoot analysis in 2015 (after Mantzouki et al., 2018b).

4.3. Producers of cyanotoxins and non-ribosomal peptides

Bloom-forming cyanobacteria species are distinguished by different abilities to produce toxic and bioactive compounds. Toxin production during blooms varies in space and time (Sabart et al., 2010; Mantzouki et al., 2018a) and it is hard to predict from cyanobacteria diversity and abundance analysis (Huisman et al., 2018). The current study determined seasonal dynamics of *mcy*E synthetase gene copy number of *Planktothrix agardhii* in the environment samples that mainly concur with the species biomass and MCs concentration (Fig. 3.5.). Similarly, high positive correlation between *P. agardhii* biomass and total MCs concentration has been observed in some other European lakes (Dolman et al., 2012; Papadimitriou et al., 2013; Grabowska et al., 2014). The obtained results can be explained due to findings of high 93% proportion of toxic *P. agardhii* strains. This is consistent with previous studies by Kurmayer et al. (2004) and Yéprémian et al. (2007), where 88% and 52% rate of toxic *P. agardhii* strains isolated from Austrian and Swiss lakes has been detected. Meanwhile, Briand et al.

(2008b) have suggested that *Planktothrix* proportion of MCs producer genotypes in blooms is higher than for *Microcystis*.

Determination of toxin producers based on dominant species in bloom samples may often lead to misidentification, therefore, synthetase gene detection and/or evaluation of intracellular toxin in the isolate's biomass should support identification of the real producer in a particular water body. A total of 274 strains of 15 species were tested for the presence of cyanotoxins and NRPs compounds in the current study. *Planktothrix agardhii* was confirmed as the main species that contributed to MCs production in Lake Širvys, because most isolates contained *mcy*E synthetase gene and had ability to produce MCs. This species is widely known as a producer of various MCs variants (Yéprémian et al., 2007; Kosol et al., 2009). The percentage of toxic genotypes in *P. agardhii* population from Lake Širvys was high and varied insignificantly during the growth season that supported findings of the other researchers on high stability of *Planktothrix* genetic structure in the population (Kurmayer et al., 2016). Apart *P. agardhii* other species such as *Microcystis aeruginosa*, *M. flos-aquae* and *M. viridis* were confirmed as MCs producers in the studied lakes as well. *Microcystis* is the best-known genus for its ability to produce the MCs (Harke et al., 2016). In general, *P. agardhii* and *Microcystis* spp. strains were able to produce four MCs variants that were also determined from the biomass in studied lakes: dmMC-RR, MC-RR, dmMC-LR and MC-YR (Table 3.3.).

Besides MCs, high amounts of NRPs in Lake Širvys environmental samples were detected with the dominant APs and AERs which production for *Planktothrix agardhii* was confirmed based on strain analysis (Table 3.3.). The strains of *P. agardhii* and *Microcystis* spp. were rich in NRPs. Similarly, other studies showed that *P. agardhii* and *Microcystis* species can synthesize a great variety of bioactive compounds (Grabowska et al., 2014; Harke et al., 2016; Kurmayer et al., 2016). Five APs, AER and planktocyclin were present in field samples where *Planktothrix agardhii* dominated (Grabowska et al., 2014). The current study confirmed NRPs synthesis of the *Aphanizomenon gracile*, *Dolichospermum lemmermannii*, *Planktothrix agardhii* and *Microcystis* spp. strains, however, particular oligopeptide production was species and strain specific. Other researches have also referred these genera as producers of NRPs with exception of *A. gracile* (Welker et al., 2004; Sivonen and Börner, 2008).

The neurotoxin STX concentrations coincided with the greatest biomass of *A. gracile*, and those of ATX-a – with *Cuspidothrix issatschenkoi* biomass

in the lakes (Figs. 3.1., 3.3.). However, ATX-a producers from various cyanobacteria species tested were not confirmed after strain assessment. Toporowska et al. (2016) have found connection between intracellular ATX relationship and the biomass of *C. issatschenkoi* as well. ATX-a production in *C. issatschenkoi* strains has been confirmed by Wood et al. (2007) and Ballot et al. (2010b). *Aphanizomenon gracile* is well recognized STX producer in European freshwaters (Pereira et al., 2004; Ballot et al., 2010a; Ledreux et al., 2010). In the current study, 123 strains were tested for neurotoxins, but STX synthesis was confirmed only for *A. gracile* of which 17% (of 63 tested strains) contained *sxt*A synthetase gene. Similar data have been obtained for *A. gracile* strains from German lakes (Ballot et al., 2010a). However, low *sxt*A gene activation has been detected in *A. gracile* strains isolated from freshwaters in France (Ledreux et al., 2010). Partial *sxt*A gene sequences of *A. gracile* strains were highly homogenous among the strains in the studied lakes (Fig. 3.8.). According to Ledreux et al. (2010), *A. gracile* strains from Spain and Germany are also grouped within a monospecific and highly supported cluster.

In the current study, alien species *Sphaerospermopsis aphanizomenoides*, *Chrysosporum bergii* and *Raphidiopsis mediterranea* were not determined as the producers of cyanotoxins, even they are known able to synthesise toxins (Schembri et al., 2001; Namikoshi et al., 2003; Sabour et al., 2005; Ledreux et al., 2010). This phenomenon has also been reported to other alien species *Cylindrospermopsis raciborskii*, which is widespread species through the continents, however, CYN production has not been detected in Europe (Rzymski and Poniedziałek, 2015).

So, two dominant species *Planktothrix agardhii* and *Aphanizomenon gracile* were confirmed as main producers of MCs, NRPs and STX in the studied lakes. Other species *Microcystis aeruginosa*, *M. flos-aquae*, *M. viridis* and *Dolichospermum lemermannii* likely contributed in low rate to the total amount of cyanotoxins and bioactive compounds due to negligible biomass detected in the lakes.

4.4. Environmental variables important for native and alien cyanobacteria proliferation and production of cyanotoxins

The world has recently been undergoing global changes and the most important challenges are warming and anthropogenical eutrophication (Huisman et al., 2018). Temperature rise will have a significant influence on already existing phytoplankton communities and support alien species

establishment (Sukenik et al., 2012). Over-enrichment of nutrients during recent decades has been associated with agricultural, urban and industrial development that accelerate eutrophication processes in aquatic ecosystems resulting in increased rate of primary production, cyanobacteria community changes and proliferation of harmful blooms (Huisman et al., 2005; Paerl and Fulton, 2006). Heisler et al. (2008) have highlighted that combination of field and experimental studies are critical for further understanding of environmental variable role in harmful algae bloom trends and allow better prediction of their occurrence and selection strategy for mitigation. Laboratory studies of bloom-forming cyanobacteria species is an instrument to understand a particular species characteristics, ecophysiological response to nutrient forms, concentrations and temperature regimes, species-specific differences of growth characteristics and toxigenesis (Heisler et al., 2008).

Bloom-forming cyanobacteria thrived in shallow ecosystems such as the studied Lakes Širvys and Jieznas, comprising over 87% of total phytoplankton biomass. The results of field study in those two lakes revealed that nutrients (IP and N:P ratio) and to some extent temperature were the most significant factors for the amount of cyanobacteria biomass and production of cyanotoxins. (Fig. 3.6., 3.7.). Therefore, the experimental part of the current study brought to light how changes in nutrients (IP, IN, N:P) and temperature rise affect the growth rate of particular dominant in the studied lakes native bloom-forming species (*Planktothrix agardhii* and *Aphanizomenon gracile*), the production of toxins and the estabishment of alien cyanobacteria (*Sphaerospermopsis aphanizomenoides* and *Chrysosporum bergii*).

4.4.1. Temperature effect on bloom-forming species growth and cyanotoxin production

In Europe, during the last decade the temperature has increased by 1.3 ± 0.11°C (Kovats et al., 2014) and is expected to rise by 4°C close to 2100 (Brown and Caldeira, 2017). It causes concern associated with formation of cyanobacteria blooms and alien species distribution, also success of their establishment in new habitats. Native cyanobacteria *Planktothrix agardhii* formed bloom in Lake Širvys under temperature range of 14–20.8°C, and *Aphanizomenon gracile* formed biomass peak in Lake Jieznas, when the temperature reached 22.2°C. Experimental study showed that the temperature had a significant effect on the tested cyanobacteria growth rate (section 3.10.1.**)**. The field study and monoculture laboratory experiments

revealed similar growth characteristics of native *P. agardhii* and *A. gracile* under wide range of temperatures. In the studied lakes, during summer and autumn both species appeared at temperature range of 7.9–26.1°C that is in agreement with similar range of temperature (10.3–22.6°C) in other temperate lakes (Mischke and Nixdorf, 2003; Toporowska et al., 2010, 2016; Karosienė et al., 2019 under review). Toporowska et al. (2010) and Walls et al. (2018) have observed *P. agardhii* and *A. gracile* successful growth at low ≤ 4°C temperatures in temperate lakes. The experiment showed that temperature did not modify significantly native species growth rate at tested 18–30°C temperatures and it was more favoured at 20–28°C. This is partially consistent with other researches were 27°C optimal temperature for *P. agardhii* (Lürling et al., 2013; Gomes et al., 2015) and 28°C for *A. gracile* (Mehnert et al., 2010) have been assessed.

Sphaerospermopsis aphanizomenoides and *Chrysosporum bergii* are alien cyanobacteria species recently dispersed to Europe (Meriluoto et al., 2017; Kokociński and Soininen, 2019), which are much less studied than the other non-native species. Selected for experiments alien species often co-occur with native *P. agardhii* and *A. gracile* in the lakes (Koreivienė and Kasperovičienė, 2011; Budzyńska et al., 2019; Kokociński and Soininen, 2019). *C. bergii* has been recorded since 2008 in Lithuanian Lake Gineitiškės, but biomass remains low up to 0.26 mg L^{-1} (Koreivienė and Kasperovičienė, 2011). During this study, *S. aphanizomenoides* was detected in Lithuania for the first time, where it formed the highest biomass up to 1.03 mg L^{-1} in Lake Jieznas and single individuals in Lake Širvys. The northernmost point to Europe of both species has been recorded in Lithuanian lakes (Koreivienė and Kasperovičienė, 2011; Savadova et al., 2018). Usually these species are found in small amounts in different European lakes (Stüken et al., 2006; Ledreux et al., 2010; Kokociński and Soininen, 2019). However, *S. aphanizomenoides* seems to be established in the new habitats and forms biomass up to 22.3 mg L^{-1} (comprised 62% of total phytoplankton biomass) in the Rusałka Reservoir in Poland (Budzyńska and Gołdyn, 2017). The field and monoculture experimental studies have revealed differences in case of two alien species growth characteristics. The experimental results have shown greater growth rate under higher temperatures of both species, however, temperature range favourable for growth of *S. aphanizomenoides* is wider (20–30°C) compared to *C. bergii* (26–30°C). The results are in agreement with the findings by Mehnert et al. (2010), where optimum growth temperature for *S. aphanizomenoides* has been determined 29°C and for *C. bergii* 26°C. It

could be concluded that *S. aphanizomenoides* is highly strong invader under lower temperatures, and both species will gain advantage under warming conditions.

It is crucial to evaluate not only the impact of global warming on formation of cyanobacteria blooms, but also on bloom toxicity. Some studies have revealed that under warming growth of toxic strains is favoured over non-toxic (Davis et al., 2009; Dziallas and Grossart, 2011). Davis et al. (2009) have revealed that elevated temperature induces the growth rate of toxic *Microcystis* strains by 83% and non-toxic strain by 33%, and consequently more toxic blooms are expected. In the studied lakes, temperature was not determined as a significant factor for variation of cyanotoxin concentrations and only a positive correlation with ATX-a was found (Fig. 3.7.). The laboratory experiments with species monocultures revealed that temperature was important for MCs and NRPs production by *P. agardhii* as a higher amount of toxins was detected under the lower tested temperatures 18–24°C (Fig. 3.9, 3.10). This coincide with Lürling et al. (2017) and Bui et al. (2018), who have revealed a decline of MCs concentration in *Microcystis* at warmer temperatures. However, it is in contrary to the results of the study by Gianuzzi et al. (2016), were higher MCs cell quota in *M. aeruginosa* strain has been detected at higher (29°C) than at lower (26°C) temperature. Also, temperature influences the type of toxin produced in *Dolichospermum* spp. with enhanced production of MC-RR at higher than 25°C and favours MC-LR at lower temperatures (Figueiredo et al., 2004; Katırcıoğlu et al., 2004). However, in the current study, the proportion of MCs variants under different tested temperatures remained similar. It is likely that cyanotoxin profile is species and/or strain-specific (Table 3.3.), so probably differences exist not only in the strain growth rate characteristics, but also in cyanotoxin production.

