Fakulta rybářství
a ochrany vod
Faculty of Fisheries
and Protection
of Waters

Jihočeská univerzita
v Českých Budějovicích
University of South Bohemia
in České Budějovice

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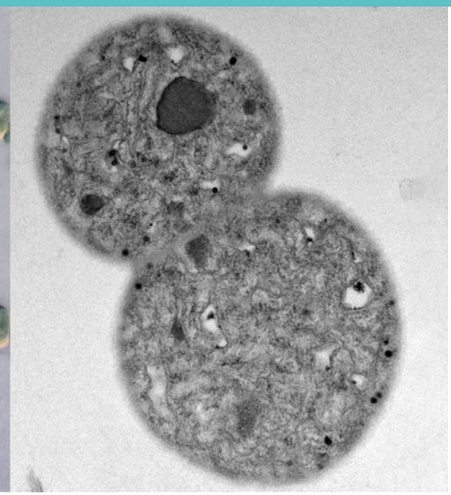
The taxonomical and physiological diversity of cyanobacteria from water environment

Taxonomická a fyziologická diverzita sinic z vodního prostředí



Doctoral thesis

The taxonomical and physiological diversity of cyanobacteria from water environment

Doctoral thesis by
Ivanova Anna Pavlovna

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Czech Republic, Vodňany, 2023

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CONTENT

CHAPTER 1

GENERAL INTRODUCTION

1.1. General introduction

Cyanobacteria are a group of gram-negative prokaryotes representing an essential part of phototrophic microorganisms. The first signs of their presence on the Earth are as old as 3.5 billion years (Summons et al., 1999; Cavalier-Smith, 2006). Cyanobacteria were responsible for changing the entire Earth's environment from an early reducing atmosphere to an oxidizing one during the Great Oxidation Event (GOE) (Schirrmeister et al., 2015). These changes made possible the evolution and diversification of the organisms. Cyanobacteria play a significant role in the Earth's biogeochemical cycles. They are one of the primary contributors to the formation of the Earth's lithosphere through the creation of stromatolites (Altermann et al., 2006).

Behind their magnificent success and superb spread lies the long time of their evolution. During the last few billions of years, those simple microscopic bacteria had enormous time to evolve to virtually any sunlit environment on Earth. A lot of cyanobacteria have developed a way to convert atmospheric nitrogen into a usable form in specialized cells known as heterocysts. This process enables the availability of nitrogen in a a wide range of ecosystems (Fay et al., 1968). Cyanobacteria can form symbioses with various organisms (e.g. algae, bryophytes, fungi, cycads, and plants) due to their ability to fix nitrogen and carbon, providing a competitive advantage in nitrogen-limited habitats (Rai et al., 2002).

Cyanobacteria are widespread. They are typical representatives of phytoplankton in freshwater reservoirs, marine environments, soil, and the surface of rocks (Bahl et al., 2011; Whitton, 2012; Hauer et al., 2015). Cyanobacteria can tolerate extreme temperature, salinity, desiccation, acidity, and irradiance, giving them an extraordinary competitive advantage in these ecosystems (Whitton, 2012). Furthermore, due to their ability to produce diverse secondary metabolites (e.g. UV-protective pigments: scytonemin and mycosporine-like amino acids), this prokaryotic group successfully survives in extreme environmental conditions (Oren and Gunde-cimerman, 2007; Kultschar and Llewellyn, 2018). Cyanobacteria are also called "pioneers" for their ability to colonize barren environments enabling the future condition for a succession of other species (Kulasooriya, 2011). Secondary metabolites of cyanobacteria are widely used in different fields such as medicine, agriculture, cosmetics, and biofuel production (Mishra and Pabbi, 2004; Nozzi et al., 2013; Singh et al., 2017; Morone et al., 2019; Nowruzi et al., 2020).

However, cyanobacteria form blooms in eutrophic waters and produce toxic compounds (O'Neil et al., 2012). Water blooms deteriorate water quality, including foul odors and tastes, causing hypoxia and anoxia of water (O'Neil et al., 2012). Cyanobacterial toxins can affect animals, including humans, causing mild skin rash, serious illness, or death in rare circumstances (Dittmann et al., 2013; Breinlinger et al., 2021). Nowadays, the rising of cyanobacterial blooms has become a critical issue, especially with climate change and eutrophication of the water environment. Therefore, it is necessary to learn as much as possible about aspects of cyanobacterial life, their diversity, ecology, dynamic in freshwater water bodies, and their identification and classification.

1.2. Eutrophication of fishponds

Ecosystems can be described in terms of their supplies of growth-limiting nutrients (Table 1). Water environments with poor nutrient supply are referred to as oligotrophic. Those with moderate nutrient levels are known as mesotrophic, and those with abundant nutrient supplies are categorized as eutrophic (Smith et al., 1999). Eutrophication, which can furthermore progress to hypertrophication is the condition of water bodies with excessive

nutrient inputs mainly of human origin (Wetzel, 1975). Eutrophication can affect not only freshwater lakes and reservoirs but also rivers and marine waters (Edmondson, 1995). The external supply of nutrients (phosphorus and nitrogen) to aquatic ecosystems proceeds from various sources, such as groundwater, rivers, and atmospheric inputs (Smith et al., 1999). Natural eutrophication is the slow aging process of lakes (Rast and Thornton, 1996). However, it can be significantly accelerated by human intervention in the natural cycling of nutrients (Rast and Thornton, 1996). It is primarily caused by wastewaters, agriculture, and industrial plants (Dokulil and Teubner, 2011).

Table 1. *Average characteristics of lakes of different trophic states (Nürnberg, 1996).*

Trophic state	TN (μg L^{-1})	TP (μg L^{-1})	Chl-a (μg L^{-1})	Secchi disk transparency (m)
Oligotrophic	<350	<10	<3.5	>4
Mesotrophic	350–650	10–30	3.5–9	2–4
Eutrophic	651–1,200	31–100	9–25	1–2
Hypertrophic	>1,200	>100	>25	<1

Phosphorus (P), the main limiting element of primary production in water environments, is the pivotal point of ecosystem status (Schindler et al., 2008). A high concentration of P >100 μg L^{-1}, known as hypertrophication, is expected to become a standard feature in various lakes and rivers in the near future (Jones and Brett, 2014) due to continual P supplementation by human activity, accelerated by climate change connected to decreased rainfall in affected regions (Sinha et al., 2017; Charlton et al., 2018). This issue is global as increasing input of nutrients, mainly from sewage water and intensive fish farming changes the trophic status of a wide spectrum of water bodies (Lewis and Wurtsbaugh, 2008). Although the concentration of various nutrients such as nitrogen (N) in water must be considered, reducing its inputs to water bodies increasingly favors hardly edible and nitrogen-fixing cyanobacteria as a response by the phytoplankton community (Schindler et al., 2008).

Fishponds, i.e., shallow lakes with relatively low water volume and well-known fish abundance, serve as outstanding natural laboratories for ecological studies. Fishponds represent the most common type of stagnant water bodies in the Czech Republic (Pechar, 2004). By area, fishponds take up 58% of the stagnant waters in the Czech Republic. More than 60% of fishponds are located in the South Bohemian region, being dominant water bodies covering 41,080 ha (Adámek et al., 2012). Recent high nutrient input from their watersheds and supplement of additional nutrients in the form of manure or fish feed makes them ideal sites for trophic studies.

Accessible historical data already documented their change from mesotrophic to the eutrophic state during the last century. Since the 1980s, intensive fish production practices connected with using mineral fertilizers changed the structure and seasonal dynamics of plankton in the Czech fishpond ecosystems (Pechar, 2000). Advanced fish breeding, fish stock densities reaching 300–600 kg ha^{-1}, along with enormous nutrient loads have resulted in a eutrophic and hypertrophic status of the majority of fishponds (Potužák et al., 2007). The main symptoms of this state are massive phytoplankton growth, fluctuation in pH and oxygen concentration, and a large excess of various products of the biodegradation, which lead to destabilizing of the fish ponds (Pechar, 2000). Therefore, ecological control of the functioning of fishponds, nutrient concentration, and phytoplankton biomass is desirable (Bíró, 1995; Pechar, 2000).

1.3. Cyanobacteria in fish ponds

Eutrophication has dramatically transformed the ecosystems of lakes and rivers worldwide. The main consequence of eutrophication is uncontrollable cyanobacterial growth , widespread in many fish ponds growth (Hallegraeff, 1993). They constitute the greater part of the phytoplankton during the summer (Sevrin-Reyssac and Pletikosic, 1990). Cyanobacteria can better than algae, sustain higher biomass under low-light conditions. The other competitive advantage is the vertical migration of cyanobacteria due to buoyancy regulation and so connected phosphorus uptake at the anoxic bottom layers of eutrophicated ponds. Cyanobacteria play a significant role in fish-culture ponds, not only because of their prolific development and spread but also due to their effects on the environment and on aquatic organisms. Cyanobacteria bloom significantly influences an ecosystem's functioning, such as deterioration in the aquatic environment, changes in biodiversity, lighting conditions, or oxygen concentrations (Schindler et al., 2008). The cyanobacteria bloom can create a significant water quality problem.

The harmful effect of cyanobacteria on fish can occur in various ways. Cyanobacteria have low nutritional value for feeding zooplankton and release toxic secondary metabolites. This leads to the massive death of fish and the poisoning of water, animals, and humans (Sevrin-Reyssac and Pletikosic, 1990). Fish are affected by a combination of factors such as cyanobacterial toxins, decreased oxygen levels due to excessive respiration by the high amount of cyanobacterial biomass during the night, and elevated levels of ammonia. Studies that examined the tissue abnormalities in fish mortalities during cyanobacterial blooms found that the major reason for the deaths was the harm caused to the gills, digestive tract, liver, heart, kidney, and skin (Drobac et al., 2016).

Also, cyanobacteria can have harmful effects on animals and birds living near polluted water. Every year there are reports of the death of cattle contaminated with cyanobacteria water (Zanchett et al., 2013). But domestic ducks are immune to toxic elements produced by cyanobacteria. They regurgitate toxins before they reach the digestive tube (Gorham, 1960).

In humans, exposure to cyanotoxins can occur in various ways. However, the oral route is the most common. This is mainly through drinking water or by eating contaminated foods (Zanchett and Oliveira-Filho, 2013). Also, it may even involve ingesting water during recreational activities. In humans, poisoning with cyanobacterial toxins can cause irritation of mucous membranes, allergic reactions, conjunctivitis, as well as gastrointestinal disorders, and spasms (Hilborn and Beasley, 2015).

The important feature concerning fishery management is production of taste and odor causing compounds. Cyanobacteria produce such compounds as geosmin or 2-methylisoborneol. These substances are responsible for fish's muddy smell, reducing its value. At least 11 cyanobacteria in the genera *Oscilatoria, Symploca , Anabaena* and *Lyngbya* produce geosmin, 2- methylisoborneol (Izaguirre et al., 1982).

The key species, the large colony-forming cyanobacteria genera like *Microcystis, Limnothrix, Planktothrix, Oscillatoria, Anabaena, Aphanizomenon, Dolichospermum,* which grow with vast numbers in water blooms, have received particular attention because of their dominance of the plankton in eutrophic fresh waters (e.g. Lyra et al., 2001; Rajaniemi et al., 2005; Moustaka-Gouni et al., 2010; van Gremberghe et al., 2011). Some cyanobacteria that were newly observed in warmed temperate zones are supposed to be invasive species (Briand et al., 2004). *Cylindrospermopsis raciborskii* with toxic effects is the most famous example of tropic cyanobacterium spreading into the temperate zone of Europe and North America (Moreira et al., 2015).

1.4. Phytoplankton dynamic in fishponds

General patterns in the seasonal succession of lake plankton are relatively well documented (Sommer et al., 1986), although many processes are involved. All fundamental ecological interactions, grazing, predation, competition, symbiosis, or parasitism, occur in plankton's seasonal development (Sommer et al., 1986). A combination of these ecological processes with unsteady abiotic conditions plays their role in plankton succession, making it unpredictable on a detailed level reflected in the plankton paradox, i.e., continually changing species composition (Hutchinson, 1961).

Thirty-five years ago, the repeated waxing and waning of planktonic populations in oligotrophic lakes were described by the plankton ecology group (PEG) model proposed by Sommer et al. (2012). Phytoplankton peaked in spring, followed by the summer maximum of zooplankton. Later, this model was altered for eutrophic lakes with two phytoplankton peaks in early spring and late summer, among zooplankton peak in late spring (Sommer et al., 2012).

The seasonal behavior of plankton might be overridden by fluctuations in weather conditions making the yearly comparisons even more challenging. To overcome all these complications, the long-term data from multiple lakes are usually evaluated for the robust general pattern of seasonal dynamics of lake plankton (Sommer et al., 1986, 2012).