Temperature was not suggested as an important environmental factor for neurotoxin STX production in the field studies of Lakes Širvys and Jieznas (Fig. 3.7.) and during the experiments (Fig. 3.9.). Consistent to the results of the current study, Casero et al. (2014) have detected STX production in *A. gracile* under wide range of temperatures was similar (15–28°C). Contrary, Dias et al. (2002) and Cirés et al. (2017) have found the rise of STX cell quota under warming. Temperature has a strong negative relationship with STX amount in benthic cyanobacteria *Microseira* (=*Lyngbya*) *wollei* (Smith et al., 2019). The ambiguous results of the studies have shown that temperature is not always significant factor for STX production.

4.4.2. Effect of nutrients on bloom-forming species growth and cyanotoxin production

Phosphorus and nitrogen may control the occurrence and intensity of the blooms (Paerl et al., 2008; Paerl and Huisman, 2009) and the particular situation depends on the specific response of potentially harmful cyanobacteria species. *Planktothrix agardhii* was the single dominant species that formed prolonged bloom in Lake Širvys, where IP ranged from 0.011 to 0.052 mg P L^{-1} and IN from 0.17 to 0.33 mg N L^{-1}. Similarly, the species tend to dominate at IP concentrations 0.020–0.158 mg P L^{-1} in other European lakes (Yéprémian et al., 2007; Toporowska et al., 2018). According to Mischke and Nixdorf (2003), *P. agardhii* suffers from nitrogen limitation when IN concentration is lower than 0.05 mg L^{-1}. Thus, concentration of nutrients in Lake Širvys was surplus for successful species proliferation, especially favoured by higher concentrations of phosphorus (Kokociński et al., 2010). The current study experiments proved that species growth was the best under eutrophic-hypertrophic conditions at N:P ratio 30:1 (Fig. 3.11.).

Another native species *Aphanizomenon gracile* was the dominant in Lake Jieznas, when IP was 0.021 mg P L^{-1}, and IN concentration 0.070 mg N L^{-1}. Statistical analysis in the studied lakes and nutrient experiment showed that IP had the strongest possitive relationship to biomass of *A. gracile* (Fig. 3.6, 3.11.). *A. gracile* growth rate gradually raised with increasing IP concentrations, however, N:P ratio effect was insignificant for species growth (Fig. 3.11.). According to Huszar et al. (2003), *A. gracile* occurs in shallow lakes with phosphorus concentrations from 0.01 to 0.03 PO_4^- L^{-1}. Diazotrophic cyanobacteria belonging to Nostocales, such as *A. gracile*, usually thrive under nitrogen deficient conditions and form blooms in the beginning of summer (Mischke and Nixdorf, 2003). However, Dolman et al. (2012) have found that *A. gracile* reaches the highest biomass in lakes with high N:P ratio. Also, that species grow well over a wide range of N and P concentrations that correspond to water bodies with high-N-low-P and low-N-high-P concentrations. It is suggesting that *A. gracile* is highly adapted species to various environmental conditions. The species is referred as typical cyanobacterium for temperate region and is characterized as dominant accompanied by other co-occurring prevailing species (Mischke and Nixdorf, 2003).

The nutrient experiment revealed that IP was significant factor for *Sphaerospermopsis aphanizomenoides* growth rate increase and the effect

was greater than for *Chrysosporum bergii* (Fig. 3.11.). This is in agreement with Sabour et al. (2009), who have shown experimentaly the maximum growth rate of *S. aphanizomenoides* under the highest nutrient concentrations. This also supports the findings by Budzyńska and Gołdyn (2017) from the field studies as *S. aphanizomenoides* is characterized as high nutrient demand species. The analysis of environmental studies performed by Kokociński and Soininen (2019), also have shown *C. bergii* preference at higher total phosphorus concentration. However, the performed experiments in the current study revealed species indifference to the increase of nutrients. N:P ratio that varied from 7 to 30 was not significant factor for growth rate of both tested alien species. Sabour et al. (2009) have shown that *S. aphanizomenoides* reaches optimal growth rate at N:P ratios from 1 to 15.

Stoichiometric theory of phytoplankton toxin regulation (Van der Waal et al., 2014) have stated that N limitation causes a reduction of N-rich toxins, P shortage causes an increase in the most N-rich STX and the limitation of both nutrients promotes the C-rich toxins. However, prediction of toxin type and concentrations in nutrient surplus conditions possibly do not follow those rules and is even more difficult to predict. Several studies have suggested that under N enrichment conditions, toxic blooms are more expected due to faster growth of toxic strains than non-toxic (Vézie et al., 2002) and higher MCs production (Welker et al., 2007; Davis et al., 2010). The studies in Lakes Širvys and Jieznas revealed that N:P ratio was a significant factor for the amount of cyanotoxins and NRPs (Fig. 3.6.), and correlated negatively with total MCs, dmMC-RR, dmMC-LR, APs and AERs. Similarly, experimental testing of *Planktothrix agardhii* demonstrated strong negative relationship of IP concentration and the amount of dmMC-RR, APs and CPs (Figs., 3.13., 3.14.). So, basically both nutrients could be significant for MCs and NRPs production. According to Vézie et al. (2002), MCs production in *Microcystis* spp. seems to be influenced by variation in N and P concentrations with different responses depending on the considered strain. However, a single variable can't fully describe the synthesis of toxins (Graham et al., 2004). Overall, complex environmental conditions rather than nutrients alone regulate *mcy* genes expression, and probably MC production is mostly related to cell division and growth (Orr and Jones, 1998; Briand et al., 2005; Kurmayer et al., 2016).

Information on factors that regulate neurotoxic saxitoxin production in cyanobacteria is very limited. Kellmann et al. (2008) have analysed STX gene cluster in cyanobacteria and concluded that probably the target toxin production may be regulated at the transcriptional level in response to the

availability of phosphate and other environmental factors. The results of the current study showed that STX positively correlated with IP concentration based on field samples (Fig 3.7.). Similarly, in the experimental study, STX concentrations were generally higher at the lowest tested N:P ratio 7:1 (Fig. 3.12.). However, the regression analysis did not reveal a relationship of *Aphanizomenon gracile* growth rate, IP concentrations, N:P ratio to STX concentrations. Smith et al. (2019) have studied benthic cyanobacteria *Microseira* (=*Lyngbya*) *wollei* and found that changes in nitrogen content are related to changes in STXs concentration.

4.5. Combined effect of environmental factors on interspecies competition

Eutrophication and climate warming simultaneously affect cyanobacteria community in natural ecosystems (O'Neil et al., 2012), therefore, more complex controlled experimental systems with multiple factors and mixes of species could give better understanding about the role of abiotic and biotic variables for the harmful algae blooms. Usually, the effect testing of environmental variables on strains is perfomed as monoculture experiments (Lürling et al., 2013; Gomes et al., 2015). Oberhaus et al. (2007) have revealed that growth of tested *Planktothrix agardhii* and *P. rubescens* species significantly differ in monoculture and mixed-culture competition experiments. Therefore, in order to elucidate competitive abilities between native bloom-forming and alien cyanobacteria species, strains were co-cultured under eutrophic and hypertrophic conditions at ambient (20°C) and warming scenario (24°C) simultaneously.

The performed experiments revealed that nutrient concentration had the greatest effect on species biomass formation and interspecies competition, but temperature and species origin were important at some extent also (Fig. 3.15.). The similar type of experiments are very limited, therefore, the obtained results are further compared to the data from field studies performed by other researchers. Wagner and Adrian (2009) have found that total phosphorus concentration is the principal force driving cyanobacteria proliferation, but also temperature positively influences cyanobacteria dominance in polymictic eutrophic lakes in Germany. The multi-lake study performed by Rigosi et al. (2014) has proved nutrients as a more powerful predictor of cyanobacterial biomass than temperature, while cyanobacteria community appears to be more sensitive to the interaction of nutrients and temperature in eutrophic and hyper-eutrophic lakes.

P. agardhii and *Aphanizomenon gracile* usually co-occur together and dominate at a particular conditions in lakes (Dolman et al., 2012; Toporowska et al., 2016). In the performed experiment, native *P. agardhii* and *A. gracile* species competed mainly for nutrients. It is likely that nutrients predetermined the dominant species and *P. agardhii* would be outcompeted by *A. gracile* under nutrients limited conditions. According to Dolman et al. (2012), both species grow well over a wide range of N and P concentrations, however, *A. gracile* forms the highest biomass at high nitrogen relative to phosphorus concentration. To withstand nutrient limitation, non-diazotroph *P. agardhii* is able to store N as phycocyanin (Van de Waal et al., 2010) and P as polyphosphate (Reynolds, 2006).

Ma et al. (2015) have found that allelopathic effect is likely to play a role in driving the seasonal alteration of dominant cyanobacteria species. Native *A. gracile* cyanobacteria suppressed biomass formation of native *P. agardhii* and both alien species likely due to alelopathic effect (Fig. 3.15.). Competition more accurately than monoculture experiments reflected the situation in the lakes, showing that alien species establishment was mainly suppressed by native species. Nevertheless, there are several studies where importance of secondary metabolites to interspecies competition among cyanobacteria has been proved (Rzymski and Poniedziałek, 2014; Ma et al., 2015; Briand et al., 2019). The conducted experiments demonstrated for the first time possible allelopathic effect of STX produced by *A. gracile* strain on other tested species isolated from the lakes in Lithuania. The current finding is confirmed by Kokociński and Soininen (2019), who have found negative relationship of *Chrysosporum bergii* biomass with the biomass of native *A. gracile* from field data analysis.

CONCLUSIONS

1. Cyanobacteria biomass in Lakes Širvys and Jieznas exceeded high alert level for bathing waters (up to 29.2 and 24.7 mg L^{-1}, respectively) and species belonging to the Nostocales and Oscillatoriales formed blooms from July to September/October. Nineteen species of potential cyanotoxin producers altogether constituted up to 76–98% of total phytoplankton biomass. *Planktothrix agardhii* was a single dominant (up to 28.1 mg L^{-1}) in Lake Širvys, whereas several species *Aphanizomenon gracile* (up to 12.7 mg L^{-1}), *Limnothrix planctonica* (up to 7.5 mg L^{-1}) and *Planktolygbya limnetica* (up to 5.5 mg L^{-1}) composed the bloom in Lake Jieznas.

2. Maximum concentration of the particular intracellular cyanotoxin did not exceed recommended values for freshwaters; however, the effect of co-occurring cyanotoxins can pose threat to humans and biota. Hepatotoxins microcystins (MCs) reached up to 16.72 µg L^{-1} in Lake Širvys (dominant dmMC-RR) and up to 0.96 µg L^{-1} in Lake Jieznas (dominant MC-RR) in September/October. Saxitoxin (STX), anatoxin-a (ATX-a), cylindrospermopsin (CYN) and bioactive non-ribosomal peptides (NRPs) were detected in Lithuanian lakes for the first time. Concentrations of STX (up to 1.06 µg L^{-1}), ATX-a (up to 0.97 µg L^{-1}) and cytotoxin CYN (up to 0.38 µg L^{-1}) were the highest in July/August. Total amount of NRPs was 3.5 times higher in Lake Širvys. Anabaenopeptins and aeruginosins prevailed in the lakes.

3. Investigations of the environmental samples and the strains confirmed that *Planktothrix agardhii* and *Aphanizomenon gracile* were the main producers of MCs and STX, respectively. *mcy*E gene was found in 93% of the tested *P. agardhii* strains, while 17% of *A. gracile* strains contained *sxt*A gene and produced toxin. MCs production was also detected in *Microcystis viridis*, *M. aeruginosa* and *M. flos-aquae* strains. NRPs synthesis was confirmed for *A. gracile*, *Dolichospermum lemmermannii*, *P. agardhii* and *Microcystis* spp. Producers of ATX-a were not determined. Alien to Europe cyanobacteria *Sphaerospermopsis aphanizomenoides*, *Raphidiopsis mediterranea*, *Chrysosporum bergii* were non-toxic.

4. Native cyanobacteria species were characterized by the similar growth rate under experimentally tested temperatures from 18°C to 30°C. Alien species reached maximum growth rate at the highest temperature, however, *Sphaerospermopsis aphanizomenoides* had wide temperature tolerance range (20°C–30°C). Under all tested nutrient concentrations, the highest growth rate was determined for *Aphanizomenon gracile* and *S. aphanizomenoides*

followed by *Chrysosporum bergii* strains. All species grew apparently better at elevated inorganic phosphorus concentrations, and only *Planktothrix agardhii* growth was suppressed by nutrient limitation and low N:P ratio.

5. Significant negative correlation between the temperature and the amount of total MCs and NRPs in the biomass of *Planktothrix agardhii* was determined during laboratory experiments. No relationship was detected between STX concentrations in the biomass of *Aphanizomenon gracile* with the tested temperature and nutrients concentration.

6. Nutrient concentration had the greatest effect on interspecies competition, whereas temperature and species itself were important to some extent also. Native *Planktothrix agardhii* and *Aphanizomenon gracile* competed mainly for nutrients, but native and alien species co-cultured acted differently. *A. gracile* suppressed the growth of native and alien species. Also, *P. agardhii* growth was suppressed by alien *Sphaerospermopsis aphanizomenoides*.

7. Overall, interrelated field and laboratory studies confirmed inorganic phosphorus as more significant factor than the temperature for growth and biomass formation of potential cyanotoxin producers. Both nutrients and temperature modified the profile of cyanotoxins and NRPs.

REFERENCES

Anne O., Bugajev D., Koreivienė J., 2015. Determining optimal growth conditions for the highest biomass microalgae species in Lithuanian part of the Curonian Lagoon for further cultivation. – International Journal of Environmental Research, 9(1): 233–246.

Antunes J.T., Leão P.N., Vasconcelos V.M., 2015. *Cylindrospermopsis raciborskii*: review of the distribution, phylogeography, and ecophysiology of a global invasive species. – Frontiers in Microbiology, 6: 1–13.

Balevičius A., 2009. Restauruotinų Lietuvos ežerų nustatymas ir preliminarus restauravimo priemonių parinkimas šiems ežerams, siekiant pagerinti jų būklę. – Aplinkos apsaugos agentūra. Probleminių ežerų restauravimo planai; galutinė ataskaita, 1, 2 dalys. – Vilnius.

Ballot A., Cerasino L., Hostyeva V. et al., 2016. Variability in the *sxt* gene clusters of PSP toxin producing *Aphanizomenon gracile* strains from Norway, Spain, Germany and 460 North America. – PLoS ONE, 11(12): e0167552.

Ballot A., Fastner J., Lentz M. et al., 2010b. First report of anatoxin-a-producing cyanobacterium *Aphanizomenon issatschenkoi* in northeastern Germany. – Toxicon, 56(6): 964–971.

Ballot A., Fastner J., Wiedner C., 2010a. Paralytic shellfish poisoning toxin-producing cyanobacterium *Aphanizomenon gracile* in Northeast Germany. – Applied and Environmental Microbiology, 76(4): 1173–1180.

Bartoli M., Zilius M., Bresciani M. et al., 2018. Drivers of Cyanobacterial Blooms in a Hypertrophic Lagoon. – Frontiers in Marine Science, 5: 434.

Bernard C., Ballot A., Thomazeau S. et al., 2017. Cyanobacteria associated with the production of cyanotoxins. – In: Meriluoto J., Spoof L., Codd G.A. (eds.), Handbook of Cyanobacterial Monitoring and Cyanotoxin Analysis. – John Wiley & Sons, Ltd., pp. 501–525.

Beversdorf L.J., Miller T.R., McMahon K.D., 2015. Long-term monitoring reveals carbon-nitrogen metabolism is key to microcystin production in eutrophic lakes. – Frontiers in Ecology, 6: 1–12.

Bláha L., Babica P., Maršálek B., 2009. Toxins produced in cyanobacterial water blooms – toxicity and risks. – Interdisciplinary Toxicology, 2(2): 36–41.

Bláhová L., Oravec M., Maršálek B. et al., 2009. The first occurrence of the cyanobacterial alkaloid toxin cylindrospermopsin in the Czech Republic as determined by immunochemical and LC/MS Methods. – Toxicon, 53: 519–524.

Bogially S., Bruno M., Curini R. et al., 2006. Monitoring algal toxins in lake water by liquid chromatography tandem mass spectrometry. – Environmental Science and Technology, 40: 2917–2923.

Bonilla S., Aubriot L., Soares M.C. et al., 2012. What drives the distribution of the bloom-forming cyanobacteria *Planktothrix agardhii* and *Cylindrospermopsis raciborskii*? – FEMS Microbiology Ecology, 79(3): 594–607.

Briand E., Gugger M., François J.-C. et al., 2008a. Temporal variations in the dynamics of potentially microcystin-producing strains in a bloom-forming *Planktothrix agardhii* (Cyanobacterium) population. – Applied and Environmental Microbiology, 74(12): 3839–3848.

Briand E., Reubrecht S., Mondeguer F. et al., 2019. Chemically mediated interactions between *Microcystis* and *Planktothrix*: impact on their growth, morphology and metabolic profiles. – Environmental Microbiology, 21(5): 1552–1566.

Briand E., Yéprémian C., Humbert J.F. et al., 2008b. Competition between microcystin- and non-microcystin-producing *Planktothrix agardhii* (cyanobacteria) strains under different environmental conditions. – Environmental Microbiology, 10(12): 3337–48.

Briand J.F., Jacquet S., Flinois C. et al., 2005. Variations in the microcystin production of *Planktothrix rubescens* (Cyanobacteria) assessed from a four-year survey of Lac du Bourget (France) and from laboratory experiments. – Microbial Ecology, 50: 418–428.

Briand J.F., Leboulanger C., Humbert J.-F. et al., 2004. *Cylindrospermopis raciborskii* (Cyanobacteria) invasion at mid latitudes: selection, wide physiological tolerance, or global warming? – Journal of Phycology, 40: 231–238.

Brient L., Lengronne M., Bormans M. et al., 2009. First occurrence of cylindrospermopsin in freshwater in France. – Environmenral Toxicology, 24: 415–420.

Brown P.T., Caldeira K., 2017. Greater future global warming inferred from Earth's recent energy budget. – Nature, 552: 45–52.

Bruno M., Barbini D.A., Pierdominici E. et al., 1994. Anatoxin-a and a previously unknown toxin in *Anabaena planctonica* from blooms found in Lake Mulargia (Italy). – Toxicon, 32: 369–373.

Bubik A., Sedmak B., Novinec M. et al., 2008. Cytotoxic and peptidase inhibitory activities of selected non-hepatotoxic cyclic peptides from cyanobacteria. – Biological Chemistry, 389(10): 1339–1346.

Budzyńska A., Gołdyn R., 2017. Domination of invasive Nostocales (Cyanoprokaryota) at 52°N latitude. – Phycological Research, 65: 322–332.

Budzyńska A., Rosińska J., Pełechata A. et al., 2019. Environmental factors driving the occurrence of the invasive cyanobacterium *Sphaerospermopsis aphanizomenoides* (Nostocales) in temperate lakes. – Science of the Total Environment, 650: 1338–1347.

Bui T., Dao T.S., Vo T.G. et al., 2018. Warming affects growth rates and microcystin production in tropical bloom-forming *Microcystis* strains. – Toxins, 10: 123.

Bumke-Vogt C., Mailahn W., Chorus I., 1999. Anatoxin-a and neurotoxic Cyanobacteria in German lakes and reservoirs. – Environmental Toxicology, 14: 117–125.

Buratti F.M., Manganelli M., Vichi S. et al., 2017. Cyanotoxins: producing organisms, occurrence, toxicity, mechanism of action and human health toxicological risk evaluation. – Archives of Toxicology, 91: 1049.

Calado S.L.M., Santos G.S., Wojciechowski J. et al., 2019. The accumulation dynamics, elimination and risk assessment of paralytic shellfish toxins in fish from a water supply reservoir. – Science of The Total Environment, 651(2): 3222–3229.

Callieri C., Bertoni R., Contesini M. et al., 2014. Lake level fluctuations boost toxic cyanobacterial "oligotrophic blooms". – PLoS ONE, 9(10): e109526.

Capelli C., Ballot A., Cerasino L. et al., 2017. Biogeography of bloom-forming microcystin producing and non-toxigenic populations of *Dolichospermum lemmermannii* (Cyanobacteria). – Harmful Algae, 67: 1–12.

Carey C.C., Ibelings B.W., Hoffmann E.P. et al., 2012. Eco-physiological adaptations that favour freshwater cyanobacteria in a changing climate. – Water Research, 46: 1394–1407.

Carmichael W.W., 2008. A world overview – one-hundred-twenty-seven years of research on toxic cyanobacteria – where do we go from here? In, Cyanobacterial Harmful Algal Blooms, State of the Science and Research Needs. H. K. Hudnell (Ed.), Springer New York. 619: 105–126.

Carmichael W.W., Azevedo S.M.F.O., An J.S. et al., 2001. Human fatalities from cyanobacteria: chemical and biological evidence for cyanotoxins. – Environmental Health Perspectives, 109: 663–668.

Carneiro R.L., Venâncio dos Santos M.E., Furlanetto Pacheco A.B. et al., 2009. Effects of light intensity and light quality on growth and circadian rhythm of saxitoxins production in *Cylindrospermopsis raciborskii* (Cyanobacteria). – Plankton Research, 31(5):4 81–488.

Carrasco D., Moreno E., Paniagua T. et al., 2007. Anatoxin-a occurrence and potential cyanobacterial anatoxin-a producers in Spanish reservoirs. – Journal of Phycology, 43(6) 1120–1125.

Casero M.C., Ballot A., Agha R. et al., 2014. Characterization of saxitoxin production and release and phylogeny of *sxt* genes in paralytic shellfish poisoning toxin-producing *Aphanizomenon gracile*. – Harmful Algae, 37: 28–37.

Catherine A., Bernard C., Spoof L. et al., 2017. Microcystins and Nodularins. In Handbook of Cyanobacterial Monitoring and Cyanotoxin Analysis, (Eds. Meriluoto J., Spoof L., Codd G. A.). – John Wiley & Sons, Ltd.

Chaffin J.D., Davis T.W., Smith D.J. et al., 2018. Interactions between nitrogen form, loading rate, and light intensity on *Microcystis* and *Planktothrix* growth and microcystin production. – Harmful Algae, 73: 84–97.

Chernova E., Sidelev S., Russkikh I. et al., 2017. *Dolichospermum* and *Aphanizomenon* as neurotoxins producers in some Russian freshwaters. – Toxicon, 130: 47–55.

Chia M.A., Kramer B.J., Jankowiak J.G. et al., 2019. The individual and combined effects of the cyanotoxins, anatoxin-a and microcystin-LR, on the growth, toxin production, and nitrogen fixation of prokaryotic and eukaryotic algae. – Toxins, 11: 43.

Chiswell R.K., Shaw G.R., Eaglesham G.K. et al., 1999. Stability of cylindrospermopsin, the toxin from the cyanobacterium *Cylindrospermopsis raciborski*: effects of pH, temperature and sunlight on decomposition. – Environmental Toxicology, 14: 155–161.

Chlipala G.E., Mo S., Orjala J., 2011. Chemodiversity in freshwater and terrestrial cyanobacteria - a source for drug discovery. – Current Drug Targets, 12(11): 1654e1673

Chorus I., Bartram J., (Eds.), 1999. Toxic cyanobacteria in water: a guide to their public health consequences, monitoring, and management. – E & FN Spon, London, UK.

Cirés S., Ballot A., 2016. A review of the phylogeny, ecology and toxin production of bloom-forming *Aphanizomenon* spp. and related species within the Nostocales (cyanobacteria). – Harmful Algae, 54: 21-43.

Cirés S., Delgado A., González-Pleiter M. et al., 2017. Temperature Influences the Production and Transport of Saxitoxin and the Expression of sxt Genes in the Cyanobacterium *Aphanizomenon gracile*. – Toxins, 9: 322.

Cirés S., Wörmer L., Ballot A. et al., 2014. Phylogeography of cylindrospermopsin and paralytic shellfish toxin-producing Nostocales cyanobacteria from Mediterranean Europe (Spain). – Applied and Environmental Microbiology, 80(4): 1359–1370.

Codd G.A., Azevedo S.M.F.O., Bagchi S.N. et al., 2005. CYANONET A Global Network for Cyanobacterial Bloom and Toxin Risk Management Initial Situation Assessment and Recommendations. – Technical Documents in Hydrology, 76 p.

Da Gomes A.M., Azevedo S.M.F.O., Lürling M., 2015. Temperature effect on exploitation and interference competition among *Microcystis aeruginosa*, *Planktothrix agardhii* and *Cyclotella meneghiniana*. – The Scientific World Journal, 2015: 834197.

Davis T.W., Berry D.L., Boyer G.L. et al., 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.

Davis T.W., Harke M.J., Marcoval M.A. et al., 2010. Effects of nitrogenous compounds and phosphorus on the growth of toxic and non-toxic strains of *Microcystis* during cyanobacterial blooms. – Aquatic Microbial Ecology, 61: 149–162.

Davis T.W., Orr P.T., Boyer G.L. et al., 2014. Investigating the production and release of cylindrospermopsin and deoxy-cylindrospermopsin by *Cylindrospermopsis raciborskii* over a natural growth cycle. – Harmful Algae, 31: 18–25.

De Figueiredo D.R., Azeiteiro U.M., Esteves S.M. et al., 2004. Microcystin-producing blooms—a serious global public health issue. – Ecotoxicology and Environmental Safety, 59: 151–163.

De la Cruz A.A., Antoniou M.G., Pelaez M. et al., 2011. Can we effectively degrade microcystins? – implications for impact on human health status. – Anti-Cancer Agents in Medicinal Chemistry, 11(1): 19–37.

De la Cruz A.A., Hiskia A., Kaloudis T. et al., 2013. A review on cylindrospermopsin: the global occurrence, detection, toxicity and degradation of a potent cyanotoxin. – Environmental Science: Processes & Impacts, 15(11): 1979–2003.