In a nutshell, the seasonal dynamics of the plankton in a lake can be described by five typical features. (1) The spring increase of light is quickly followed by a rapid increase in primary production – phytoplankton growth with fast doubling time-spanning hours to days (Sommer, 2009). The phytoplankton species compete for resources, but the ultimate amount of carbon fixed by phytoplankters strictly subjects to Liebig's Law of the Minimum, with the limiting element being mostly phosphorus (P) (Schindler, 1977). (2) Zooplankton grazes on phytoplankton, using it as an energy source both for its growth and offspring in doubling time in the frame of days to weeks (Ranta et al., 1993). Zooplankton, especially big Daphnia species, can effectively eliminate any phytoplankton in a lake, being able to filter the entire volume more than once a day (Thompson et al., 1982) as well as scrape out the colonial species. (3) As the water temperature increases above ~17 °C the key planktivorous fish species as common carp (*Cyprinus carpio*), roach (*Rutilus rutilus*), or topmouth gudgeon (*Pseudorasbora parva*) increase their feeding rate and preferentially consume the zooplankton according its size and visibility (Jamet, 1994; Chang et al., 2004; Roy et al., 2019). (4) As the big species of zooplankton are consumed by fish at the end of spring, their grazing on phytoplankton drops down. The amount of zooplankton is controlled by fish stock until the water temperature falls to ~17 °C. (5) The total biomass of phytoplankton grows again until the light intensity decreases in autumn. (6) Physical control leads to lower growth rates of plankters and fish yielding low biomasses of both zooplankton and phytoplankton until spring when the cycle starts over (1).

The natural seasonal dynamics of plankton have been observed in fishponds in the late 1970s. Their seasonal plankton patterns corresponded to one of the predictable scenarios for shallow lakes not influenced by human activities.

In contrast to oligotrophic, mesotrophic, and eutrophic waters, the seasonal development of plankton in hypertrophic fishponds is not yet well understood. These ponds indicated puzzling and unpredictable seasonal variability under the short term studies (Potužák et al., 2007). The study of biological processes in hypertrophic water bodies is particularly critical due to their increasing spread in the near future.

1.5. Phytoplankton communities in lakes with different trophy level

Phytoplankton are a diverse group of microscopic, photosynthetic organisms that form the base of the aquatic food chain. They are found in both freshwater and marine ecosystems and are essential to the health and productivity of these environments.

The trophic level of a lake refers to the amount of nutrients, particularly nitrogen and phosphorus, available in the water. Lakes can be classified into three broad categories based on their trophic status: oligotrophic, mesotrophic, and eutrophic (Nürnberg, 1996).

Oligotrophic lakes are low in nutrients, have clear water, and support relatively low levels of phytoplankton. The phytoplankton communities in these lakes are typically dominated by small, nutrient-limited species such as green algae and diatoms. These phytoplankton communities are adapted to low-nutrient environments and are often highly competitive for the limited nutrients available in oligotrophic lakes (Kirk, 2010).

Mesotrophic lakes have moderate levels of nutrients and support a more diverse and productive phytoplankton community than oligotrophic lakes. The phytoplankton in mesotrophic lakes include a mix of small and large species, including diatoms, green algae, and cyanobacteria (Scheffer et al., 1993). The phytoplankton community in mesotrophic lakes is often dominated by one or a few species, but overall species richness is higher than in oligotrophic lakes.

Eutrophic lakes are high in nutrients and support a very productive phytoplankton community. The high levels of nutrients in these lakes lead to the growth of large, fast-growing phytoplankton species, such as cyanobacteria (Smith et al., 1999). These large species can form dense, visible blooms, which can have negative impacts on water quality, including decreased dissolved oxygen levels and increased risk of harmful algal blooms.

Overall, the phytoplankton communities in lakes with different trophic levels are adapted to the different nutrient conditions and have distinct species compositions and growth characteristics. These communities play important roles in the ecology of lakes and can have significant impacts on the water quality and overall health of these ecosystems (Jeppesen et al., 2005).

The increasing eutrophication of lakes due to human impact has become a severe environmental problem in Europe. As a consequence, not only the quality of water is decreasing, but also all aquatic organisms are affected. Initial changes in aquatic communities due to increased eutrophication start with changes in species composition, abundance, or phytoplankton production sequences (Alexander et al., 2017). Eutrophication may cause a catastrophic shift in the aquatic ecosystem, leading to biodiversity loss (Scheffer et al., 2001). Therefore the information obtained from phytoplankton communities can substantially contribute to evaluating eutrophication levels in freshwater water bodies.

There is a well-known, positive relationship between nutrient loading and productivity in lakes (Schindler, 1978). Increasing nutrient loads with eutrophication leads to the growth of phytoplankton biomass (Nicholls and Dillon, 1978). Species composition and richness are constantly changing due to biotic and abiotic changes and internal and exogenous drivers (Reynolds et al., 1993). Various studies have shown that phytoplankton biomass, species composition, and diversity change with increasing nutrient concentration (Smith, 1982; Watson et al., 1997). These changes are related to differences among species in nutrient uptake, storage, growth, and loss rates (Reynolds, 1984). However, there are missing data for comparisons of changes in the average biomass of major phytoplankton taxa, and species composition with nutrient levels among lakes with different trophy levels. To understand how eutrophication affects the quantity and types of phytoplankton present, a comprehensive evaluation of all the community components is essential, and this can be accomplished by analyzing long-term data sets.

1.6. A polyphasic approach to the cyanobacterial classification

Cyanobacteria are a phylogenetically very old group of prokaryotic microorganisms. Cyanobacteria were, together with algae, originally classified as primitive plants (Haeckel, 1866), applying the botanical classification based on their photosynthetic pigments, size and morphology. The first mention of cyanobacteria as a separate group and their systematic were dating back to the 19th century (Wallroth, 1833; Thuret, 1875; Bornet and Flahault, 1888; Gomont, 1892). Early phycologists used a light microscope and classified cyanobacteria based only on their morphology and rarely ecological criteria. In the the 20th century, cyanobacteria were divided based on their particular features, such as the presence of sheath, branching, and special cells (akinetes, heterocysts, hormogonia) (Geitler, 1925, 1932, 1942). Even though the classification of cyanobacteria based only on morphological and ecological trait were used for a long time, in modern days, it appears to be insufficient. The situation changed dramatically during the second half of the 20th century with the introduction of modern laboratory techniques such as electron microscopy (Rippka et al., 1974), flow cytometry (Chisholm et al., 1988) and molecular methods (Nübel et al., 1997).

In particular the use of 16S rRNA sequences and the adoption of mathematical algorithms to infer relationships of individual species, offering a simple method to analyze the prokaryotic phylogeny (Giovannoni et al., 1988; Boyer et al., 2001, Komárek, 2010). The main result of this "molecular revolution" was an abandonment of the artificial botanical classification and the clear establishment of Cyanobacteria as an independent phylum within domain Bacteria (Woese, 1987). Despite the fact that the original wide acceptance of 16S rRNA derived phylogenies, it was soon recognized that they do not represent an "ultimate" solution. Naturally, any phylogeny based on a single gene can only provide a partial and incomplete picture. The combination of traditional methods, together with their molecular biology and ultrastructure, helped researchers to re-evaluate their previous opinions about species distribution (Hoffmann, 1988; Komarek, 2003). This polyphasic approach was used for the systematic revaluation of cyanobacterial taxa (Anagnostidis and Komárek, 1985, 1988, 1990). Despite the great effort of re-evaluation of cyanobacteria classification, only a small amount of cyanobacteria diversity was analyzed using a polyphasic approach.

Most scientists favouring to study of bloom-forming species. Unfortunately, nowadays, there have been limited studies into cyanobacterial diversity in extreme climates. Consequently, it is crucial to study species diversity for many non-investigated environments, where climatic changes present a risk for future sustainability. Classic phycology approaches based on morphology evaluation and sequencing hold strong potential for bringing new knowledge about cyanobacteria's community structure and diversity. These methods provided to discover two new genera and six species of cyanobacteria in the Alaska North Slope (Strunecky et al., 2020). These findings were already used as background for further detailed taxonomical studies and recently published in Strunecký et al. (2022).

1.7. Objectives of the thesis

The current study was devoted to the comprehensive investigation of cyanobacteria. We used a long-term data set from 9 fishponds in the South Bohemian region to study phytoplankton dynamics and species composition. We study how nutrient load, zooplankton biomass and high fish stock influence phytoplankton succession and cyanobacteria dominance in summer. Also we discover new species of cyanobacteria in previously non-investigated environments.

To reach our goal, the following objectives were pursued:

1. To study the plankton dynamic, species composition, and nutrients concentration in 9 fishponds in the South Bohemian region;
2. To find out the relationship between phytoplankton and nutrients, zooplankton biomass, and high fish stock;
3. To investigate the difference in phytoplankton communities ponds with different trophic state;
4. To identify and isolate cyanobacterial species from the Alaskan North Slope.

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CHAPTER 2

CYANOBACTRIAL DOMINANCE IN HYPERTROPHIC FISHPONDS IS NOT DRIVEN BY ZOOPLANKTON

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Cyanobactrial dominance in hypertrophic fishponds is not driven by zooplankton

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Abstract

In order to understand the processes of plankton succession in hypertrophic water bodies, we analyzed 8-year data from six hypertrophic fishponds in Czechia collected in the period 2008–2016. Supplemental fish feed was identified as the important source of phosphorus resulting in an overall mean of 150 μg L–1 in fishpond water. The dominance of cyanobacteria in the phytoplankton closely mirrored the overall phosphorus level in the water. While algae flourished until nitrogen became scarce, cyanobacteria were able to thrive due to their ability to fix nitrogen. The top-down pressure plays a significant role. Fish biomass of 300–900 kg ha^{-1} disrupted the plankton food web by top-down elimination of zooplankton. Lower fish biomass increases the phosphorus transfer from phytoplankton to fish via zooplankton, potentially improving water quality. Such specific conditions of hypertrophic fishponds are reflected in a seasonal plankton succession that previous ecological studies have not described.

Keywords: *cyanobacteria; hypertrophication; fish stock; nitrogen; phosphorus; plankton dynamics.*

Introduction

Cultural eutrophication has dramatically transformed the ecosystems of lakes and rivers worldwide. It is widely accepted that the main limiting element of primary production in aquatic environments is phosphorus (P) which acts as the primary driver of ecosystem status (Schindler et al., 2008). Hypertrophy, defined by a concentration of P higher than 100 μg L^{-1}, is expected to become a feature of many lakes due to continuous P input by human activity (Jones and Brett, 2014). The study of biological processes in hypertrophic water bodies is thus of crucial relevance due to their extent in the near future.

The most infamous trait in eutrophic and hypertrophic water bodies is the dominance of cyanobacteria, often resulting in harmful cyanobacterial blooms. A rising number of studies link such cyanobacterial dominance to three factors: (1) low concentration of soluble nitrogen in water (Gao et al., 2014; Ivanova et al., 2022) favoring the nitrogen-fixing species of cyanobacteria (Jones and Brett, 2014; Di Cesare et al., 2018); (2) specific cyanobacterial features such as the buoyancy in species with gas vesicles (Porat et al., 2001; Chu et al., 2007), together with superb low-light utilization (Gao et al., 2014, Nedoma and Nedbalová,

2006); and (3) zooplankton feeding preference for algae, leading to the algae elimination that opens a free niche for extensive development of cyanobacteria.

These assumptions are hard to test, particularly in the natural environment where the combination of ecological processes and unstable abiotic conditions drives plankton succession, making it unpredictable at a population level. This phenomenon is reflected in the 'paradox of plankton,' i.e., the continual change of species composition and community structure (Hutchinson, 1961). However, a specific situation in South Bohemian fishponds enabled us to study one particular variable, i.e. item (3) above: zooplankton's influence on phytoplankton's seasonal succession.

These fishponds were surveyed in the 1960s and 1970s (Kořínek, 1967; Fott et al., 1980) when the natural seasonal dynamics of plankton communities followed predictable scenarios for shallow lakes not influenced by human activity. The seasonal dynamics of the plankton succeeded in several typical phases: (1) A rapid increase in primary production – phytoplankton growth with fast doubling time-spanning hours to days – followed by the increase of light in early spring. The phytoplankton species competed for resources, but the ultimate amount of carbon fixed by phytoplankters was strictly subjected to the limiting element, which was most probably phosphorus (Schindler, 1977). (2) Zooplankton grazed on phytoplankton, using it as an energy source for its growth and offspring production with doubling time in the frame of days to weeks (Ranta et al., 1993). Zooplankton, especially large *Daphnia* species, effectively eliminated most edible phytoplankton in fishponds in late spring (Kořínek, 1967). (3) As the water temperature increased, the key planktivorous fish species as common carp (*Cyprinus carpio*) and roach (*Rutilus rutilus*) with moderate fish biomass below 300 kg ha^{-1} (Fott et al., 1980) increased their feeding rate and preferentially consumed the zooplankton according to its size and visibility (Chang et al., 2004, Jamet, 1994, Zemanová et al., 2020). (4) When fish consumed the large species of zooplankton at the end of spring, their grazing on phytoplankton dropped. The amount of zooplankton was controlled by fish stock until the water temperature decreased (Pechar, 2000; Potužák et al., 2007). (5) The total phytoplankton biomass grew again until the light intensity decreased in autumn. (6) Low light and temperature during winter limited phytoplankton and zooplankton biomasses until next spring when the cycle starts over (1).

Since the 1980s, increased nutrient input from the watershed and intensified fish management caused additional eutrophication of South Bohemian fishponds (Pechar, 2000). Further nutrient loads from watersheds boosted by supplementary feeding of fish reaching biomasses around 1,000 kg ha^{-1} (Francová et al., 2019a) resulted in hypertrophic conditions. Simultaneously a high fish predation pressure causes a low abundance of zooplankton (Potužák et al., 2007), altering the seasonal succession of phytoplankton.

Considering the knowledge gaps in food webs connections in freshwater bodies, particularly the effect of the top-down forcing on seasonal plankton succession (Diniz et al., 2019, Ersoy et al., 2019, Jeppesen et al., 2020, Rettig and Smith, 2021), we analyzed the chemical and biological variables in six hypertrophic fishponds. As the annual pattern of plankton succession is often overridden by fluctuations in weather, hampering year-to-year comparisons, data from ten seasons were evaluated, providing altogether 404 data points for chemistry data, 253 for phytoplankton, and 172 for zooplankton. These data were analyzed to establish the general pattern of seasonal plankton succession, emphasizing the drivers of excessive development of cyanobacteria in hypertrophic water bodies.

Material and methods

Study area

Six fishponds in the Czech Republic were studied. Their size ranged from 68 to 242 ha, water volume from 1.3 to 5.9 10^6 m^3, and mean depth from 1.0 to 2.4 m. (Table 1; Fig. S1). Fishponds Rožmberk (RZ), Dehtář (DH), Svět (SV), Horusický (HO), Ratmírovský (RT) located in the upper Vltava River basin were monitored in the context of EU Water Framework Directive by the Vltava River Authority, State Enterprise. Due to high fish abundance in all studied ponds the historical data of fishponds with lower fish density and known zooplankton biomass are included in the analysis (Hrbáček, 1962). Fishponds Smyslov (SM) and Velký Pálenec (VP) are located in the same region. Historically, they shared fishery management as studied ponds due to the only single government-enabled methodology prior to 1989 (Fig. 1). The duration of hydrobiological sampling season was set from April to August, and it is referred to throughout the text as 'season'.

Table 1. *Overview of the basic characteristics of the fishponds including their location, size, depth parameters and the number of sapmles of basic chemical analyses (TP, DP, TN, N-NO3, N-NH4, POC, TOC) with Chlorophyll-a, and transparency generally in two weeks intervals; Samples of phytoplankton, and zooplankton were measured generally bi-weekly or monthly from spring to autumn with a sampling periods.*

Site	GPS coordinates	Surface area (ha)	Volume (10^6 m^3)	Depth average/ max (m)	Chem. Parametrs	Phyto-plankton	Zoo-plankton	Sampling frequency and duration
Rožmberk (RŽ)	49°2′51.073″N 14°45′42.357″E	449	5.94	1.2/4.1	99 (2008–2015)	58 (2009–2015)	47 (2008–2015)	2008–2009, 2015–monthly; 2010–2014–bi–weekly
Dehtář (DH)	49°0′29.909″N 14°18′21.541″E	228	4.71	2.4/6.0	103 (2008–2015)	66 (2009–2016)	40 (2008–2015)	2008–2009, 2013–2014, 2015– monthly; 2010–2012, 2015–bi–weekly
Svět (SV)	48°59.942'N 14°45.864' E	214	3.39	NA/3	80 (2008–2015)	50 (2009–2015)	36 (2008–2015)	2008–2012–monthly; 2013–2015–bi–weekly
Horusický (HR)	49°9′54.198″N 14°41′30.608″E	415	3.97	1.0/6.0	48 (2012–2015)	26 (2012–2015)	21 (2012–2015)	2012–2013–bi–weekly; 2014–2015–monthly
Ratmírovský (RT)	49°8′36.091″N 15°7′12.991″E	78	1.30	1.7/6.0	38 (2008–2009, 2011–2012)	27 (2009–2012)	16 (2008–2012)	2008–2009, 2011– monthly; 2012–bi–weekly.
Hejtman–Hamerský (HH)	49°8′44.757″ N 15°9′57.915″ E	68	1.60	2.3/6.0	36 (2008–2009, 2011–2012)	26 (2009–2012)	12 (2008–2011)	2008–2009,2012–monthly; 2010–2011–bi–weekly.

Physical and chemical parameters

Each fishpond was sampled biweekly or monthly using Bayer tube sampler at the deepest site between 08:00 and 10:00 h from April to October and monthly during the ice-free period (from November to March) from 2008 to 2016. The sampling period varied among ponds (Table 1). The integrated sample of the euphotic layer (from 0–1 m depth) was collected for chemical analyses.

Water temperature, dissolved oxygen (DO) concentration, pH, electrical conductivity, and turbidity were measured in situ using a field multimeter equipped with a multi-parameter probe (YSI 6600 V2-4, YSI, USA). Water transparency was examined by Secchi disc. Total phosphorus (TP) and soluble reactive phosphorus (SRP) were analyzed by inductively coupled plasma spectrometry (Agilent 8800 ICP-QQQ) (ISO-EN, 2003). The concentration of total nitrogen (TN) was established by the determination of bound nitrogen following oxidation of nitrogen oxides (Analytic Jena multi N/C 2100, Germany) according to ISO (2003). The concentration of total carbon (TC), total organic carbon (TOC), and dissolved organic carbon (DOC) were analysed after filtration on GFC filter (Whatman, USA) by high-temperature catalytic oxidation (Analytic Jena multi N/C 2100, Germany). For determination of the concentration of ammonium (NH4-N) and nitrate (NO3-N), spectrometry and liquid chromatography were used (Shimadzu UV-1650PC, Shimadzu Corporation, Japan; Dionex ICS-1000, ThermoFisher Scientific, USA) (ISO-SFSEN, 2009).

Chlorophyll-a (Chl-a) concentrations were analyzed spectrophotometrically after extraction by hot ethanol (Shimadzu UV-1650PC, Shimadzu Corporation, Japan) (Hungarian_Standard, 1992).

Phytoplankton

Phytoplankton samples were taken monthly from April through October as an unfiltered subsample in the 0–1 m depth layer using a Van Dorn sampler simultaneously with chemical samples (253 analyzed communities) (Table 1). Phytoplankton was preserved with Lugol solution and counted after species determination in Utermohl's chambers under an inverted microscope (Olympus IX 71) (Lund et al., 1958). The biovolume was calculated using the algal cell dimensions according to Komárková and Cronberg (1994) and converted to fresh biomass using factor of 1.0 of specific gravity (Hillebrand et al., 1999). Phytoplankton biomass was converted to carbon using the conversion factor of 0.2 (Menden-Deuer and Lessard, 2000).

Zooplankton

Sampling of zooplankton was carried out simultaneously with phytoplankton (Table 1). Zooplankton was collected using an Apstein plankton net (net mesh = 40 μm) by vertical hauls from the bottom to the surface. The sample was divided by sieves with mesh sizes of 0.71 mm and 0.42 mm (Seda and Dostalkova, 1996). Samples were preserved with formaldehyde and analyzed using optical microscopy to determine species composition and abundance in sedimentation chambers (Seda and Dostalkova, 1996). The taxon-specific regression was used to recalculate the dry weight of zooplankton to carbon equivalent (Dumont et al., 1975; Bottrell et al., 1976; Rosen, 1981; McCauley, 1984; Culver et al., 1985; Wetzel and Likens, 2000). Altogether, 172 samples of zooplankton were determined and counted.

Fish and feed

Detailed data provided by fishery enterprises covered 5 ponds in studied years, data for RT were available for 2011 only. Each fish harvest and fish supplement throughout the year course with an estimated growth rate of fish were recalculated to monthly data to agree with the final fish harvest every second year or every year in the case of RZ. The biomass of fish was recalculated from t ha^{-1} to mg L^{-1} using pond volumes (Table 1). Feed, mainly as whole grain wheat or barley was recalculated from whole year consumption data according to the generally used practice by South Bohemian fishery enterprises (Hartman and Regenda, 2016). Values were further converted to TP using a conversion ratio of 3.3 g of P to 1 kg whole grain (Potužák et al., 2016).

Data and statistical analyses

The concentrations of TP, SRP, TN, NO_3-N, NH_4-N, Chl-*a*, TOC, and POC, along with transparency, were used as parameters for the description of the habitat variables and pond trophy. To investigate the seasonal trends in physical and chemical parameters, phytoplankton (CPhy), zooplankton (CZoo), and fish biomass density, and cumulative supplemental feed data were fitted by locally weighted smoothing (LOESS) with a span of 95%. Relationships among CPhy and CZoo and habitat variables were evaluated using mixed-effect models, which allowed flexible accounting for repeated measurements in the same ponds and temporal autocorrelation in the investigated time series (Pinheiro and Bates, 2006). Linear mixed models (LMM) were employed to test the relationship among CPhy, CZoo, and other eutrophication traits (Chl-*a*, transparency, TP, POC). The parameters were analyzed independently from each other. Additive mixed models (AMM) with penalty tensor products of cubic regression splines (Wood, 2006) were tested to fit nonlinear relationships. We also evaluated time-lagged relationships of CPhy *vs*. TN, NO_3-N, NH_4-N, TP, and SRP; CZoo *vs.* CPhy; and CZoo *vs.* fish biomass. In the mixed effect models, we first specified complex random effect structure involving slopes and intercepts varying among ponds and years within each pond (Barr et al., 2013). The random effect structure was subsequently simplified using Akaike's information criterion, and only the most parsimonious models were furtherly interpreted. Assumptions of the models were carefully checked using diagnostic plots of residuals and correlograms (Bjørnstad and Falck, 2001). If needed, data were log-transformed to improve normality and homogeneity of variance. In the case of significant temporal autocorrelation patterns in the residuals, the models were re-fitted using a continuous autoregressive correlation structure (Pinheiro and Bates, 2006). The predictive accuracy of each model was assessed using leave-one-out cross-validated relative median absolute error (RMAE).

The analyses were performed in R v. 3.6.0 (R_Core_Team, 2019) using the packages gamm4 (Wood et al., 2017), ggplot2 (Wickham, 2016), ncf (Bjørnstad and Cai, 2019), and nlme (Pinheiro et al., 2019). Adobe Illustrator was used for the final graphing of figures.

Results

Physical and chemical parameters

The mean and standard deviation (SD) of TP concentration in the six fishponds were 0.15 ± 0.09 mg L^{-1} (in a range of 0.03–0.61 mg L^{-1}) (Table S1). The concentration of TP in SV was significantly lower than in other ponds ($P<0.05$) (Table S2; Fig. S2a). The locally weighted concentration of TP in the studied fishponds increased from April to August (Fig. 1a). Total

phosphorous was positively correlated with POC (Table S3; Fig. S3d) (P<0.0001, R^2 = 0.45), likely reflecting the fishpond primary productivity.

The mean and standard deviation (SD) of SRP was 0.03 ± 0.03 mg L^{-1} (in a range of 0.01–0.16 mg L^{-1}) (Table S1). The concentration of SRP in DH was significantly higher and in SV lower than in other ponds (P<0.05) (Table S2; Fig. S2b). The concentration of SRP in DH, RT, HH, and SV ponds reached maximum values in the early summer, whereas it peaked in mid- and late summer in RZ and HO (Fig. S4a).

Mean concentration of TN was 1.8 ± 0.7 mg L^{-1}, peaking from July through September, except in RT and HH where the spring concentration rapidly decreased until June. (Table S1; Fig. 1b). The concentration of TN in HO, RT, and HH was significantly higher than in other ponds (P<0.05) (Table S2; Fig. S2c). The mean concentration of $N\text{-}NO_3$ was 0.3 ± 0.5 mg L^{-1} with a spring peak 3.6 mg L^{-1} (Table S1; Fig. 5a). The mean concentration of $N\text{-}NH_4$ was 0.2 ± 0.2 mg $L^{-1,}$ with maximum values of 0.3 mg L^{-1} during May and September (Table S1; Fig. S5b). The concentration of $N\text{-}NH_4$ in HO was significantly higher than in other ponds (P<0.05) (Table S2, Fig. S2d).

The mean concentration of Chl-*a* was 0.08 ± 0.07 mg L^{-1} (Table S1). In July, maximum values of Chl-*a* 0.5 mg L^{-1} were measured through August during intensive growth of phytoplankton, while minimum values of Chl- *a* appeared during spring 0.001 mg L^{-1} (Fig. 1c, 6, Fig. S3b). Transparency was typically low (<1.0 m) and significantly positively correlated with CPhy (Fig. 1c, d; Fig. S3a). RZ had significantly lower transparency, and DH had the highest transparency among ponds (Table S2; Fig. S2 f).

Over the spring and summer, the total organic carbon (TOC) level in the ponds gradually rose (Fig. S4b). Meanwhile, the amount of particulate organic carbon (POC) steadily increased until August, after which it dropped slightly in all the ponds (Fig. 6; Fig. S4c).