De Nobel W.T., Huisman J., Snoep J.L. et al., 1997. Competition for phosphorus between the nitrogen-fixing cyanobacteria *Anabaena* and *Aphanizomenon*. – FEMS Microbiology Ecology, 24: 259–267.

De Senerpont Domis L.N., Mooij W.M., Hülsmann S. et al., 2007. Can overwintering versus diapausing strategy in Daphnia determine match–mismatch events in zooplankton–algae interactions? – Oecologia, 150: 682–698.

Dembowska E., 2011. Cyanobacterial blooms in shallow lakes of the Iławskie Lake District. – Limnological Review, 11(2): 69–79.

Descy J.-P., Pirlot S., Verniers G. et al., 2009. Cyanobacterial blooms: toxicity, diversity, modelling and management "B-Blooms 2". – Final Report Phase 1. Brussels: Belgian Science Policy, 59 p.

Devlin J., Edwards O., Gorham P. et al., 1977. Anatoxin-a, a toxic alkaloid from *Anabaena flos-aquae* NCR-44h. – Canadian Journal of Chemistry, 55: 1367–1371.

Dias E., Pereira P., Franca S., 2002. Production of paralytic shellfish toxins by *Aphanizomenon* sp. LMECYA 31 (Cyanobacteria). – Journal of Phycology, 38: 705–712.

Dolman A.M., Rücker J., Pick F.R. et al., 2012. Cyanobacteria and cyanotoxins: the influence of nitrogen versus phosphorus. – PLoS ONE, 7(6): e38757.

Dolman A.M., Wiedner C., 2015. Predicting phytoplankton biomass and estimating critical N:P ratios with piecewise models that conform to Liebig's law of the minimum. – Freshwater Biology, 60: 4.

Donald D.B., Bogard M.J., Finlay K. et al., 2013. Phytoplanktonspecific response to enrichment of phosphorus-rich surface waters with ammonium, nitrate, and urea. – PLoS ONE, 8: e53277–e53277.

Downing J.A., Watson S.B., McCauley E., 2001. Predicting Cyanobacteria dominance in lakes. – Canadian Journal of Fisheries and Aquatic Sciences, 58(10): 1905–1908.

Duy T.N., Lam P.K.S., Shaw G.R. et al., 2000. Toxicology and risk assessment of freshwater cyanobacterial (blue-green algal) toxins in water. – Reviews of Environmental Contamination and Toxicology, 163:113–186. In: Ibelings B.W., Chorus I. (Eds.), 2007. Accumulation of cyanobacterial toxins in freshwater

‘‘seafood’’ and its consequences for public health: a review. – Environmental Pollution, 150: 177–192.

Dyble J., Tester P.A., Litaker R.W., 2006. Effects of light intensity on cylindrospermopsin production in the cyanobacterial HAB species *Cylindrospermopsis raciborskii*. – African Journal of Marine Science, 28: 309–312.

Dziallas C., Grossart H.P., 2011. Increasing Oxygen Radicals and Water Temperature Select for Toxic *Microcystis* sp. – PLoS ONE, 6: e25569.

Edwards C., Beattie K.A., Scrimgeour C.M. et al., 1992. Identification of anatoxin-a in benthic cyanobacteria (blue-green algae) and in associated dog poisonings at Loch Insh, Scotland. – Toxicon, 30: 1165–1175.

El-Shehawy R., Gorokhova E., Fernández-Piñas F. et al., 2012. Global warming and hepatotoxin production by cyanobacteria: What can we learn from experiments? – Water Research, 46(5): 1420–1429.

Faassen E.J., Harkemab L., Begemanb L. et al., 2012. First report of (homo)anatoxin-a and dog neurotoxicosis after ingestion of benthic cyanobacteria in The Netherlands. – Toxicon, 60: 378–384.

Faassen E.J., Lürling M., 2013. Occurrence of the Microcystins MC-LW and MC-LF in Dutch Surface Waters and Their Contribution to Total Microcystin Toxicity. – Marine Drugs, 11(7): 2643–2654.

Farkas O., Gyémant G., Hajdú G. et al., 2014. Variability of microcystins and its synthetase gene cluster in *Microcystis* and *Planktothrix* waterblooms in shallow lakes of Hungary. – Acta Biologica Hungarica, 65(2): 227–39.

Fastner J., Erhard M., von Döhren H., 2001. Determination of oligopeptide diversity within a natural population of *Microcystis* spp. (Cyanobacteria) by typing single colonies by matrix-assisted aaser desorption ionization–time of flight mass spectrometry. – Applied and Environmental Microbiology, 67(11): 5069–5076.

Fastner J., Heinze R., Humpage A.R. et al., 2003. Cylindrospermopsin occurrence in two German lakes and preliminary assessment of toxicity and toxin production of *Cylindrospermopsis raciborskii* (Cyanobacteria) isolates. – Toxicon, 42: 313–321.

Fawell J.K., Mitchell R.E., Hill R.E. et al., 1999. The toxicity of cyanobacterial toxins in the mouse: II anatoxin-a. – Human and Experimental Toxicology, 18: 168e173.

Ferranti P., Fabbrocino S., Chiaravalle E. et al., 2013. Profiling microcystin contamination in a water reservoir by MALDI-TOF and liquid chromatography coupled to Q/TOF tandem mass spectrometry. – Food Research International, 54(1): 1321–1330.

Fitzgerald D.J., Cunliffe D.A., Burch M.D., 1999. Development of health alerts for cyanobacteria and related toxins in drinking water in South Australia. – Environmental Toxicology, 14: 203e209.

Funari E., Manganelli M., Sinisi L., 2012. Impact of climate change on waterborne diseases. – Annali dell’Istituto Superiore di Sanita, 48(4): 473–87.

Furey A., Crowley J., Shuilleabhain A.N. et al., 2003. The first identification of the rare cyanobacterial toxin, homoanatoxin-a, in Ireland. – Toxicon, 41: 297–303.

Gademann K., Portmann C., Blom J.F. et al., 2010. Multiple toxin production in the Cyanobacterium *Microcystis*: isolation of the toxic protease inhibitor cyanopeptolin. – Journal of Natural Products, 73(5): 980–984.

Gągała I., Izydorczyk K., Skowron A. et al., 2010. Appearance of toxigenic cyanobacteria in two Polish lakes dominated by *Microcystis aeruginosa* and *Planktothrix agardhii* and environmental factors influence. – Ecohydrology & Hydrobiology, 10(1): 25–34.

Geada P., Gkelis S., Teixeira J.A. et al., 2017. 17 - Cyanobacterial toxins as a high value-added product. In Gonzalez-Fernandez C., Muñoz R., Microalgae-Based Biofuels and Bioproducts - From Feedstock Cultivation to End-products, Woodhead Publishing, pp. 401–428.

Gianuzzi L., Krock B., Crettaz Minaglia M.C. et al., 2016. Growth, toxin production, active oxygen species and catalase activity of *Microcystis aeruginosa* (Cyanophyceae) exposed to temperature stress. – Comparative Biochemistry and Physiology - Part C, 189: 22–30.

Giardino C., Bresciani M., Pilkaityte R. et al., 2010. In situ measurements and satellite remote sensing of case 2 waters: first results from the Curonian Lagoon. – Oceanologia 52 (2): 197–210.

Gkelis S., Lanaras T., Sivonen K., 2006. The presence of microcystins and other cyanobacterial bioactive peptides in aquatic fauna collected from Greek freshwaters. – Aquatic Toxicology, 78(1): 32–41.

Gkelis S., Lanaras T., Sivonen K., 2015. Cyanobacterial toxic and bioactive peptides in freshwater bodies of Greece: concentrations, occurrence patterns, and implications for human health. – Marine Drugs, 13(10): 6319–6335.

Gkelis S., Zaoutsos N., 2014. Cyanotoxin occurrence and potentially toxin producing cyanobacteria in freshwaters of Greece: a multi-disciplinary approach. – Toxicon 78: 1–9.

Gobler C.J., Davis T.W., Coyne K.J. et al., 2007. Interactive influences of nutrient loading, zooplankton grazing, and microcystin synthetase gene expression on cyanobacterial bloom dynamics in a eutrophic New York lake. – Harmful Algae, 6: 119–133.

Grabowska M., Kobos J., Toruńska-Sitarz A. et al., 2014. Non-ribosomal peptides produced by *Planktothrix agardhii* from Siemianówka Dam Reservoir SDR (northeast Poland). – Archives of Microbiology, 196(10): 697–707.

Grabowska M., Mazur-Marzec H., 2011. The effect of cyanobacterial blooms in the Siemianówka Dam Reservoir on the phytoplankton structure in the Narew River. – Oceanological and Hydrobiological Studies, 40: 19–26.

Graham J.L., Jones J.R., Jones S.B. et al., 2004. Environmental factors influencing microcystin distribution and concentration in the Midwestern United States. – Water Research, 38: 4395–4404.

Greer B., McNamee S.E., Boots B. et al., 2016. A validated uplc–ms/ms method for the surveillance of ten aquatic biotoxins in european brackish and freshwater systems. – Harmful Algae, 55: 31–40.

Gugger M., Lenoir S., Berger C. et al., 2005. First report in a river in France of the benthic cyanobacterium *Phormidium favosum* producing anatoxin-a associated with dog neurotoxicosis. – Toxicon, 45: 919–928.

Guillard R.R.L., 1973. Division rates. In: Stein J.R. (ed.), Handbook of Phycological Methods: Culture Methods and Growth Measurements. – Cambridge University Press, London, UK, pp. 289–311.

Häggqvist K., Akçaalan R., Echenique-Subiabre I. et al., 2017. Case Studies of Environmental Sampling, Detection, and Monitoring of Potentially Toxic Cyanobacteria. In Handbook of Cyanobacterial Monitoring and Cyanotoxin Analysis, (Eds. Meriluoto J., Spoof L., Codd G. A.). – John Wiley & Sons, Ltd.

Hall T.A., 1999: BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. – Nucleic Acids Symposium Series, 41: 95–98.

Hanessian S., Del Valle J.R., Xue Y. et al., 2006. Total Synthesis and Structural Confirmation of Chlorodysinosin A. – Journal of the American Chemical Society, 128(32): 10491– 766.

Harada K., Fujii K., Shimada T. et al., 1995. Two cyclic peptides, anabaenopeptins, a third group of bioactive compounds from the cyanobacterium *Anabaena flos-aquae* NRC 525-17. – Tetraedron Letters, 36(9): 1511–1514.

Harke M.J., Steffen M.M., Gobler C.J. et al., 2016. A review of the global ecology, genomics, and biogeography of the toxic cyanobacterium, *Microcystis* spp. – Harmful Algae, 54: 4–20.

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.

Hawkins P.R., Runngar M.T.C., Jakcson A.R.B. et al., 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(5): 1292–1295.

Heath M.W., Wood S.A., Barbieri R.F. et al., 2014. Effects of nitrogen and phosphorus on anatoxin-a, homoanatoxin-a, dihydroanatoxin-a and dihydrohomoanatoxin-a production by *Phormidium autumnale*. – Toxicon, 92: 179–185.

Heisler J.P., Gilbert J., Burkholder J. et al., 2008. Eutrophication and harmful algal blooms: a scientific consensus. – Harmful Algae, 8: 3–13.

Ho L., Sawade E., Newcombe G., 2012. Biological treatment options for cyanobacteria metabolite removal - A review. – Water Research, 46: 1536–1548.

Horst G.P., Sarnelle O., White J.D. et al., 2014. Nitrogen availability increases the toxin quota of a harmful cyanobacterium, *Microcystis aeruginosa*. – Water Research, 54: 188–198.

Huisman J., Codd G.A., Paerl H.W. et al., 2018. Cyanobacterial blooms. – Nature Reviews Microbiology, 16(8): 471–483.

Huisman J.M., Matthijs H.C.P., Visser P.M., 2005. Harmful Cyanobacteria. Springer Aquatic Ecology Series 3. Springer, Dordrecht, The Netherlands.

Humbert J.F., Fastner J., 2017. Ecology of cyanobacteria. In: Meriluoto J., Spoof L., Codd G.A. (Eds.), Handbook on Cyanobacterial Monitoring and Cyanotoxin Analysis. – John Wiley & Sons, Ltd., pp. 11–18.

Huszar V., Kruk C., Caraco N., 2003. Steady-state assemblages of phytoplankton in four temperate lakes (NE USA). – Hydrobiologia, 502: 97–109.

Ibelings B.W., Havens K.E., 2008. Cyanobacterial toxins: a qualitative meta-analysis of concentrations, dosage and effects in freshwater, estuarine and marine biota. – Advances in Experimental Medicine Biology, 619: 675–732.

Ibelings B.W., Mur L.R., Kinsman R. et al., 1991. *Microcystis* changes its buoyancy in response to the average irradiance in the surface mixed layer. – Archiv für Hydrobiologie, 120: 385–401.

Jaanus, A., Andersson, A., Olenina, I. et al., 2011. Changes in phytoplankton communities along a north–south gradient in the Baltic Sea between 1990 and 2008. – Boreal Environment Research, 16(A): 191–208.