Phytoplankton

The CPhy continuously increased until June or July in all ponds, peaking in summer with maximum values ranging from 9.72 to 11.54 C mg L^{-1} (Fig. 6). In total, 260 taxa of phytoplankton were observed: 123 taxa of Chlorococcales, 50 Cyanobacteria, 36 Bacillariophyceae, 11 Desmidiaceae, 11 Volvocales, 8 Cryptophyceae, 10 Euglenopyceae, 7 Dinophyceae, and 4 Chrysophyceae. Cyanobacteria, Chlorococcales, Bacillariophyceae, Cryptophyceae represented the dominant fraction of total phytoplankton biomass. Cyanobacteria prevailed at RZ and SV, with *Planktothrix, Microcystis, Anabaena,* and *Aphanizomenon* accounted for approximately 63% of total CPhy in RZ and 67 % in SV, with mean concentration of 4.2 ± 5.5 C mg L^{-1} and 2.9 ± 6.4 C mg L^{-1}, respectively (Fig. 2a). Bacillariophyceae (*Ulnaria acus, Aulacoseira* sp., *Nitzschia* sp.) were dominant phytoplankton group, with 32% and 43% and mean C concentrations of 0.5 ± 1.3 mg L^{-1} and 1.3 ± 1.4 mg L^{-1}, at DH and HH in spring (Fig. 2b). DH and HH were dominated by cyanobacteria in summer and autumn, with mean CPhy concentrations 1.0 ± 3.35 C mg L^{-1} at DH and 3.5 ± 6.3 C mg L^{-1} at HH (Fig. 2a). Cyanobacteria contributed the major portion of CPhy at RT in spring and summer representing 52% of total CPhy and mean concentration of 2.5 ± 2.7 mg L^{-1} (Fig. 2a). During autumn, Bacillariophyceae were the dominant group with a mean concentration of 1.3 ± 1.36 C in mg L^{-1} (Fig. 2b). At HO, Chlorococcales were the major group of phytoplankton from April to August with mean CPhy of 2.8 ± 10.3 mg L^{-1} represented by *Botryococcus braunii, Oocystis marssonii,* and *Pseudopediastrum boryanum* (Fig. 2c); Cryptophyceae formed the second most dominant group in this period, represented by *Cryptomonas marssonii, Cryptomonas reflexa,* and *Chroomonas minuta* with mean CPhy ~0.3 ± 0.3 mg L^{-1} from April to August (Fig. 2d). Cyanobacteria dominated in HO during September and October, accounting for a CPhy concentration of ~10 mg L^{-1}.

CPhy was positively correlated with TP in all ponds ($P < 0.0001$, $R^2 = 0.35$) (Fig. 4; Fig. S3c). The results of the additive mixed models indicated a P-limitation of phytoplankton growth in the system (Fig. 4). After accounting for the effect of P in the additive model, the relationship between TN and CPhy was not significant ($P = 0.0579$) (Fig. 4). The CPhy was highest at N:P from 10:1 to 25:1 and simultaneously with TP higher than ~0.2 mg L^{-1} (Fig. 4). Under these conditions, cyanobacteria contributed the major portion of CPhy. Only 2.9% of CPhy values were found in conditions of TN:TP <10:1, in which cyanobacteria formed 50% and Bacillariophyta 36% of phytoplankton biomass. With TP under 0.002 mol L^{-1} (0.06 mg L^{-1}) and TN:TP >25:1, the dominant species were Chlorophyta and Bacillariophyta. This scenario was observed in spring at DH, HO, RT, and HH (Fig. 2).

Anticipated significant positive correlation was found between Chl-*a* and CPhy (Chl-*a* = 22.6 × $CPhy^{1.4}$, $P < 0.0001$, and RMAE = 35%). The CPhy was also significantly positively correlated with Chl-*a*, TP, and transparency (CPhy = 0.1 × Chl-*a* $^{0.8}$, $P < 0.0001$, RMAE = 39%; CPhy = 16 × TP, $P < 0.0001$, RMAE = 41%; and CPhy = 0.9 × $Trans^{-1.4}$, $P < 0.0001$, RMAE = 38.6%) (Figure S3b,c,e). POC was positively correlated with CPhy but not correlated with TN (CPhy = 1.1 × 0.4 TN, $P = 0.0205$, RMAE = 49.1%). We did not find immediate ($F\_1,167 = 0.09$, $P = 0.763$) or time-lagged ($F\_1,1367 = 0.76$, $P = 0.386$) response of CZoo to CPhy.

Zooplankton

The mean concentration of total CZoo was 0.5 ± 0.8 C in mg L^{-1}, maximum values ranged from 1.1 to 5.9 C in mg L^{-1}in HO, DH, and SV in April. Minimum values ranged from 0.004 to 0.01 mg L^{-1} in RZ, DH, SV, and RT in April (Table S1).

CZoo showed a clear seasonal pattern in the studied period, with the maximum in May and June in all ponds (Fig. 6). CZoo primarily consisted of large daphnids such as *Daphnia cucullata, D. galeata,* and *D. magna* contributing from 50% to 80% of total CZoo giving maximum values of 0.5 to 2.5 mg L^{-1} (Fig. 3a).

Small *Daphnia* species such as *D. ambigua* or *D. parvula* were found only in SV, DH, and HO in 26 cases. The maximum Czoo of this group was observed in April in SV at 1.38 mg L^{-1}. Small number of data did not allow to provide graphs and include them into statistical analyses. Other Cladocera as *Bosmina longirostris, Moina micrura,* and *Chydorus sphaericus* peaked in spring with a maximum C value of 1.3 mg L^{-1} in May in RZ (Fig. 2b). CZoo of adult Copepoda (*Cyclops vicinus, Thermocyclops crassus, Mesocyclops leuckarti*) was always less than 0.4 mg L^{-1} with its maximum in late summer (Fig. 2c). The average Czoo of Copepoda nauplii was 0.008 ± 0.008 mg L^{-1}, with the highest concentration, 0.05 mg L^{-1}, observed in DH in April (Fig. 2d). Rotifers included 60 species with abundances to 665 900 ind. L^{-1}; however, their CZoo was always lower than 0.02 mg L^{-1}. The main species in all studied ponds were *Keratella cochlearis, Brachionus angularis,* and *Polyarthra dolichoptera*.

A significant negative relationship (CZoo = 1.22-0.24 × ln(Fish in mg L^{-1}), $P = 0.015$, RMAE = 70.1%) between fish biomass and CZoo was found (Fig. 5).

Fish and feed supplement

The total biomass of fish in studied ponds consicted of 90% Common carp (*Cyprinus carpio* L.), and the rest is bighead and silver carp (*Hypophthalmichthys nobilis* Richardson 1845 and *Hypophthalmichthys molitrix* Val.), grass carp (*Ctenopharyngodon idella* Valenciennes 1844), northern pike (*Esox lucius* L.), pike-perch (*Sander lucioperca* L.), tench (*Tinca tinca* L.), Prussian carp (*Carassius gibelio* Bloch), common and silver bream (*Abramis brama* L., *A. bjoerkna* L.). The fish biomass at the start and at the fish harvest and the quantity of feed supplied are shown in Fig. S6.

Discussion

The six studied fishponds represent specific environment constrained by extreme nutrient load and intense predation pressure from farmed and weed fish. Weekly feeding is considered as an important P source to the pond ecosystem for maximizing fish production (Rutegwa et al., 2019a,b). High fish density impairs the trophic food web by eliminating zooplankton. The results of this study describe plankton succession in fishponds and the high influence of top-down forcing.

The currently accepted paradigm for eutrophic lakes with fish activity is summarized by the revised PEG model that describes two to three modal phytoplankton and zooplankton curves peaking in late spring, middle of the summer, and autumn (Sommer et al., 2012). The spring production of phytoplankton is followed by zooplankton grazing reducing phytoplankton biomass. The summer phytoplankton peak in the PEG model is attributed to the development of inedible phytoplankton species represented mainly by cyanobacteria.

However, such a pattern of plankton succession was not observed at our sites. Although the concentration of phytoplankton varied between fishponds, its general seasonal development remained very similar. The CPhy rose continuously to maximal values from 0.14 mg C L^{-1} to 11.54 mg C L^{-1} from spring to autumn without any modes or antimodes (Figs. 2 and 6, Fig. S7). Although cyanobacterial Cphy was lower during the first part of the season, cyanobacteria prevailed from July till the end of the season (Fig. S7).

The grazing pressure of zooplankton is considered as a key factor regulating phytoplankton biomass (Hrbáček et al., 1961). The zooplankton biomass was reduced by fish predation in the studied fishponds, and populations of large Cladocerans did not grow fast enough to influence the phytoplankton.

In addition, visually oriented predators, such as undesirable cyprinid fish, consume preferentially large *Daphnia* with multiple and most developed offsprings (Zemanová et al., 2020). The absence of antimodes in CPhy reflected a low density of zooplankton that was effectively suppressed by fish (Fig. 3, Fig. S4). The top-down control triggered by high density of planktivorous fish (Carpenter and Kitchell, 1988) opened a niche for uncontrolled phytoplankton growth.

Fish density alone could not explain the fluctuations in CZoo, which varied in a range 0.6 ± 0.5 mg L^{-1} with similar fish density (Fig. 5). Datapoints on August 1 of all years, both in the first and second year of the production cycle (Fig. 5), suggest that fish concentration higher than 20 mg L^{-1} (~280 kg ha^{-1}) suppresses zooplankton growth from spring through autumn (Fig. 5). In contrast historical data reveal that fish densities below 5 mg L–1 (~70 kg ha^{-1}) enable natural phytoplankton succession in fishponds, characterized by a spring peak followed by zooplankton grazing producing a clear-water phase (Fott et al., 1980, Hrbáček, 1962, Pechar, 2000). For acceptable ecosystem function of fishponds, fish density should not exceed 300 kg ha^{-1}. Such stocking density prevents the ingrowth of aquatic vegetation and sediment filling while simultaneously enabling sustainable fish production (Francová et al., 2019a,b).

The well known key role of P in phytoplankton growth (Schindler, 1977) was confirmed by our data and corresponded to the summer peak of TP concentration (Figs. 1a, 2, 4, 6, and Fig. S5). As cyanobacteria fix inorganic carbon as well as molecular nitrogen dissolved in water, the concentration of NH_4-N and NO_3-N in water is not critical to their proliferation (S. Fig. 3).

Nitrogen input from the watershed and added manure (Rutegwa et al., 2019b) was not denitrified during winter and early spring, resulting in high NO_3-N and corresponding TN concentrations in late spring. Rising temperatures in spring triggered the denitrification that effectively released N from fishponds, including that from added feed. (Figs. 1b and

7, Fig. S3 and S4). The reported rate of denitrification in eutrophic fishponds ranges from ~150 to 700 mg N m^{-2} d^{-1} (Jensen et al., 1992, Olah et al., 1994, Piña-Ochoa and Álvarez-Cobelas, 2006). At this rate, all nitrogen would be effectively released from the studied ponds in 3–21 days, especially taking into account the high quantity of available DOC (Table S1). Despite rapid denitrification, the TN levels remained high in the studied hypertrophic ponds, with N:P lower than 10:1 rarely observed (Fig. 4). Moreover, most of both main nutrients are particulated, i.e. they are bound in plankton biomass (Fig. 1b). The high P concentration thus mitigates the relevance of low stoichiometric N:P ratios in a hypertrophic system dominated by cyanobacteria (Fig. 4).

A long-standing debate exists over whether nitrogen effects on phytoplankton growth are dependent on the type of water body, trophic status, season (Downing and McCauley, 1992, Kolzau et al., 2014), and the stoichiometric ratio supporting cyanobacterial dominance (Smith, 1983). Data obtained during this study indicate that eutrophication of the water bodies could not be controlled by reducing nitrogen input. Soluble nitrogen was almost absent in studied fishponds (Figs. 1b and 4). A plausible explanation of cyanobacterial prevalence in nutrient-rich water may be the higher N turnover rate by bacteria using high levels of organic carbon connected with rapid denitrification and, counterintuitively, the limitation of algae by low levels of inorganic nitrogen during summer and autumn. Fixation of nitrogen by cyanobacteria is then assumed to explain their dominance during summer and autumn in hypertrophic water bodies.

Phytoplankton biomass was driven by the high P concentration. Whereas CZoo moderately increased in spring until temperatures >17 °C allowed fish feeding, suppressing zooplankton throughout the season, and efficiently disconnected the entire food web. In consequence, zooplankton was not able to suppress the proliferation of phytoplankton. Therefore, the seasonal plankton dynamics in hypertrophic fishponds might be constrained by the concentration of P and fish stock density. Maximizing phosphorus transfer from phytoplankton through zooplankton to fish at their densities below 300 kg ha^{-1} would be a simple measure to reduce phytoplankton and improve water quality. Under such densities, the amount of P added by supplementary feed in the most impacted ponds would decrease by ½ resulting in a 30% decrease in the overall P load. Simultaneously, a reduced fish stock pressure would allow the development of zooplankton, increasing the transfer of P from phytoplankton through zooplankton to fish biomass, which is regularly removed from the fishponds' environment. Such measures increase fishponds' ecological status without a hardly achievable decrease in phosphorus input from watersheds.