James K.J., Crowley J., Hamilton B. et al., 2005. Anatoxins and degradation products, determined using hybrid quadrupole time-of-flight and quadrupole ion-trap mass spectrometry: forensic investigations of cyanobacterial neurotoxin poisoning. – Rapid Communications in Mass Spectrometry, 19: 1167–1175.

James K.J., Furey A., Sherlock I.R. et al., 1998. Sensitive determination of anatoxina, homoanatoxin-a and their degradation products by liquid chromatography with fluorimetric detection. – Journal of Chromatography A, 798: 147–157.

Jančula D., Strakov, L., Sadílek J. et al., 2014. Survey of cyanobacterial toxins in Czech water reservoirs – the first observation of neurotoxic saxitoxins. – Environmental Science and Pollution Research International, 21: 8006–8015.

Jankavičiūtė G., 1962. Keturių didesnių Žemaitijos ežerų palyginamieji augalijos ir fitoplanktono bruožai. – Lietuvos TSR MA darbai, C 1(27): 3–18.

Jankavičiūtė G., 1963. Duomenys apie Dysnų ežero fitoplanktoną. – Lietuvos TSR MA darbai, C 3(32): 53–68.

Jankavičiūtė G., 1996. Lietuvos vandenų vyraujantys dumbliai. – Vilnius.

Jankavičiūtė G.J., 1986. Kačestvennaja i količestvennaja ocenka fitoplanktona oz. Drūkšiai v 1979–1981. – Trudy AN LSR, B 2 (94): 15–25.

Janssen E.M.-L., 2019. Cyanobacterial peptides beyond microcystins - A review on co-occurrence, toxicity, and challenges for risk assessment. – Water Research, 15(151): 488–499.

Jones G.J., Negri A.P., 1997. Persistence and degradation of cyanobacterial paralytic shellfish poisons (PSPs) in freshwaters. – Water Research, 31: 525–533.

Kaas H., Henriksen P., 2000. Saxitoxins (PSP toxins) in Danish lakes. – Water Research, 34(7): 2089–2097.

Kaebernick M., Neilan B., Börner T. et al., 2000. Light and the transcriptional response of the microcystin biosynthesis gene cluster. – Applied and Environmental Microbiology, 66(8): 3387–3392.

Kardinaal W.E.A., Janse I., Kamst-van Agterveld M. et al., 2007. *Microcystis* genotype succession in relation to microcystin concentrations in freshwater lakes. – Aquatic Microbial Ecology, 48: 1–12.

Karosienė J., Savadova K., Toruńska-Sitarz A. et al., 2019. Production of saxitoxins and anatoxin-a by cyanobacteria from Lithuanian lakes. – Under review.

Kasperovičienė J., 2001. The summer phytoplankton structure of some lakes located in Lithuanian protected areas. – Biologija, 2: 80–83.

Kasperovičienė J., 2007. Assessment of ecological status of the Dovinė river catchment area lakes according to phytoplankton data. – Ekologija, 53(2): 13–21.

Kasperovičienė J., 2008. Planktono melsvabakterių sklaida vandens telkiniuose ir jų toksiškumas. – Projekto ataskaita.

Kasperovičienė J., Koreivienė J., Paškauskas R., 2005. Cyanoprokaryotes and microcystins dynamics in shallow hypertrophic lake (south-eastern Lithuania). – Oceanological and hydrobiological studies, 34(3): 93–104.

Kaštovský J., Hauer T., Mareš J. et al., 2010. A review of the alien and expansive species of freshwater cyanobacteria and algae in the Czech Republic. – Biological Invasions, 12: 3599–3625.

Katırcıoğlu H., Akın B.S., Atıcı T., 2004. Microalgal toxin(s): characteristics and importance. – African Journal of Biotechnology, 3 (12): 667–674.

Kavaliauskienė, J., 1996. Algae of Lithuanian Lakes; Institute of Geography. – Vilnius, Lithuania, 148 p.

Kellmann R., Mihali T.K., Jeon Y.J. et al., 2008. Biosynthetic Intermediate Analysis and Functional Homology Reveal a Saxitoxin Gene Cluster in Cyanobacteria. – Applied and Environmental Microbiology, 74(13): 4044–4053.

Kellmann R., Ploux O., Neilan B.A., 2013. Neurotoxic alkaloids from cyanobacteria. In: Ramawat, K.G., Me' rillon, J.M. (Eds.), Natural products. Phytochemistry, botany and metabolism of alkaloids, phenolics and terpenes. – Springer Publishers, Heidelberg, pp. 39–83.

Kimura M., 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. – Journal of Molecular Evolution, 16: 111–120.

Kiss T., Vehovszky A., Hiripi L. et al., 2002. Membrane effects of toxins isolated from a cyanobacterium, *Cylindrospermopsis raciborskii*, on identified molluscan neurones. – Comparative Biochemistry and Physiology Part C: Toxicoly & Pharmacology, 131: 167–176.

Klausmeier C.A., Litchman E., Daufresne T. et al., 2004. Optimal nitrogen-to-phosphorus stoichiometry of phytoplankton. – Nature, 429: 171–174.

Kobos J., Błaszczyk A., Hohlfeld N. et al., 2013. Cyanobacteria and cyanotoxins in Polish freshwater bodies. – Oceanological and Hydrobiological Studies, 42(4): 358–378.

Kodani S., Suzuki S., Ishida K. et al., 1999. Five new cyanobacterial peptides from water bloom materials of lake Teganuma (Japan). – FEMS Microbiology Letters, 178(2): 343–348.

Kohler E., Grundler V., Haeussinger D. et al., 2014. The toxicity and enzyme activity of a chlorine and sulfate containing aeruginosin isolated from a non-microcystin-producing *Planktothrix* strain. – Harmful Algae, 39: 154–160.

Kokociński M., Akçaalan R., Salmaso N. et al., 2017a. Expansion of Alien and Invasive Cyanobacteria. In Handbook of Cyanobacterial Monitoring and Cyanotoxin Analysis; Meriluoto J., Spoof L., Codd G.A., Eds.; Wiley: Hoboken, NJ, USA, pp. 28–39.

Kokociński M., Dziga D., Spoof L. et al., 2009. First report of the cyanobacterial toxin cylindrospermopsin in the shallow, eutrophic lakes of Western Poland. – Chemosphere, 74: 669–675.

Kokociński M., Gągała I., Jasser I. et al., 2017b. Distribution of invasive *Cylindrospermopsis raciborskii* in the East-Central Europe is driven by climatic and local environmental variables. – FEMS Microbiology Ecology, 93.

Kokociński M., Mankiewicz-Boczek J., Jurczak T. et al., 2013. *Aphanizomenon gracile* (Nostocales), a cylindrospermopsin producing cyanobacterium in Polish lakes. – Environmental Science and Pollution Research, 20: 5243–5264.

Kokociński M., Soininen J., 2019. New insights into the distribution of alien cyanobacterium *Chrysosporum bergii* (Nostocales, Cyanobacteria). – Phycological Research.

Kokociński M., Stefaniak K., Mankiewicz-Boczek J. et al., 2010. The ecology of the invasive cyanobacterium *Cylindrospermopsis raciborskii* (Nostocales, Cyanophyta) in two hypereutrophic lakes dominated by *Planktothrix agardhii* (Oscillatoriales, Cyanophyta). – European Journal of Phycology, 45(4): 365–374.

Komárek J., 2013. Cyanoprocaryota. Heterocytous Types. Süßwasserflora von Mitteleuropa (19/3). – Springer-Verlag: Berlin, Heidelberg.

Komárek J., Anagnostidis K., 1998. Cyanoprocaryota 1. Teil: *Chroococcales*. Süßwasserflora von Mitteleuropa, 19(1). – Jena–Stuttgart–Lübek–Ulm.

Komárek J., Anagnostidis K., 2005. Cyanoprocaryota 2. Teil: *Oscillatoriales*. Süßwasserflora von Mitteleuropa, 19(2). – München.

Komárek J., Komárková J., 2006: Diversity of *Aphanizomenon*-like cyanobacteria. – Czech Phycology (Fottea), 6: 1–32.

Komárek J., Zapomělová E., 2008. Planktic morphospecies of the cyanobacterial genus *Anabaena* = subg. *Dolichospermum* – 2. part: straight types. – Fottea. 8: 1–14.

Koreivienė J., Kasperovičienė J., 2011. Alien cyanobacteria *Anabaena bergii* var. *limnetica* Couté et Preisig from Lithuania: Some aspects of taxonomy, ecology and distribution. – Limnologica, 41: 325–333.

Koreivienė J., Kasperovičienė J., Savadova K. et al., 2016. Collection of pure cultures of algae and cyanobacteria for research, teaching and biotechnological applications (Nature Research Centre, Lithuania). – Botanica Lithuanica, 22(1): 87–92.

Koskenniemi K., Lyra C., Rajaniemi-Wacklin P. et al., 2007. Quantitative real-time PCR detection of toxic *Nodularia* cyanobacteria in the Baltic Sea. – Applied and Environmental Microbiology, 73(7): 2173–2179.

Kosol S., Schmidt J., Kurmayer R., 2009. Variation in peptide net production and growth among strains of the toxic cyanobacterium *Planktothrix* spp. – European Journal of Phycology, 44(1): 49–62.

Kosten S., Huszar V.L.M., Bécares E. et al., 2012. Warmer climates boost cyanobacterial dominance in shallow lakes. – Global Change Biology, 18: 118–126.

Kovats R.S., Valentini R., Bouwer L.M. et al., 2014. Europe. In Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part B: Regional Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change; Barros V.R., Field C.B., Dokken D.J., Mastrandrea M.D., Mach K.J., Bilir T.E., Chatterjee M., Ebi K.L., Estrada Y.O., Genova R.C. et al., Eds.; Cambridge University Press: Cambridge, UK; New York, NY, USA, pp. 1267–1326.

Kuiper-Goodman T., Falconer I.R., Fitzgerald J., 1999. Human health aspects. In: Chorus, I., Bartram, J. (Eds.), Toxic Cyanobacteria in Water: A Guide to their Public Health Consequences, Monitoring and Management. E & FN Spon, London, pp. 113–153.

Kumar S., Stecher G., Tamura K., 2016. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. – Molecular Biology and Evolution, 33(7): 1870–1874.

Kurmayer R., Christiansen G., Fastner J. et al., 2004. Abundance of active and inactive microcystin genotypes in populations of the toxic cyanobacterium *Planktothrix* spp. – Environmental Microbiology, 6: 831–841.

Kurmayer R., Christiansen, 2009. The genetic basis of toxin production in Cyanobacteria. – Freshwater Reviews, 2(1): 31–50.

Kurmayer R., Deng L., Entfellner E., 2016. Role of toxic and bioactive secondary metabolites in colonization and bloom formation by filamentous cyanobacteria *Planktothrix*. – Harmful Algae, 54: 69–86.

Lebret K., Kritzberg S., Figueroa R. et al., 2012: Genetic diversity within and genetic differentiation between blooms of a microalgal species. – Environmental Microbiology, 14(9): 2395–2404.

Ledreux A., Thomazeau S., Catherine A. et al., 2010. Evidence for saxitoxins production by the cyanobacterium *Aphanizomenon gracile* in a French recreational water body. – Harmful Algae, 10: 88–97.

Lepère C., Wilmotte A., Meyer B., 2000. Molecular diversity of *Microcystis* strains (Cyanophyceae, Chroococcales) based on 16S rDNA sequences. – Systematics and Geography of Plants, 70(2): 275–283.

Lepistö, L., Holopainen, A.-L., 2008. Are distinct morphotypes of the genera *Anabaena* and *Aphanizomenon* a response to environmental variation in boreal and arctic lakes in Finland? – Archiv fur Hydrobiologie. Supplementband: Algological Studies, 127: 1–13.

Lesutienė J., Ložys L., Dainys J. et al., 2018. Migratory patterns and cyanotoxin concentrations of pikeperch (Sander lucioperca) in the coastal waters of the Baltic Sea. – Boreal Environment Research, 23: 315–327.

Lifshits M., Carmeli S., 2012. Metabolites of *Microcystis aeruginosa* bloom material from Lake Kinneret, Israel. – Journal of Natural Products, 75(2): 209–219.

Liu X., Lu X., Chen Y., 2011. The effects of temperature and nutrient rations on *Microcystis* bloms in Lake Taihu, China: an 11-year investigation. – Harmful Algae, 10: 337–343.

Lopes V.R., Ramos V., Martins A. et al., V.M., 2012. Phylogenetic, chemical and morphological diversity of cyanobacteria from Portuguese temperate estuaries. – Marine Environmental Research, 73: 7–16.

Lürling M., Eshetu F., Faassen E.J. et al., 2013. Comparison of cyanobacterial and green algal growth rates at different temperatures. – Freshwater Biology, 58: 552–559.

Lürling M., Faassen E.J., 2013. Dog Poisonings Associated with a *Microcystis aeruginosa* Bloom in the Netherlands. – Toxins, 5(3): 556–567.