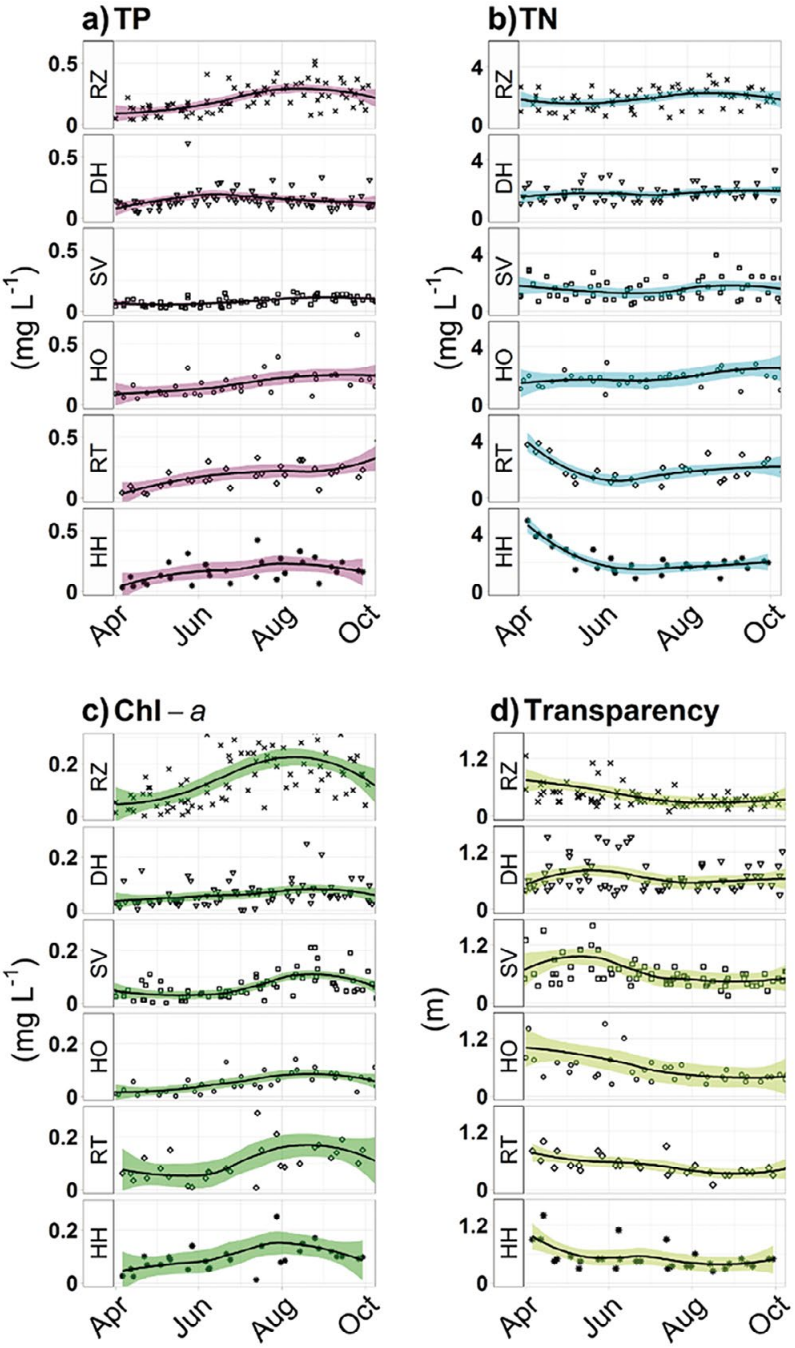

Figure 1. *Concentration of a) TP, b) TN, c) Chl-a, and d) values of water transparency measured at the studied fishponds during the studied period. The black line is based on the locally weighted smoothing (LOESS) function. The coloured area represents the 95% confidence interval. Each symbol represents an individual measurement. Rožmberk (RZ), Dehtář (DH), Svět (SV), Horusický (HO), Ratmírovský (RT), Hejtman Hamerský (HH).*

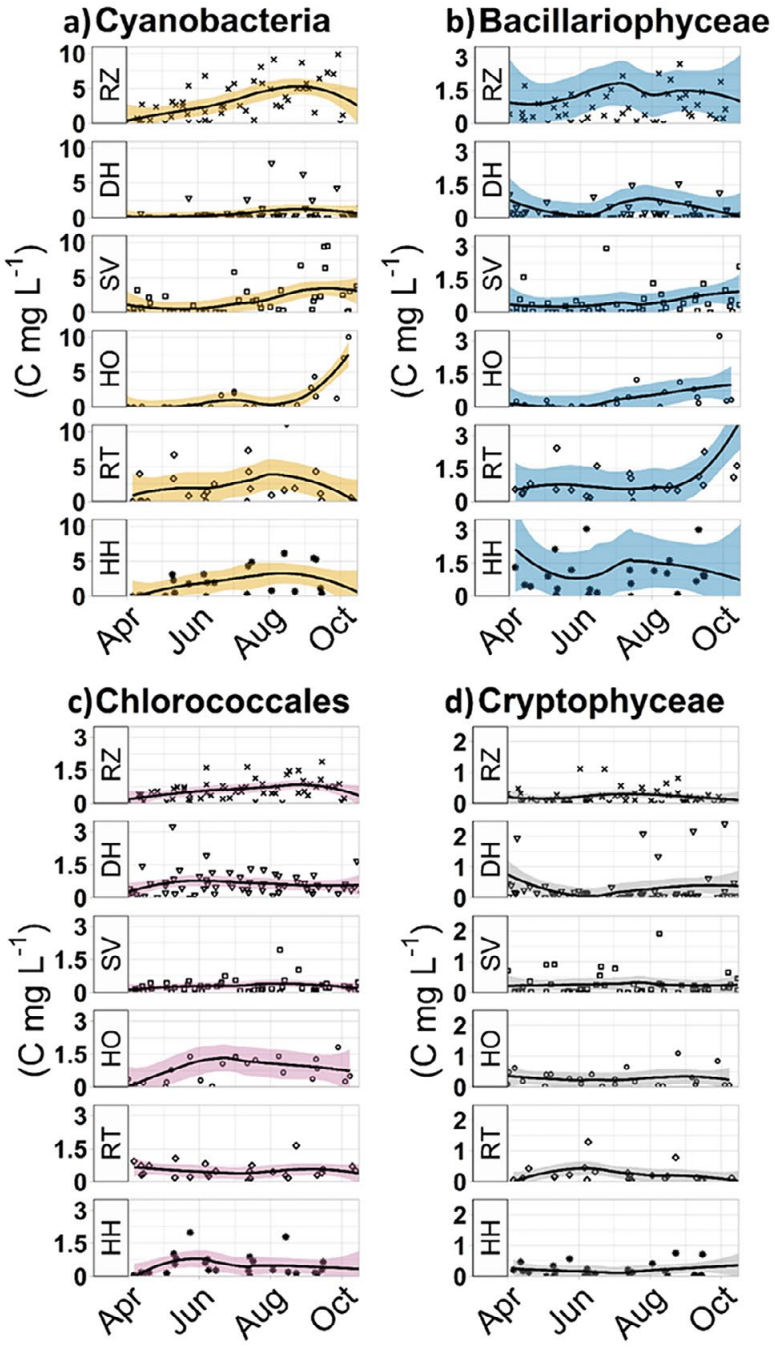

Figure 2. *Seasonal development/variability of CPhy. The line, intervals and points are the same as in Fig. 1. Rožmberk (RZ), Dehtář (DH), Svět (SV), Horusický (HO), Ratmírovský (RT), Hejtman Hamerský (HH).*

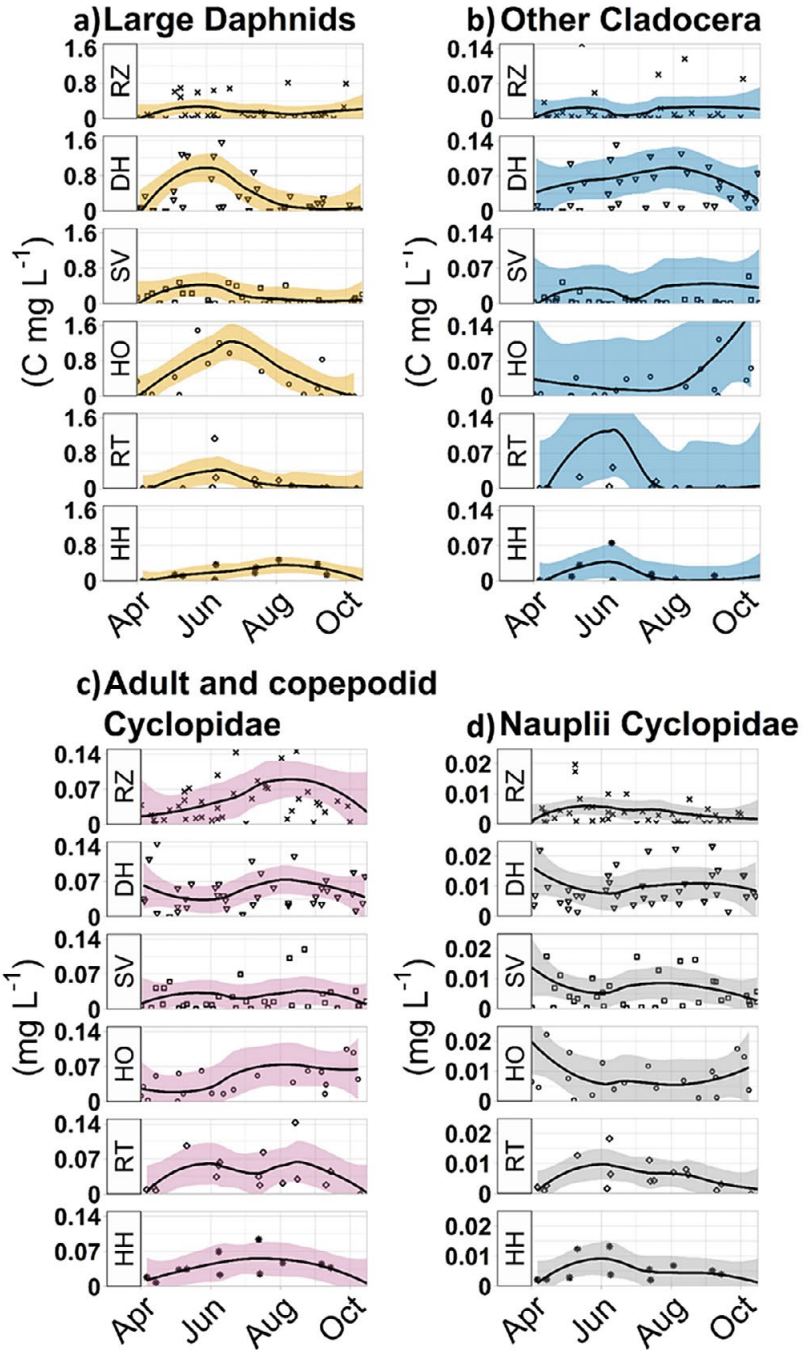

Figure 3. *Seasonal development/variability of CZoo. The line, intervals and points are the same as in Fig.1. Rožmberk (RZ), Dehtář (DH), Svět (SV), Horusický (HO), Ratmírovský (RT), Hejtman Hamerský (HH). The species within small Daphia group were identified in three ponds in 26 cases. Small number of data did not allow to provide graphs and include them into statistical analyses.*

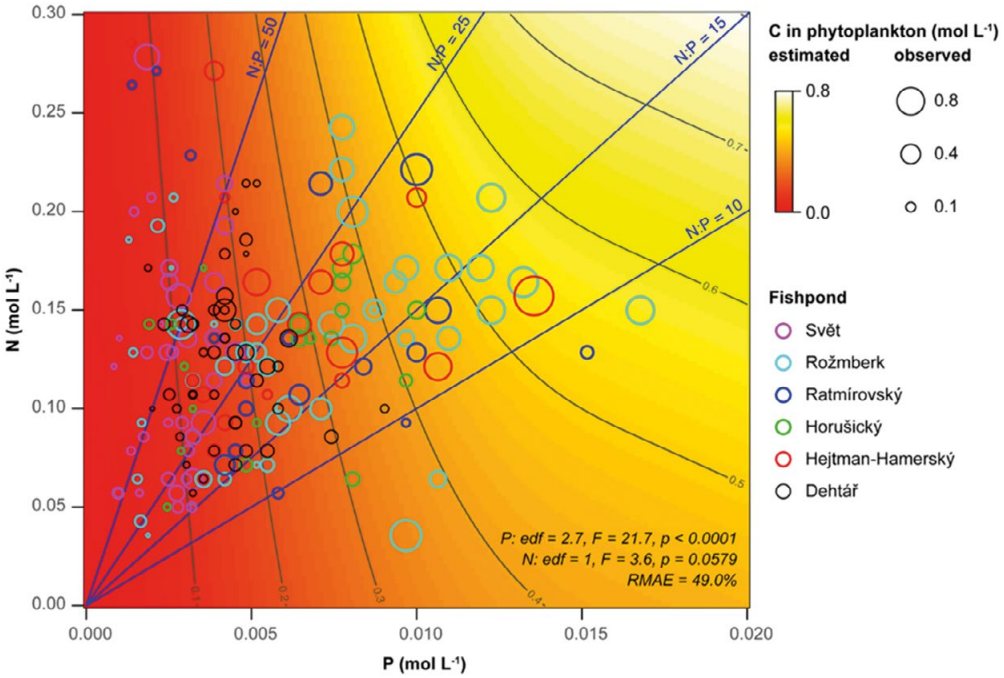

Figure 4. *TP:TN ratio relative to level of carbon in phytoplankton (CPhy). Blue lines indicate N:P ratios. Coloured circles represent ponds, with size reflecting CPhy values. The AMM model includes estimated degrees of freedom (edf), test statistics (F), probability (p), and measure of predictive accuracy (RMAE).*

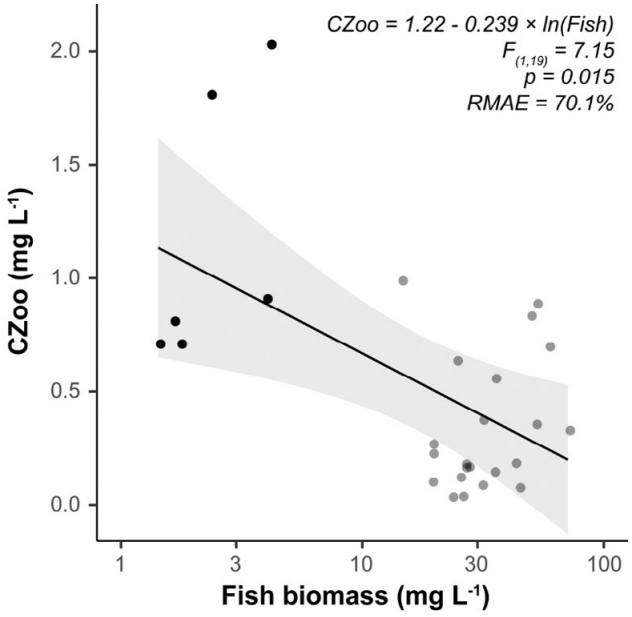

Figure 5. *The relationship between fish biomass and CZoo as predicted by LMM (line ± 95% confidence bands in grey). The test statistics ($F_{(df1,df2)}$), probability (p), and measure of predictive accuracy (RMAE) are displayed. Datapoints show the fish and zooplankton biomass on August 1st in each pond for every year. Black points indicate historical data from Hrbáček et al. (Hrbáček, 1962).*

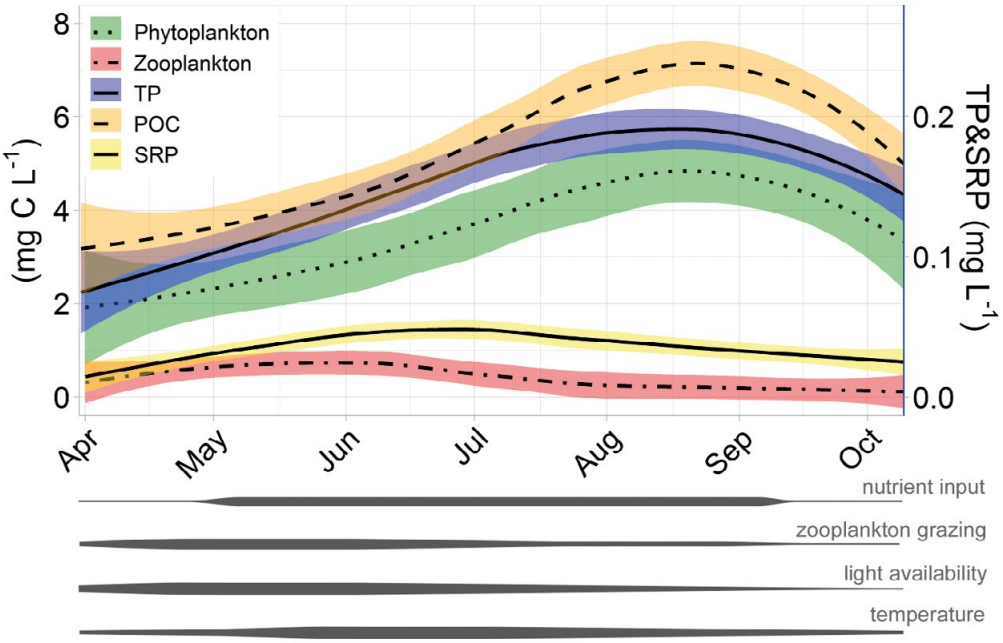

Figure 6. *Seasonal dynamic of CPhy and CZoo, TP, POC, and SRP. The curve is fitted curve using the locally weighted smoothing (LOESS) function. The coloured area around the curve represents a 95% confidence interval for the fitted curve. The thickness of the horizontal bars indicates the seasonal change relative importance of nutrient input, zooplankton grazing, light intensity, and temperature.*

Conclusion

Nutrient concentration, phytoplankton and zooplankton composition, together with fish stock densities, were evaluated in a study of the seasonal plankton succession in 6 hypertrophic fishponds in the Czech Republic. The reduction of zooplankton by high fish stock densities resulted in the uncontrolled growth of phytoplankton. Phytoplankton composition was driven by high phosphorus levels associated with low concentrations of mineral nitrogen in the water, which was reflected in the dominance of nitrogen-fixing cyanobacteria during summer and autumn. Decreasing fish densities below 300 kg ha^{-1} would reduce the overall phosphorus load to fishponds by 30%. Such a measure will simultaneously lower the P concentration in the environment by allowing efficient transfer of P from phytoplankton through zooplankton to the fish biomass regularly removed from the fishponds.

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Conflict of interest

No potential conflict of interest was reported by the authors

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Supplementary materials

Table S1. *Mean ± standard deviation (SD), maximum, and minimum values of water components concentrations measured in this study.*

Parameters	Mean ± SD	Minimum	Maximum
TP (mg L^{-1})	0.15 ± 0.09	0.03 (SV)	0.61(DH)
SRP (mg L^{-1})	0.03 ± 0.03	0.01 (SV)	0.16 (DH)
TN (mg L^{-1})	1.83 ± 0.73	0.50 (RZ, SV)	6.80 (HO)
N-NO_3 (mg L^{-1})	0.33 ± 0.51	0.03 (DH, RT)	3.60 (HH)
N-NH_4 (mg L^{-1})	0.17 ± 0.2	0.02 (RZ, DH, SV,HO)	1.30 (HO,)
POC(mg L^{-1})	5.43 ± 3.63	0.22 (SV)	21.24 (RZ)
TOC (mg L^{-1})	17.46 ± 5.3	8.10 (HH)	48 (RZ)
Chl-a (mg L^{-1})	0.08 ± 0.07	0.001(HO)	0.49 (RZ)
Transparency (m)	0.6 ± 0.36	0.10 (RZ, RT)	3.50 (SV)
CPhy (mg C L^{-1})	3.46 ± 3.53	0.14 (RZ)	12.27 (RT)
CZoo (mg C L^{-1})	0.45 ± 0.76	0.004 (RZ)	5.87 (SV)

Table S2. *Results of Tukey's HSD post-hoc tests for pair-wise comparison of studied ponds. Significant P-values are indicated. In all analyses, the significance level was set as 0.05.*

Water component	contras	estimate	SE	df	t.ratio	p.value
TP	DH-SV	0.5203	0.0821	398	6.341	<.0001
TP	HH-SV	0.5448	0.1105	398	4.931	<.0001
TP	HO-SV	0.6251	0.1005	398	6.218	<.0001
TP	RT-SV	0.6459	0.1085	398	5.954	<.0001
TP	RZ-SV	0.7055	0.0828	398	8.523	<.0001
SRP	RZ-DH	−0.4092	0.0963	193	−4.251	0.0005
SRP	RZ-SV	0.5253	0.0995	193	5.282	<.0001
SRP	DH-SV	0.9345	0.099	193	9.441	<.0001
SRP	DH-HO	0.3888	0.1245	193	3.122	0.0249
SRP	DH-RT	0.5667	0.1344	193	4.215	0.0005
SRP	DH-HH	0.6447	0.1316	193	4.898	<.0001
SRP	SV-HO	−0.5457	0.127	193	−4.297	0.0004
TN	SV-HO	−0.2709	0.0819	300	−3.308	0.0133
TN	SV-RT	−0.26639	0.0895	300	−2.976	0.0369
TN	SV-HH	−0.37301	0.0905	300	−4.121	0.0007
N-NH_4	RZ-SV	−0.4253	0.144	337	−2.953	0.0391
N-NH_4	RZ-HO	−0.7931	0.173	337	−4.593	0.0001
N-NH_4	DH-HO	−0.501	0.173	337	−2.89	0.0468
N-NH_4	HO-RT	0.6378	0.216	337	2.957	0.0388
Chl-*a*	RZ-DH	0.08819	0.0102	338	8.656	<.0001
Chl-*a*	RZ-SV	0.08301	0.0105	338	7.886	<.0001
Chl-*a*	RZ-HO	0.09388	0.0126	338	7.464	<.0001
Chl-*a*	RZ-HH	0.04449	0.014	338	3.168	0.0206
Chl-*a*	DH-RT	−0.04957	0.0139	338	−3.572	0.0054
Chl-*a*	DH-HH	−0.0437	0.014	338	−3.112	0.0245
Chl-*a*	SV-RT	−0.04439	0.0141	338	−3.143	0.0223
Chl-*a*	HO-RT	−0.05526	0.0157	338	−3.517	0.0065
Chl-*a*	HO-HH	−0.0494	0.0159	338	−3.114	0.0243
Transparency	RZ-DH	−0.5053	0.0734	332	−6.888	<.0001
Transparency	RZ-SV	−0.4431	0.0744	332	−5.957	<.0001
Transparency	RZ-HO	−0.3652	0.0889	332	−4.109	0.0007
Transparency	DH-RT	0.2945	0.099	332	2.974	0.0369

Table S3. *Statistical relationships between studied variables.*

Statistical relationships between studied variables	
CPhy vs Chl-*a*	CPhy = 0.1 × Chl-*a* $^{0.8}$, *P* < 0.0001, RMAE= 39%
CPhy vs TP	CPhy = 16 × TP, *P* < 0.0001, RMAE = 41%
CPhy vs Transparency	CPhy = 0.9 × Trans$^{-1.4}$, *P* < 0.0001, RMAE=38.6%
POC vs TP	POC = 18.64 × TP$^{0.7}$, P < 0.0001, RMAE = 25.1%
CZoo vs CPhy	F_1,167 = 0.09, *P* = 0.763 F_1,1367 = 0.76, *P* = 0.386
Fish biomass and CZoo	CZoo=1.22-0.24*ln(Fish in mg L^{-1}), *p* = 0.015, RMAE = 70.1%

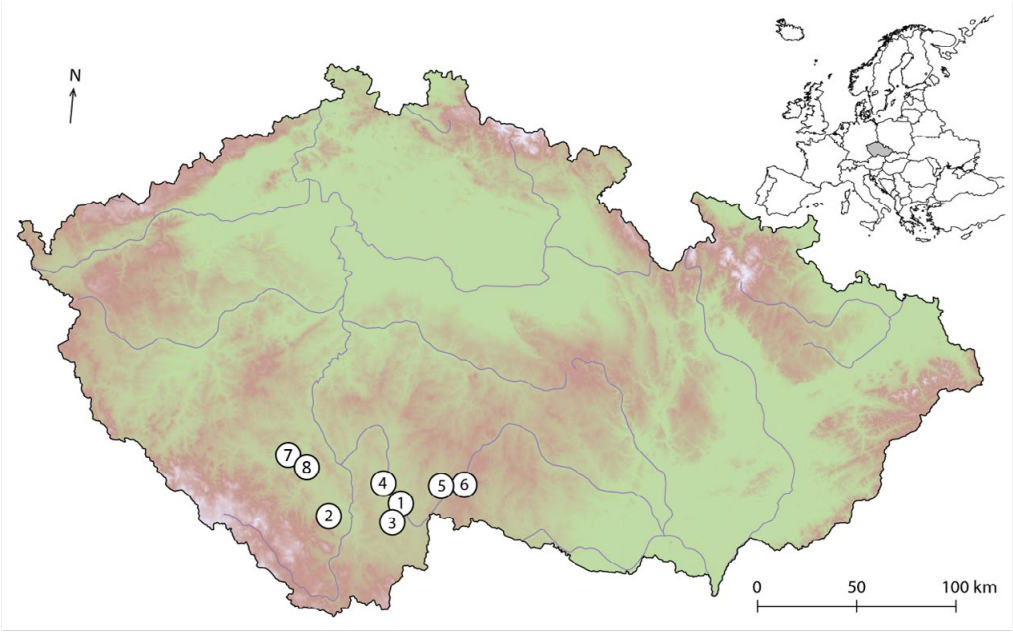

Figure S1. *Location of monitored fishponds. Pond names and their abbreviations are as follows: Rožmberk (RZ), Dehtář (DH), Svět (SV), Horusický (HO), Ratmírovský (RT), Hejtman Hamerský (HH), Smyslov (SM), Velký Pálenec (VP).*

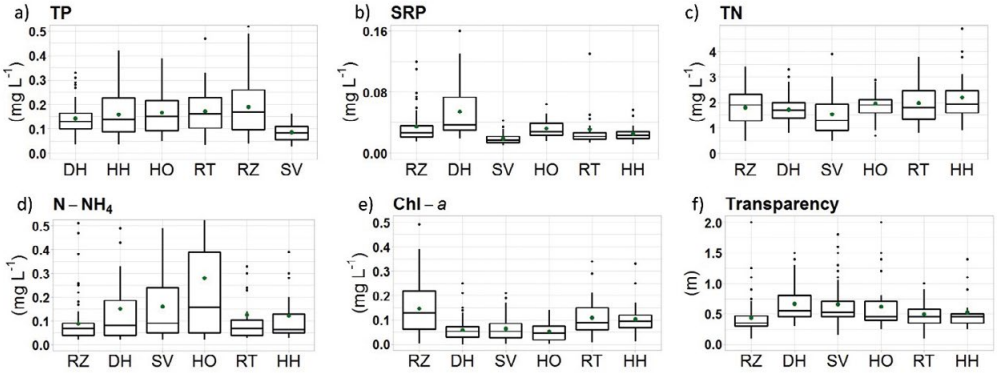

Figure S2. *Box-plot comparisons of a) TP, b) SRP, c) TN, d) N-NH4, e) Chl-aconcentration. The boxes show the 25th, 50th (median), 75th percentiles, and mean concentration (green dot) in studied ponds.*