Lürling M., Mello M.M., van Oosterhout F. et al., 2018. Response of natural cyanobacteria and algae assemblages to a nutrient pulse and elevated temperature. – Frontiers in Microbiolgy, 9: 1851.

Lürling M., van Oosterhout F., Faassen E., 2017. Eutrophication and warming boost cyanobacterial biomass and microcystins. – Toxins, 9: 64.

Ma Z.L., Fang T.X., Thring R.W. et al., 2015. Toxic and non-toxic strains of *Microcystis aeruginosa* induce temperature dependent allelopathy toward growth and photosynthesis of *Chlorella vulgaris*. – Harmful Algae, 48: 21–29.

Maileht K., Nõges T., Nõges P. et al., 2013. Water colour, phosphorus and alkalinity are the major determinants of the dominant phytoplankton species in European lakes. – Hydrobiologia, 704(1): 115–126.

Manti G., Mattei D., Messineo V. et al., 2005. First report of *Cylindrospermopsis raciborskii* in Italy. – Harmful Algae News, 28: 8–9.

Mantzouki E., Campbell J., van Loon E. et al., 2018b. A European multi lake survey dataset of environmental variables, phytoplankton pigments and cyanotoxins. – Scientific data, 5: 180226.

Mantzouki E., Lürling M, Fastner J. et al., 2018a. Temperature effects explain continental scale distribution of cyanobacterial toxins. – Toxins, 10(4): 156.

Marinho M.M., Souza M.B.G., Lürling M., 2013. Light and phosphate competition between *Cylindrospermopsis raciborskii* and *Microcystis aeruginosa* is strain dependent. – Microbial Ecology, 66(3): 479–88.

Markevičienė A., 1962. Kai kurie fitoplanktono bruožai Spernios, Bambenos, Dovinės upeliuose ir Simno bei Žuvinto ežeruose. Lietuvos TSR MA Geologijos ir geografijos institutas. Moksliniai pranešimai. T. XIV. Sąs. 2. Ežerotyra, p. 171–176.

Meehl G.A., Stocker T.F., Collins W.D. et al., 2007. Global climate projections. In: Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K.B., Tignor, M., Miller, H.L. (Eds.), Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge.

Mehnert G., Leunert F., Cirés S. et al., 2010. Competitiveness of invasive and native cyanobacteria from temperate freshwaters under various light and temperature conditions. – Journal of Plankton Research, 32(7): 1009–1021.

Merel S., Clément M., Thomas O., 2010. State of the art on cyanotoxins in water and their behaviour towards chlorine. – Toxicon, 55: 677–691.

Meriluoto J., Blaha L., Bojadzija G. et al., 2017. Toxic cyanobacteria and cyanotoxins in European waters – recent progress achieved through the CYANOCOST Action and challenges for further research. – Advances in Oceanography and Limnology, 8(1): 161–178.

Messineo V., Bogialli S., Melchiorre S. et al., 2009. Cyanobacterial toxins in Italian freshwaters – Limnologia, 39: 95–106.

Mischke U., Nixdorf B., 2003. Equilibrium phase conditions in shallow German lakes: How Cyanoprokaryota species establish a steady state phase in late summer. – Hydrobiologia, 502: 123–132.

Mohamed Nor N.H., Te S.H., Mowe M.A.D. et al., 2019. Environmental factors influence cylindrospermopsin production of *Cylindrospermopsis raciborskii* (CR12). – Journal of Plankton Research, 41(2): 114–126.

Monchamp M.-E., Pick F.R., Beisner B.E. et al., 2014. Nitrogen forms influence microcystin concentration and composition via changes in cyanobacterial community structure. – PLoS ONE, 9: e85573.

Moustaka-Gouni M., Hiskia A., Genitsaris S. et al., 2017. First report of Aphanizomenon favaloroi occurrence in Europe associated with saxitoxins and a massive fish kill in Lake Vistonis, Greece. – Marine & freshwater research, 68(4): 793–800.

Moustaka-Gouni M., Kormas K.A., Vardaka E. et al., 2009. *Raphidiopsis mediterranea* Skuja represents non-heterocytous lifecycle stages of *Cylindrospermopsis raciborskii* (Woloszynska) Seenayya et Subba Raju in Lake

Kastoria (Greece), its type locality: evidence by morphological and phylogenetic analysis. – Harmful Algae, 8: 864–872.

Mur L.R., 1983. Some aspects of the ecophysiology of cyanobacteria. – Annals of Microbiology (Institute Pasteur), 134: 61–72.

Namikoshi M., Murakami T., Watanabe M.F. et al., 2003. Simultaneous production of homoanatoxin-a, anatoxin-a, and a new non-toxic 4-hydroxyhomoanatoxin-a by the cyanobacterium *Raphidiopsis mediterranea* Skuja. – Toxicon, 42(5): 533–538.

Negri A.P., Jones G.J., 1995. Bioaccumulation of paralytic shellfish poisoning (PSP) toxins from the cyanobacterium *Anabaena circinalis* by the freshwater mussel *Alathyria condola*. – Toxicon, 33: 667–678.

Neilan B.A., Jacobs D., Goodman A.E., 1995. Genetic diversity and phylogeny of toxic cyanobacteria determined by DNA polymorphisms within the phycocyanin locus. – Applied and Environmental Microbiology, 61(11): 3875–3883.

Neilan B.A., Pearson L.A., Muenchhoff J. et al., 2013. Environmental conditions that influence toxin biosynthesis in cyanobacteria. – Environmental Microbiology, 15: 1239–1253.

Nogueira I.C., Pereira P., Dias E. et al., 2004. Accumulation of paralytic shellfish toxins (PST) from the cyanobacterium *Aphanizomenon issatschenkoi* by the cladoceran *Daphnia magna*. – Toxicon, 44(7): 773–80.

Nübel U., Garcia-Pichel F., Muyzer G., 1997. PCR primers to amplify 16S rRNA genes from cyanobacteria. – Applied and Environmental Microbiology, 63(8): 3327–3332.

O'Neil J.M., Davis T.W., Burdord M.A. et al., 2012. The rise of harmful cyanobacteria blooms: The potential roles of eutrophication and climate change. – Harmful Algae, 14: 313–334.

Oberhaus L., Briand J.F., Leboulanger C. et al., 2007. Comparative effects of the quality and quantity of light and temperature on the growth of *Planktothrix agardhii* and *P. rubescens*. – Journal of Phycology, 43: 1191–1199.

Ohtani I., Moore R.E., 1992. Cylindrospermopsin: a potent hepatotoxin from the blue-green algae *Cylindrospermopsis raciborskii*. – Journal of the American Chemical Society, 114: 7941–7942.

Okino T., Matsuda H., Murakami M. et al., 1993. Microginin, an angiotensin-converting enzyme inhibitor from the blue-green alga *Microcystis aeruginosa*. – Tetrahedron Letters, 34(3): 501–504.

Olenina I., Hajdu S., Edler L. et al., 2006. Biovolumes and size-classes of phytoplankton in the Baltic Sea. – HELCOM, Baltic Sea Environment Proceedings No. 106: 144.

Olrik K., Blomquist P., Brettum P. et al., 1998. Methods for quantitative assessment of phytoplankton in freshwaters, part I: 86. – Naturvårdsverket förlag, Stockholm.

Onodera H., Oshima Y., Henriksen P. et al., 1997. Confirmation of anatoxin-a(s), in the cyanobacterium *Anabaena lemmermannii*, as the cause of bird kills in Danish lakes. – Toxicon, 35: 1645–1648.

Orr P.T., Jones G.J., 1998. Relationship between microcystin production and cell division rates in nitrogen-limited *Microcystis aeruginosa* cultures. – Limnology and Oceanography, 43: 1604–1614.

Osswald J., Rellán S., Gago-Martinez A. et al., 2009. Production of anatoxin-a by cyanobacterial strains isolated from Portuguese freshwater systems. – Ecotoxicology, 18(8): 1110–1115.

Paerl H.W., Fulton R.S., 2006. Ecology of harmful cyanobacteria. In: Graneli E., Turner J. (eds) Ecology of harmful marine algae. Springer, Berlin, pp. 95–107.

Paerl H.W., Fulton R.S., Moisander P.H. et al., 2001. Harmful freshwater algal blooms with an emphasis on cyanobacteria. – The Scientific World, 1: 76–113.

Paerl H.W., Huisman J., 2008. Blooms like it hot. – Climate, 320: 57–58.

Paerl H.W., Huisman J., 2009. Climate change: a catalyst for global expansion of harmful cyanobacterial blooms. – Environmental Microbiology Reports, 1: 27–37.

Paerl H.W., Joyner J.J., Joyner A.R. et al., 2008. Co-occurrence of dinoflagellate and cyanobacterial harmful algal blooms in southwest Florida coastal waters: a case for dual nutrient (N and P) input controls. – Marine Ecology Progress Series, 371: 143–153.

Paerl H.W., Otten T.G., 2016. Duelling 'CyanoHABs': unravelling the environmental drivers controlling dominance and succession among diazotrophic and non-N2 - fixing harmful cyanobacteria. – Environmental Microbiology, 18(2): 316–24.

Paldavičienė A., 2015b. Cyanotoxins and their accumulation in the Curonian Lagoon. PhD thesis.

Paldavičienė A., Mazur-Marzec H., Razinkovas A., 2009. Toxic cyanobacteria bloom in the Lithuanian part of the Curonian Lagoon. – Oceanologia, 51(2): 203–216.

Paldavičienė A., Zaiko A., Mazur-Marzec H. et al., 2015a. Bioaccumulation of microcystins in invasive bivalves: A case study from the boreal lagoon ecosystem. – Oceanologia, 57: 93–101.

Pantelić D., Svirčev Z., Simeunović J. et al., 2013. Cyanotoxins: Characteristics, production and degradation routes in drinking water treatment with reference to the situation in Serbia. – Chemosphere, 91: 421–441.

Papadimitriou T., Katsiapi M., Kormas K.A. et al., 2013. Artificially-born “killer” lake: Phytoplankton based water quality and microcystin affected fish in a reconstructed lake. – Science of the Total Environment, 452–453: 116–124.

Patocka J., Gupta R.C., Kuca K., 2011. Anatoxin-a(s): natural organophosphorus anticholinesterase agent. – Military Medical Science Letters, 80: 129–139.

Paul V.J., 2008. Global warming and cyanobacterial harmful algal booms. In: Hudnell, K.H. (Ed.), Cyanobacterial Harmful Algal Blooms: State of the Science

and Research Needs. – Advances in Experimental Medicine and Biology, 619(24): 239–257.

Pawlik-Skowrońska B., Toporowska M., Mazur-Marzec H., 2019. Effects of secondary metabolites produced by different cyanobacterial populations on the freshwater zooplankters *Brachionus calyciflorus* and *Daphnia pulex*. – Environmental Science and Pollution Research, 26(12): 11793–11804.

Pearson L., Mihali T., Moffitt M. et al., 2010. On the chemistry, toxicology and genetics of the cyanobacterial toxins, microcystin, nodularin, saxitoxin and cylindrospermopsin. – Marine Drugs, 8: 1650–1680.

Pekar H., Westerberg E., Bruno O. et al., 2016. Fast, rugged and sensitive ultra high pressure liquid chromatography tandem mass spectrometry method for analysis of cyanotoxins in raw water and drinking water—First findings of anatoxins, cylindrospermopsins and microcystin variants in Swedish source waters and infiltration ponds. – Jorunal of Chromatography A, 1429: 265–276.

Pełechata A., Pełechaty M., Pukacz A., 2006. Cyanoprokaryota of shallow lakes of Lubuskie Lakeland (mid-western Poland). – Oceanological and Hydrobiological Studies, 35(1): 3–14.

Pereira P., Li R., Carmichael W.W. et al., 2004. Taxonomy and production of paralytic sellfish toxins by the freshwater cyanobacterium *Aphanizomenon gracile* LMECYA40. – European Journal of Phycology, 39: 361–368.

Pierangelini M., Sinha R., Willis A. et al., 2015. Constitutive cylindrospermopsin pool size in *Cylindrospermopsis raciborskii* under different light and CO_2 partial pressure conditions. – Applied and Environmental Microbiology, 81(9): 3069–3076.

Pilkaitytė R., 2003. Fitoplanktono kaita ir gausumas eutrofinėse estuarinėse lagūnose. Daktaro disertacija.

Pitois F., Fastner J., Pagotto C. et al., 2018. Multi-toxin occurrences in ten French water resource reservoirs. – Toxins, 10(7): E283.

Pomati F., Sacchi S., Rossetti C. et al., 2000. The freshwater cyanobacterium *Planktothrix* sp. FP1: Molecular identification and detection of paralytic shellfish poisoning toxins. – Journal of Phycology, 36: 553–562.

Poulíčková A., Hašler P., Kitner M., 2004. Annual Cycle of *Planktothrix agardhii* (Gon.) Anagn. & Kom. Nature Population. – International Review Hydrobiology, 89(3): 278–288.

Puschner B., 2018. Chapter 57 – Cyanobacterial (Blue-green algae) toxins. Veterinary Toxicology (Third Edition). – Basic and Clinical Principles, pp. 763–777.

Quesada A., Moreno E., Carrasco D. et al., 2006. Toxicity of *Aphanizomenon ovalisporum* (Cyanobacteria) in a Spanish water reservoir. – European Journal of Phycology, 41(1): 39–45.

Rantala A., Rajaniemi-Wacklin P., Lyra C. et al., 2006. Detection of microcystin-producing cyanobacteria in Finnish lakes with genus-specific microcystin

synthetase gene E (mcyE) PCR and associations with environmental factors. – Applied and Environmental Microbiology, 72(9): 6101–6110.

Rantala A., Rizzi E., Castiglioni B. et al., 2008. Identification of hepatotoxin-producing cyanobacteria by DNA-chip. – Environmental Microbiology, 10(3): 653–664.

Rantala-Ylinen A., Känä S., Wang H. et al., 2011. Anatoxin-a synthetase gene cluster of the cyanobacterium *Anabaena* sp. Strain 37 and molecular methods to detect potential producers. – Applied and Environmental Microbiology, 77(20): 7271–7278.

Rapala J., Robertson A., Negri A.P. et al., 2005. First report of saxitoxin in Finnish lakes and possible associated effects on human health. – Environmental Toxicology, 20: 331–340.

Rapala J., Sivonen K., Luukkainen R. et al., 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(6): 581–591.

Rasconi S., Winter K., Kainz M.J., 2017. Temperature increase and fluctuation induce phytoplankton biodiversity loss – Evidence from a multi-seasonal mesocosm experiment. – Ecology and Evolution, 7(9): 2936–2946.

Reynolds C.S., 2006. "Photosynthesis and Carbon Acquisition in Phytoplankton." In The Ecology of Phytoplankton, edited by C. Reynolds, 93–143. Cambridge University Press: London.

Rigosi A., Carey C.C., Ibelings B.W. et al., 2014. The interaction between climate warming and eutrophication to promote cyanobacteria is dependent on trophic state and varies among taxa. – Limnology and Oceanography, 59: 99–114.

Rodríguez E., Onstad G.D., Kull T.P.J. et al., 2007. Oxidative elimination of cyanotoxins: Comparison of ozone, chlorine, chlorine dioxide and permanganate. – Water Research, 41(15):3 381–3393.

Rohrlack T., Skulberg R., Skulberg O.M., 2009. Distribution of oligopeptide chemotypes of the cyanobacterium *Planktothrix* and their persistence in selected lakes in fennoscandia. – Journal of Phycology, 45(6): 1259–1265.

Rücker J., Stüken A., Nixdorf B. et al., 2007. Concentrations of particulate and dissolved cylindrospermopsin in 21 *Aphanizomenon*-dominated temperate lakes. – Toxicon, 50: 800–809.

Rücker J., Wiedner C., Zippel P., 1997. Factors controlling the dominance of *Planktothrix agardhii* and *Limnothrix redekei* in eutrophic shallow lakes. – Hydrobiologia, 342/343:107–115.

Ryan C.N., Thomas M.K., Litchman E., 2017. The effects of phosphorus and temperature on the competitive success of an invasive cyanobacterium. – Aquatic Ecology, 51(3): 463–472.

Rzymski P., Poniedziałek B., 2014. In search of environmental role of cylindrospermopsin: a review on global distribution and ecology of its producers. – Water Research, 66: 320–327.

Rzymski P., Poniedziałek B., 2015. The surprising world of cyanobacteria: cylindrospermopsin has a soil face. – Journal of Phycology, 51: 1037–1039.

Sabart M., Pobel D., Briand E. et al., 2010. Spatiotemporal variations in microcystin concentrations and in the proportions of microcystin-producing cells in several *Microcystis aeruginosa* populations. – Applied and Environmental Microbiology, 76(14): 4750–4759.

Sabour B., Loudiki M., Oudra B. et al., 2005. Dynamics and toxicity of *Anabaena aphanizomenoides* (Cyanobacteria) water-blooms in the shallow brackish Oued Mellah lake (Morocco). – Aquatic Ecosystem Health, 8: 95–104.

Sabour B., Loudiki M., Vasconcelos V., 2009. Growth responses of *Microcystis ichthyoblabe* Kützing and *Anabaena aphanizomenoides* Forti (Cyanobacteria) under different nitrogen and phosphorus conditions. – Chemistry and Ecology, 25: 337–44.

Saker M.L., Griffiths D.J., 2000. The effect of temperature on growth and cylindrospermopsin content of seven isolates of *Cylindrospermopsis raciborskii* (Nostocales, Cyanophyceae) from water bodies in northern Australia. – Phycologia, 39(4): 349–354.

Sanseverino I., Conduto António D., Loos R. et al., 2017. Cyanotoxins: methods and approaches for their analysis and detection. – The report of Joint Research Centre, EUR 28624.

Savela H., Spoof L., Höysniemi N. et al., 2017. First report of cyanobacterial paralytic shellfish toxin biosynthesis genes and paralytic shellfish toxin production in Polish freshwater lakes. – Advances in Oceanography and Limnologu Journal, 8(1): 61–70.

Scheffer M., Rinaldi S., Gragnani A. et al., 1997. On the dominance of filamentous cyanobacteria in shallow, turbid lakes. – Ecology, 78(1): 272–282.

Scheffer M., Rinaldi S., Gragnani A. et al., 1997. On the dominance of filamentous cyanobacteria in shallow, turbid lakes. – Ecology, 78(1): 272–282.

Schembri M.A., Neilan B.A., Saint C.P., 2001. Identification of genes implicated in toxin production in the cyanobacterium *Cylindrospermopsis raciborskii*. – Environmental Toxicology, 16: 413–421.

Schindler D.W., 2012. The dilemma of controlling cultural eutrophication of lakes. – Proceedings of the Royal Society B, 279: 4322–4333.

Schreuder H., Liesum A., Lonze P. et al., 2016. Isolation, co-crystallization and structure-based characterization of anabaenopeptins as highly potent inhibitors of activated thrombin activatable fibrinolysis inhibitor (TAFIa). – Scientific Reports, 6.

Schwaderer A., Yoshiyama K., de Tezanos Pinto P. et al., 2011. Ecoevolutionary differences in light utilization traits and distributions of freshwater phytoplankton. – Limnology and Oceanography, 56: 589–598.

Sedmak B., Elersek T., Grach-Pogrebinsky O. et al., 2008. Ecotoxicologically relevant cyclic peptides from cyanobacterial bloom (*Planktothrix rubescens*) - a

threat to human and environmental health. – Radiology and Oncology, 42(2): 102–113.

Sivonen K., 2009. Cyanobacterial toxins. Encyclopedia of Microbiology, (Moselio Schaechter, Editor), pp. 290-307 Oxford: Elsevier.

Sivonen K., Börner T., 2008. Bioactive compounds produced by cyanobacteria. In: The Cyanonbacteria: Molecular Biology, Genomics, and Evolution., A. Herrero, and E. Flores, eds. (Norfolk, UK.: Caister Academic Press.), pp. 159–197.

Sivonen K., Niemelä S.I., Niemi R.M. et al., 1990. Toxic cyanobacteria (blue-green algae) in Finnish fresh and coastal Waters. – Hydrobiologia, 190: 267–275.

Skulberg O.M., Codd G.A., Carmichael W.W., 1984. Toxic blue-green algal blooms in Europe: a growing problem. – AMBIO, 13(4): 244–247.

Smith Z.J., Martin R.M., Wei B. et al., 2019. Spatial and Temporal Variation in Paralytic Shellfish Toxin Production by Benthic *Microseira* (*Lyngbya*) *wollei* in a Freshwater New York Lake. – Toxins, 11:44.

Song L., Sano T., Li R. et al., 1998. Microcystin production of *Microcystis viridis* (cyanobacteria) under different culture conditions. – Phycological Research, 46(s2).

Spoof L., Berg K.A., Rapala J. et al., 2006. First observation ofcylindrospermopsin in *Anabaena lapponica* isolated from the boreal environment (Finland). – Environmental Toxicology, 21: 552–560.

Stefaniak K., Kokociński M., 2005. Occurrence of invasive Cyanobacteria species in the polimictic lakes of the Wielkopolska region (Western Poland). – Oceanological and Hydrobiological Studies, 34(3): 137–148.

Sterner R.W., 2008. On the phosphorus limitation paradigm for lakes. – International Review of Hydrobiology, 93: 433–445.

Stevens D.K., Krieger R.I., 1991. Effect of route of exposure and repeated doses on the acute toxicity in mice of the cyanobacterial nicotinic alkaloid anatoxin-a. – Toxicon, 29: 134–138.

Stocker T.F., Qin D., Plattner G.-K. et al., 2013. IPCC, 201. Climate change 2013: the physical science basis. In: Contribution of Working Group I to the Fifth Assessment Report of the Intergovern-mental Panel on Climate ChangeCambridge University Press, Cambridge, United Kingdom and New York, NY USA, 1535 pp.

Stoyneva-Gärtner M., Descy J.-P., Latli A, et al., 2017a. Assessment of cyanoprokaryote blooms and of cyanotoxins in Bulgaria in a 15-years period (2000-2015). – Advances in Oceanography and Limnology, 8(1).

Stoyneva-Gärtner M., Uzunov B., Dimitrova P., 2017b: Pilot assessment of cyanotoxins as potential risk factors for cancer in Bulgaria. – BioDiscovery, 20: e20501.

Strickland J.D.H., Parsons T.R., 1968. Determination of dissolved oxygen. In: A Practical Handbook of Seawater Analysis. – Fisheries Research Board of Canada, Bulletin, 167: 71–75.

Sukenik A., Hadas O., Kaplan A. et al., 2012. Invasion of Nostocales (cyanobacteria) to subtropical and temperate freshwater lakes—Physiological, regional, and global driving forces. – Frontiers in Microbiology, 3: 86.

Sukenik A., Quesada A., Salmaso N., 2015. Global expansion of toxic and non-toxic cyanobacteria: effect on ecosystem functioning. – Biodiversity and Conservation, 24(4): 889–908.

Šulčius S., Alzbutas G., Kvederavičiūtė K., et al., 2015b. Draft genome sequence of the cyanobacterium *Aphanizomenon flos-aquae* strain 2012/KM1/D3, isolated from the Curonian Lagoon (Baltic Sea). – Genome Announcements, 3(1): e01392-14.

Šulčius S., Pilkaitytė R., Mazur-Marzec H., et al., 2015a. Increased risk of exposure to microcystins in the scum of the filamentous cyanobacterium Aphanizomenon flos-aquae accumulated on the western shoreline of the Curonian Lagoon. – Marine Pollution Bulletin, 99: 264–270.

Svirčev Z., Drobac D., Tokodi N. et al., 2017. Toxicology of microcystins with reference to cases of human intoxications and epidemiological investigations of exposures to cyanobacteria and cyanotoxins. – Archives of Toxicology, 91: 621-650.

Svirčev Z., Obradović V., Codd G.A. et al., 2016. Massive fish mortality and *Cylindrospermopsis raciborskii* bloom in Aleksandrovac Lake. – Ecotoxicology, 25(7): 1353–1363.

Tamura K., 1992. Estimation of the number of nucleotide substitutions when there are strong transition-transversion and G+C-content biases. – Molecular Biology and Evolution, 9(4): 678–687.

Tan X., Gu H., Zhang X. et al., 2019. Effects of phosphorus on interspecific competition between two cell-size cyanobacteria: *Synechococcus* sp. and *Microcystis aeruginosa*. – Bulletin of Environmental Contamination and Toxicology, 102(2): 231–238.

Teneva I., Mladenov R., Belkinova D. et al., 2010. Phytoplankton community of the drinking water supply reservoir Borovitsa (South Bulgaria) with an emphasis on cyanotoxins and water quality. – Central European Journal of Biology, 5(2): 231–239.

Testai E., Scardala S., Vichi S. et al., 2016. Risk to human health associated with the environmental occurrence of cyanobacterial neurotoxic alkaloids anatoxins and saxitoxins. – Critical Reviews in Toxicology, 46(5): 385–419.

Tonk L., Visser P.M., Christiansen G. et al., 2005. The microcystin composition of the cyanobacterium *Planktothrix agardhii* changes toward a more toxic variant with increasing light intensity. – Applied and Environmental Microbiology, 71(9): 5177–5181.

Toporowska M., Ferencz B., Dawidek J., 2018. Impact of lake-catchment processes on phytoplankton community structure in temperate shallow lakes. – Ecohydrology, 11(8).

Toporowska M., Pawlik-Skowrońska B., Kalinowska R., 2016. Mass development of diazotrophic cyanobacteria (Nostocales) and production of neurotoxic anatoxin-a in a *Planktothrix* (Oscillatoriales) dominated temperate lake. – Water, Air, & Soil Pollution, 227: 321.