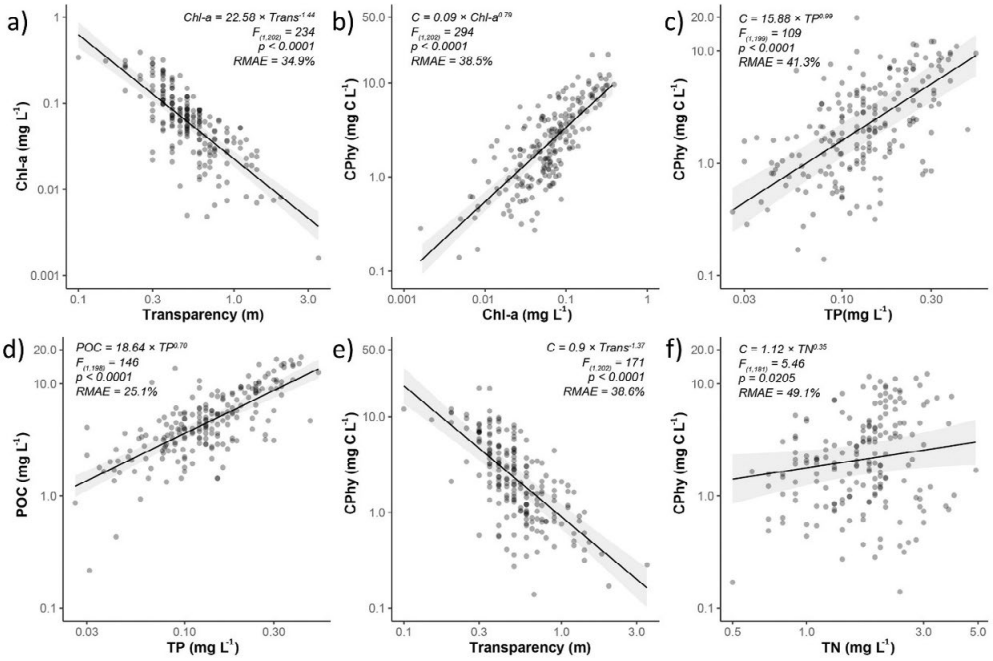

Figure S3. *Pairwise relationship of phytoplankton with environmental data. LMM estimates and 95% confidence bands are displayed as lines and grey polygons. Summary of each model includes test statistics (F(df1,df2)), probabilitiy (p), and measure of predictive accuracy (RMAE).*

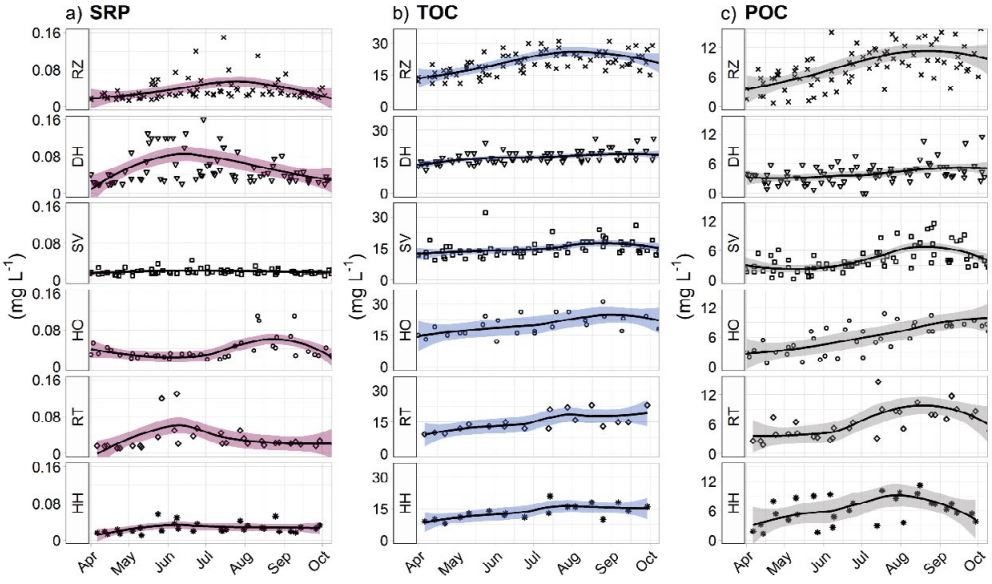

Figure S4. *Concentration of a) SRP, b) TOC, c) POC throughout the monitored period. The black line is based on locally weighted smoothing (LOESS) function. The coloured area represents 95% confidence interval. Symbols represent individual measurements.*

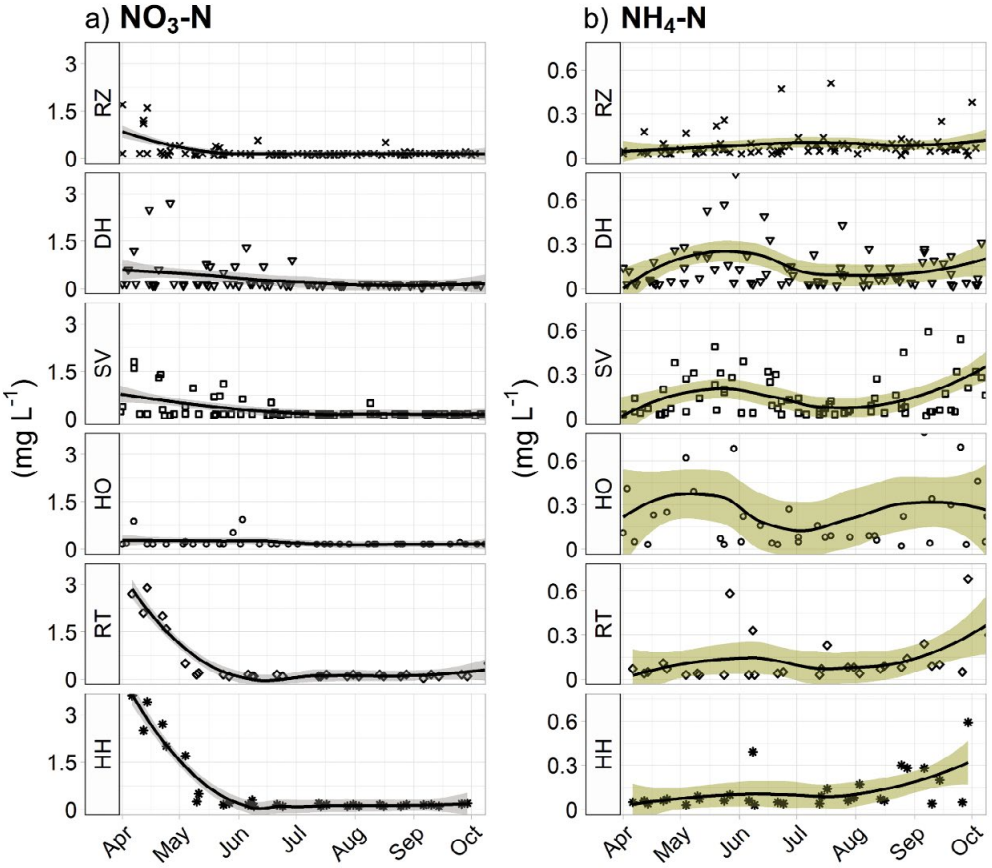

Figure S5. *Concentration of a) NO_3-N, b) NH_4-N throughout the monitored period. The black line is based on locally weighted smoothing (LOESS) function. The coloured area represents 95% confidence interval for the fitted curve. Symbols represent individual measurements.*

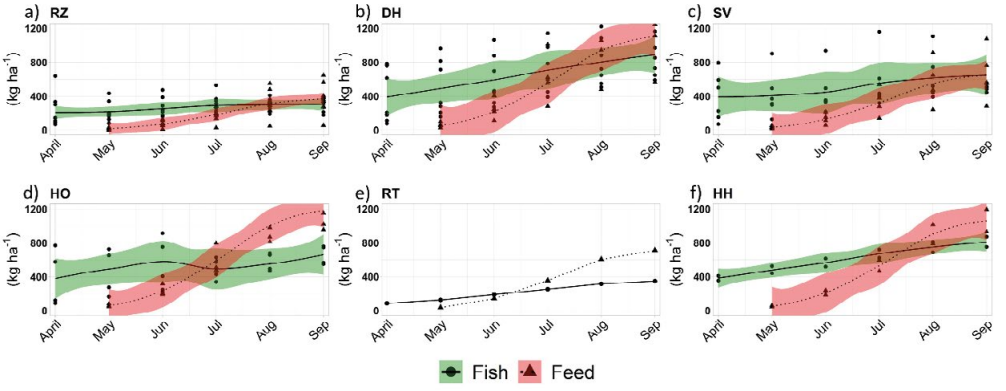

Figure S6. *Fish density and weekly cumulative feed during the season. The black lines are based on the locally weighted smoothing (LOESS) function. The colored area represents 95% confidence interval. Each symbol represents an individual measurement. Rožmberk (RZ), Dehtář (DH), Svět (SV), Horusický (HO), Ratmírovský (RT), Hejtman Hamerský (HH).*

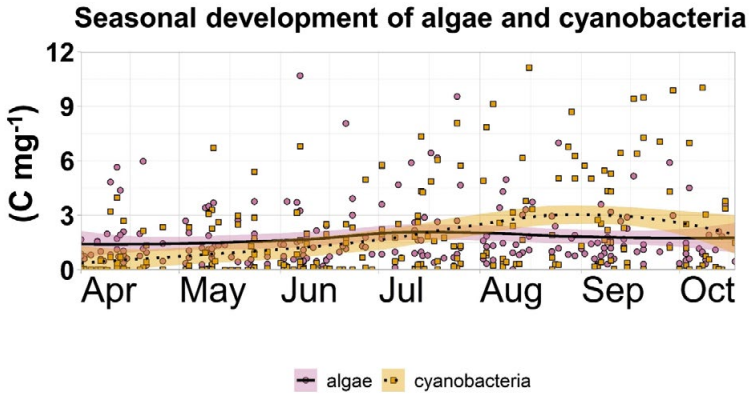

Figure S7. *Seasonal dynamic of algae and cyanobacteria in C mg^{-1} in studied ponds.The curve is fitted curve using the locally weighted smoothing (LOESS) function. The black line is based on the locally weighted smoothing (LOESS) function. The coloured area represents the 95% confidence interval. Each symbol represents an individual measurement.*

CHAPTER 3

SEASONAL DEVELOPMENT OF PHYTOPLANKTON IN SOUTH BOHEMIAN FISHPONDS

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My share on this work was about 60%.

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Seasonal Development of Phytoplankton in South Bohemian Fishponds (Czechia)

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Abstract: Fishponds with a relatively small water volume, high fish abundance, and wide range of nutrient concentrations serve as suitable models for ecological studies. Intensified fish production, together with increased input of nutrients from the watershed, resulted in hypertrophic conditions in the majority of fishponds, the most common type of lentic ecosystems worldwide. In order to understand the processes driving plankton succession, we analyzed eight-year data from nine fishponds in Czechia with differing trophic status. The mean concentration of phosphorus (P) was 200 μg L^{-1} in hypertrophic ponds, 130 μg L^{-1} in eutrophic, and 40 μg L^{-1} in mesotrophic. Correspondingly the mean concentration of phytoplankton was 14.9 mg L^{-1} in hypertrophic ponds, 7.3 mg L^{-1} in eutrophic, and 1.96 mg L^{-1} in mesotrophic. Although the fish stock of 200–900 kg ha^{-1} eliminated zooplankton in eutrophic and hypertrophic ponds the faster-growing algae did not prevail over cyanobacteria. Zooplankton grazing pressure on algae is thus not relevant in studied food webs. Due to the rapid biological denitrification in hypertrophic and eutrophic fishponds resulting in low concentration of mineral nitrogen (N), these ponds were dominated by N-fixing cyanobacteria throughout the whole season. Similarly, the faster-growing algae prevail over cyanobacteria in mesotrophic ponds until the decrease of available mineral nitrogen. The limitation by mineral N is thus the primary driver of phytoplankton composition reflected in cyanobacterial dominance, independently of the trophic status and fish density in studied fishponds.

Keywords: cyanobacteria; hypertrophication; fish stock; nitrogen; phosphorus; plankton dynamics

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1. Introduction

Cultural eutrophication has dramatically transformed the ecosystems of lakes and rivers worldwide. It is widely accepted that the main limiting element of primary production in aquatic environments is phosphorus (P) that acts as the primary driver of ecosystem status [1]. Hypertrophy, defined by concentration of P higher than 100 μg L^{-1}, is expected to become a feature of many lakes in the near future [2] due to continuous P input by human activity, accelerated by climate change connected to decreased rainfall in affected regions [3,4]. The study of biological processes in hypertrophic water bodies is of crucial relevance due to their extend in the near future.