Toporowska M., Pawlik-Skowrońska B., Krupa D. et al., 2010. Winter versus summer blooming of phytoplankton in a shallow lake: Effect of hypertrophic conditions. – Polish Journal of Ecology, 58: 3–12.

Torres C., Lürling M., Marinho M.M., 2016. Assessment of the effects of light availability on growth and competition between strains of *Planktothrix agardhii* and *Microcystis aeruginosa*. – Microbial Ecology, 71(4): 802–813.

Turner A.D., Dhanji-Rapkova M., O'Neil A. et al., 2018. Analysis of Microcystins in Cyanobacterial Blooms from Freshwater Bodies in England. – Toxins, 10(1): 39.

Turner P.C., Gammie A.J., Hollinrake K. et al., 1990. Pneumonia associated with contact with cyanobacteria. – British Medical Journal, 300: 1440–1441.

Ūselytė S., 1959. Fitoplankton zaliva Kuršių Marios i ego sezonnaja dinamika. – Knygoje: Kuršių Marios. – Vilnius, pp. 139–163

Ūselytė S., 1961. Baltijos jūros prie Lietuvos TSR krantų kompleksiniai tyrimai (Fitolanktonas ir jo ryšys su Kuršių mariomis). – Lietuvos TSR MA darbai, C 3(26): 101–113.

Utermöhl H., 1958. Zur Ver vollkommung der quantitativen phytoplankton-methodik. – Mitteilung Internationale Vereinigung Fuer Theoretische unde Amgewandte Limnologie, 9: 39 p.

Vaičiūtė D., Bresciani M., Bučas M., 2012. Validation of MERIS bio-optical products with in situ data in the turbid Lithuanian Baltic Sea coastal waters. – Journal of Applied Remote Sensing, 6: 063568-1.

Vailionis L., 1936: Nemuno ,,žydėjimas". – Kosmos, 7-12: 181–183.

Vaitomaa J., Rantala A., Halinen K. et al., 2003. Quantitative real-time PCR for determination of microcystin synthetase gene E copy numbers for *Microcystis* and *Anabaena* in lakes. – Applied and Environmental Microbiology, 69(12): 7289–7297.

Van de Waal D.B., Ferreruela G., Tonk L. et al., 2010. Pulsed nitrogen supply induces dynamic changes in the amino acid composition and microcystin production of the harmful cyanobacterium *Planktothrix agardhii*. – FEMS Microbiology Ecology, 74(2): 430–8.

Van de Waal D.B., Smith V.H., Declerck S.A.J. et al., 2014. Stoichiometric regulation of phytoplankton toxins. – Ecology Letters.

Van de Waal D.B., Verspagen J.M., Finke J.F. et al., 2011. Reversal in competitive dominance of a toxic versus non-toxic cyanobacterium in response to rising CO_2. – The ISME Journal, 5(9): 1438–50.

Van de Waal D.B., Verspagen J.M.H., Lürling M. et al., 2009. The ecological stoichiometry of toxins produced by harmful cyanobacteria: an experimental test of the carbon-nutrient balance hypothesis. – Ecology Letters, 12: 1326–1335.

Vézie C., Rapala J., Vaitomaa J. et al., 2002. Effect of nitrogen and phosphorus on growth of toxic and nontoxic Microcystis strains and on intracellular microcystin concentrations. – Microbial Ecology, 43: 443–454.

Via-Ordorika L., Fastner J., Kurmayer R. et al., 2004. Distribution of microcystin producing and non-microcystin-producing Microcystis sp. in European freshwater bodies: detection of microcystins and microcystin genes in individual colonies. Systematic and Applied Microbiology, 27: 592–602.

Vitėnaitė T., 2001. Lietuvos vandens telkinių melsvadumblių (Cyanophyta) sąvadas. – Botanica Lithuanica, 7(4): 343–364.

Wagner C., Adrian R., 2009. Cyanobacteria dominance: Quantifying the effects of climate change. – Limnology and Oceanography, 54(6): 2460–2468.

Walls J.T., Wyatt K.H., Doll J.C. et al., 2018. Hot and toxic: Temperature regulates microcystin release from cyanobacteria. – Science of the Total Environment, 610–611: 786–795.

Welker M., Brunke M., Preussel K. et al., 2004. Diversity and distribution of *Microcystis* (Cyanobacteria) oligopeptide chemotypes from natural communities studied by single-colony mass spectrometry. – Microbiology Society Journals, 150: 1785–1796.

Welker M., Šejnohová L., Némethová D. et al., 2007. Seasonal shifts in chemotype composition of Microcystis sp. Communities in the pelagial and the sediment of a shallow reservoir. – Limnology and Oceanography, 52: 609–619.

Welker M., von Döhren H., 2006. Cyanobacterial peptides - Nature's own combinatorial biosynthesis. – FEMS Microbiology Reviews, 30(4): 530–563.

Wells M.L., Trainer V.L., Smayda T.J. et al., 2015. Harmful algal blooms and climate change: Learning from the past and present to forecast the future. – Harmful Algae, 49: 68–93.

Wetzel R.G., 1983. Limnology. – 2nd Edition, Saunders College Publishing, Philadelphia.

WHO, 1998. World Health Organization. Cyanobacterial toxins: microcystin-LR. In: Guidelines for Drinking Water Quality (Ed.) Addendum to vol.2. Health Criteria and Other Supporting Information, 2nd ed. World Health Organization, Geneva, pp.95–110.

WHO, 2003. Guidelines for safe recreational water environments. Volume 1: Coastal and Fresh Waters. – World Health Organization, Geneva.

Wiedner C., Nixdorf B., Heinze R. et al., 2002. Regulation of Cyanobacteria and microcystin dynamics in polymictic shallow lakes. – Archiv fur Hydrobiologie, 155: 383–400.

Wiedner C., Visser P.M., Fastner J. et al., 2003. Effects of light on the microcystin content of *Microcystis* strain PCC 7806. – Applied and Environmental Microbiology, 69: 1475–1481.

Wiese M., D'agostino P.M., Mihali T.K. et al., 2010. Neurotoxic 675 alkaloids: saxitoxin and its analogs. – Marine Drugs, 8: 2185–2211.

Wilde S.B., Murphy T.M., Hope C.P. et al., 2005. Avian vacuolar myelinopathy (AVM) linked to exotic aquatic plants and a novel cyanobacterial species. – Environmental Toxicology, 20: 348–353.

Wiley F., Wilde S.B., Birrenkott A. et al., 2007. Investigation of the link between avian vacuolar myelinopathy and a novel species of cyanobacteria through laboratory feeding trials. – Journal of Wildlife Diseases, 4:337–344.

Wilhelm S., Adrian R., 2008. Impact of summer warming on the thermal characteristics of a polymictic lake and consequences for oxygen, nutrients and phytoplankton. – Freshwater Biology, 53: 226e237.

Winder M., Reuter J.E., Schladow S.G., 2008. Lake warming favours small-sized planktonic diatom species. – Proceedings of the Royal Society B: Biological Sciences, 276: 427e435.

Wolf H.U., Frank C., 2002. Toxicity assessment of cyanobacterial toxin mixtures. – Environmental Toxicology, 17: 395–399.

Wood S.A., Rasmussen J.P., Holland P.T. et al., 2007. First report of the cyanotoxin anatoxin-a from *Aphanizomenon issatschenkoi* (Cyanobacteria). – Journal of Phycology, 43: 356–365.

Wörmer L., Cirés S., Agha R. et al., 2011. First detection of cyanobacterial PSP (paralytic shellfish poisoning) toxins in Spanish freshwaters. –Toxicon, 57: 918–921.

Yamamoto Y., Nakahara H., 2006. Importance of interspecific competition in the abundance of Aphanizomenon flos-aquae (Cyanophyceae). – Limnology, 7(3): 163–170.

Yéprémian C., Gugger M.F., Briand E. et al., 2007. Microcystin ecotypes in a perennial *Planktothrix agardhii* bloom. – Water Research, 41(19): 4446–56.

Zębek E., 2006. Quantitative changes of *Planktolyngbya brevicellularis*, *Limnothrix redekei* and *Aphanizomenon gracile* in the annual cycle vs. physicochemical water parameters in the urban Lake Jeziorak Mały. – 35(1): 69–84.

Zervou S.-K., Christophoridis C., Kaloudis T. et al., 2017. New SPE-LC-MS/MS method for simultaneous determination of multi-class cyanobacterial and algal toxins. – Journal of Hazradous Materials, 321: 56–66.

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LIST OF PUBLICATIONS

Publications corresponded to ISI Web of Science

Savadova K., Mazur-Marzec H., Karosienė J., Kasperovičienė J., Vitonytė I., Toruńska-Sitarz A., Koreivienė J., 2018. Effect of increased temperature on native and alien nuisance cyanobacteria from temperate lakes: an experimental approach. Toxins, 10(11):445

Karosienė J., **Savadova K.**, Toruńska-Sitarz A., Koreivienė J., Kasperovičienė J., Vitonytė I., Błaszczyk A., Mazur-Marzec H., 2019. Production of saxitoxins and anatoxin-a by cyanobacteria from Lithuanian lakes. Under review

Mantzouki E., Campbell J., van Loon E. […], **Savadova K.** et al., 2018. A European multi lake survey dataset of environmental variables, phytoplankton pigments and cyanotoxins. Scientific data, 5:180226.

Mantzouki E., Lürling M, Fastner J., Senerpont Domis L., Wilk-Woźniak E., Koreivienė J., Seelen L., Teurlincx S, Verstijnen Y., Krztoń W., Walusiak E., Karosienė J., Kasperovičienė J., **Savadova K.** et al. 2018. Temperature effects explain continental scale distribution of cyanobacterial toxins. Toxins, 10(4).

Vičkačkaitė V., Lingytė A., Kasperovičienė J., Bugelytė B., Koreivienė J., **Savadova K.**, 2016. Selection of an esterification catalyst for assay of total fatty acid content in cyanobacteria and algae using gas chromatography. Chemija, 27(4):202–207.

Scientific publications in the other periodical refereed journals

Grendaitė D., Stonevičius E., Karosienė J., **Savadova K.**, Kasperovičienė J., 2018. Chlorophyll-a concentration retrieval in eutrophic lakes in Lithuania from Sentinel-2 data. Geologija, Geografija, 4(1): 15–28.

Koreivienė J., Kasperovičienė J., **Savadova K.,** Karosienė J., Vitonytė I., 2016. Collection of pure cultures of algae and cyanobacteria for research, teaching and biotechnological applications (Nature Research Centre, Lithuania). Botanica Lithuanica, 22(1): 87-92.

Abstracts of scientific conferences

Attended and presented

Savadova K., Karosienė J., Toruńska-Sitarz A., Koreivienė J., Kasperovičienė J., Vitonytė I., Błaszczyk A., Mazur-Marzec H., 2017. Cyanobacterial neurotoxins and their producers in temperate lakes in Lithuania. 11th International Phycological Congress. The challenges and opportunities of the molecular era for algal research and bioprospecting. Szczecin, Poland. P. 166-167.

Savadova K., Koreivienė J., Karosienė J., Kasperovičienė J., Vitonytė I., 2016. Recovery of shallow lake from cultural eutrophication in the light of climate change. XXXVth International Conference of the Polish Phycological Society. Algae in anthropogenically transformed ecosystems. Łódź-Stryków, Poland. P. 92.

Savadova K., Koreivienė J., Sivonen K., Kasperovičienė J., Suurnäkki S., Karosienė J., Wahlsten M., Vitonytė I., 2015. Variation of bloom forming cyanobacteria and microcystins in shallow hypertrophic lake. Sixth European phycological congress. Algae brings life to the world. London, UK. P. 204.

Presented as co-author

Koreivienė, J., Kasperovičienė, J., Karosienė, J., **Savadova, K.**, Vitonytė, I., Valčiukas, R., Staniulis D., Želvis, K., 2018. Prospecting of indigenous freshwater microalgae as a valuable regional resource. 37th International Conference of the Polish Phycological Society. Kraków-Dobczyce, Poland.

Stonevičius, E., Grendaitė, D., Karosienė, J., **Savadova, K.**, Kasperovičienė, J., 2018. Sentinel 2 data for retrieval of chlorophyll-α concentration in small lakes. 17th biennial conference ERB2018 Euromediterranean network of experimental and representative basins; Innovative monitoring techniques and modelling approaches for analysing hydrological processes in small basins. Darmstadt, Germany.

Grendaitė, D., Stonevičius, E., Karosienė, J., **Savadova, K.**, Kasperovičienė, J., 2018. Ecological water quality assessment of Lithuanian lakes using remote sensing. 61st international conference for students of physics and natural sciences. Vilnius, Lithuania.

Internships

2018/03/03–03/24	University of Gdańsk, Poland. “Production of secondary metabolites of freshwater cyanobacteria applicable in biotechnology”, supervisor Prof. dr. hab. Hanna Mazur-Marzec. Funded by COST program (ES1408 EUAlgae)
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