General patterns in the seasonal succession of plankton are complex, but relatively well documented in oligotrophic to eutrophic lakes [5]. All basic ecological interactions, including grazing, predation, competition, symbiosis, and parasitism influence plankton composition and biomass [6,7]. A combination of ecological processes and unstable abiotic conditions drives plankton succession, making it unpredictable at the population level.

This phenomenon is reflected in the 'paradox of plankton' (i.e., the continual change of species composition and community structure) [8]. The typical annual pattern of plankton succession might be overridden by fluctuations in weather, hampering year-to-year comparisons. To compensate for these difficulties, data from numerous seasons and water bodies are usually evaluated to establish the general pattern of seasonal plankton succession.

Fishponds, having relatively low water volume and high fish abundance, might serve as suitable models for ecological studies. With a combined area of 41,080 ha, they are the dominant water bodies in South Bohemia, Czech Republic. High nutrient input from their watersheds and in the form of manure and fish feed makes them ideal sites for trophic studies, as their trophic status has progressed from mesotrophic to hypertrophic within the past 60 years [9].

The natural seasonal dynamics of plankton communities were observed in Czech fishponds through the 1960s and 1970s [10,11]. Their seasonal succession corresponded to a predictable scenario for shallow lakes not influenced by human activity. The low transparency during spring driven by phytoplankton growth and later a high-transparency stage caused by filtration activity of large zooplankton, mainly *Daphnia* spp., is well reflected in the revised PEG model [6]. Seasonal dynamics of plankton has been well economized in fishery management, with moderate fish biomass below 300 kg ha^{-1} [11], providing efficient production [12]. Zooplankton played a major role in energy transfer from phytoplankton to fish, and natural fish production relied on the abundance of large *Daphnia* as keystone species [10]. Since the 1980s, intensified fish production practices, together with increased input of nutrients from the watershed, changed the status of the majority of fishponds from mesotrophic to eutrophic and altered the structure and dynamics of plankton communities [9]. Long-term nutrient loads from watersheds and fish stock biomass reaching 1000 kg ha^{-1} [13] resulted in hypertrophic conditions and impaired efficiency of fish production [12].

To better understand the influence of variable nutrient levels and fish densities on plankton behavior, we studied nine fishponds for eight years.

Hypothesis 1. (H1). *We hypothesized that the concentrations of main nutrients, both P and N, are positively correlated with the biomass of phytoplankton.*

Hypothesis 2. (H2). *We suggest that PEG model would be sufficient to describe the plankton dynamics in studied fish ponds.*

We also try to explain the seasonal development of phytoplankton and particularly the cyanobacterial dominance in eutrophic and hypertrophic fishponds.

Hypothesis 3. (H3). *We also hypothesized that zooplankton will preferentially consume algae, thus providing a free niche for the development and dominance of cyanobacteria in all studied types of fishponds.*

2. Material and Methods

2.1. Study Area

Nine fishponds in the Czech Republic, with an area from 68 to 449 ha, water volume from 1.3×10^3 to 5.9×10^3 m^3, and mean depth in the range from 1.0 to 2.6 m were selected for the study (Figure 1). They were monitored in the context of the EU Water Framework Directive by the Vltava River Authority, State Enterprise. Fishponds Rožmberk (RZ), Dehtář (DH), Svět (SV), Horusický (HO), Ratmírovský (RT), Hejtman Hamerský (HH), Bezdrev (BZ), Staňkovský (ST), and Hejtman-Koštěnický (HK) belong to the largest in the South Bohemian region and are all located in the South Bohemian basin. Basic parameters of the fishponds are shown in Table 1. The hydrobiological sampling season was set from April to August, and it is referred to throughout the text as "season". The

mean monthly temperature during the spring part of the season (April to May) rises from 8 to 17 °C, and daylight period increases from 12:56 h to 15:56 h. The summer season (June to August), is usually warm, with a mean monthly temperature ranging from 21 to 24 °C, and daylight period ranging from 16:06 h in June to 15:04 h in August. In autumn (September to October), the monthly mean temperatures decrease from 18 °C to 8 °C, and daylight period is reduced from 13:23 h to 11:37 h.

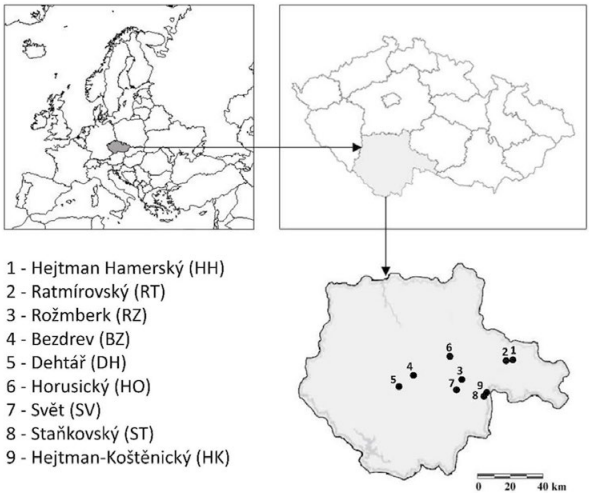

Figure 1. Location of monitored fishponds.

Table 1. Overview of the fishpond's basic characteristics including their trophic status.

Ponds:	GPS Location	Surface Area (ha)	Volume (10^6 m^3)	Depth Average/max (m)	Fishery Trophic Status
Hejtman-Hamerský (HH)	49°8′44″ N 15°9′57″ E	68	1.60	2.4/6.0	H
Ratmírovský (RT)	49°8′36″ N 15°7′12″ E	78	1.30	1.7/6.0	H
Rožmberk (RZ)	49°2′51″ N 14°45′42″ E	449	5.94	1.3/4.1	H
Bezdrev (BZ)	49°2′53″ N 14°23′10″ E	434	6.63	1.5/7.0	E
Dehtář (DH)	49°0′29″ N 14°18′21″ E	228	4.71	2.1/6.0	E
Horusický (HR)	49°9′54″ N 14°41′30″ E	415	3.97	1.0/6.0	E
Svět (SV)	48°59′ N 14°45′ E	214	3.39	1.6/3.0	E
Staňkovský (ST)	48°58′37″ N 14°57′25″ E	246	6.33	2.6/10.0	M
Hejtman-Koštěnický (HK)	48°57′45″ N 14°56′23″ E	80	1.46	1.8/6.0	M

2.2. Chemical Parameters

The upper layer (0–1 m depth) of each fishpond was sampled biweekly or monthly using a Bayer tube sampler at the deepest site in each pond between 08:00 and 10:00 h from April to October 2008 through to 2016, (Table 2, Figure 1). Ponds were also sampled at monthly intervals from November to March during the ice-free period. Sampling duration and frequency varied among ponds, as shown in Table 1.

Table 2. The sampling duration and number of measured parameters in ponds. Chemical analyses include TP, SRP, TN, NO_3-N, NH_4-N, POC, and Chlorophyll-a. Sampling frequency was two weeks or one month, as indicated in the table.

Site	Chemical Analyses	Phytoplankton	Zooplankton	Sampling Frequency and Duration
Hejtman-Hamerský (HH)	36 (2008–2009, 2011–2012)	26 (2009–2012)	12 (2008–2011)	2008–2009, 2012—monthly; 2010–2011—bi-weekly
Ratmírovský (RT)	38 (2008–2009, 2011–2012)	27 (2009–2012)	16 (2008–2012)	2008–2009, 2011—monthly; 2012—bi-weekly
Rožmberk (RŽ)	99 (2008–2015)	58 (2009–2015)	47 (2008–2015)	2008–2009, 2015—monthly; 2010–2014—bi-weekly
Bezdrev (BZ)	61 (2007–2015)	46 (2009–2015)	51 (2008–2015)	2008–2015—monthly
Dehtář (DH)	103 (2008–2015)	66 (2009–2016)	40 (2008–2015)	2008–2009, 2013–2014, 2016—monthly; 2010–2012, 2015—bi-weekly
Horusický (HR)	48 (2012–2015)	26 (2012–2015)	21 (2012–2015)	2012–2013—bi-weekly; 2014–2015—monthly
Svět (SV)	80 (2008–2015)	50 (2009–2015)	36 (2008–2015)	2008–2012—monthly; 2013–2015—bi-weekly
Staňkovský (ST)	93 (2007–2015)	59 (2009–2015)	54 (2008–2015)	2008–2009, 2012—monthly; 2010–2011—bi-weekly
Hejtman-Koštěnický (HK)	70 (2007–2012)	34 (2009–2012)	26 (2008–2011)	2008–2009, 2012–2015—monthly; 2010–2011—bi-weekly

Water samples were analysed for total P (TP) and soluble reactive P (SRP) by inductively coupled plasma spectrometry (Agilent 8800 ICP-QQQ, Santa Clara, California, USA; [14]). The concentration of total N (TN) was measured by determination of bound N following oxidation of N-oxides (Analytik Jena multi N/C 2100, Jena, Germany) according to ISO SIST [15]. Spectrometry and liquid chromatography were used for ammonium (NH_4-N) and nitrate (NO_3-N) determination (Shimadzu UV-1650PC, Shimadzu Corporation, Tokyo, Japan; Dionex ICS-1000, ThermoFisher Scientific, Waltham, MA, USA) [16]. Chlorophyll-a (Chl-*a*) concentration was determined spectrophotometrically after extraction by hot ethanol (Shimadzu UV-1650PC, Shimadzu Corporation, Tokyo, Japan) [17]. Water transparency was measured by Secchi disc.

2.3. Phytoplankton

Phytoplankton was collected simultaneously with chemical samples at monthly intervals from April to October as unfiltered subsamples from the euphotic layer using a Van Dorn sampler (392 analyzed communities in total; Table 2). Samples were preserved with Lugol's solution, stored in the dark, and counted in Utermöhl's chamber using an inverted microscope (Olympus IX 71, Tokyo, Japan) [18]. Biovolume calculation was based on the mean algal cell dimensions using the approximation of cell morphology to regular geometric shapes, according to Komárková and Cronberg [19]. The algal cell volume was converted to wet biomass, assuming a specific gravity of 1.0 [20].

2.4. Zooplankton

Sampling was carried out simultaneously with phytoplankton (Table 2), zooplankton was collected by vertical hauls from the bottom to the surface using an Apstein plankton

net (net mesh = 40 μm). Each sample was divided by two sieves of mesh size 0.71 mm and 0.42 mm [21] and fixed with 4% formaldehyde. Zooplankton species were determined and counted in a total of 386 samples. The zooplankton abundance was estimated by counts using optical microscopy in sedimentation chambers. Dry weight for each species was calculated using taxon-specific regressions [22–27].

2.5. Fish and Feed

Commercial fisheries provided fish data for five ponds in the studied years, while data for fishpond RT were available for 2011 only. Fish that were stocked into ponds at the beginning of each production cycle, together with data of fish added or harvested in corresponding dates, were calculated as monthly data consistent with fish harvest every second year or annually for RZ. The total biomass consisted of 90% common carp (*Cyprinus carpio* L.). The remainder was bighead and silver carp (*Hypophthalmichthys nobilis* Richardson 1845 and *H. molitrix* Val., respectively), grass carp (*Ctenopharyngodon idella* Valenciennes 1844), northern pike (*Esox lucius* L.), pike-perch (*Sander lucioperca* L.), tench (*Tinca tinca* L.), Prussian carp (*Carassius gibelio* Bloch), common and silver bream (*Abramis brama* L. and *A. bjoerkna* L., respectively). The weight of annual supplemental feed, mainly whole grain wheat or barley, was calculated to monthly values according to the standard practice of South Bohemian commercial fisheries [28]. Values were further converted to TP using a conversion ratio of 1 g of P to 1 kg whole grain [28].

No quantitative survey of fish abundance and feed supplied was undertaken in pond BZ and mesotrophic ponds. ST and HK were not managed and used only for recreational fishing.

2.6. Data and Statistical Analyses

The concentrations of TP, SRP, TN, NO_3-N, NH_4-N, and Chl-*a*, along with transparency, were used to describe habitat variables and pond trophic status. To investigate the seasonal trends in zooplankton (Zoo), phytoplankton (Phy), fish biomass density, and cumulative supplemental feed, data were fitted by locally weighted smoothing (LOESS) with a span of 95%. Linear mixed models (LMM) were employed to test the relationship among Phy TB vs. TP and TN. The variables were analyzed independently of one another. Assumptions of the models were checked using diagnostic plots of residuals and correlograms [29]. The data were log-transformed to improve normality. The predictive accuracy of each model was assessed using leave-one-out cross-validated relative median absolute error (RMAE).

The principal component analysis (PCA) was performed according to the similarity in the variables of concentrations of TP, NO_3-N, NH_4-N, TN, SRP, and Chl-*a* between studied ponds.

The analyses were performed in R version 3.6.0 (R Development Core Team, Vienna, Austria) [30] using the package ggplot2 [31]. Adobe Illustrator was used for the final adjusting of figures.

3. Results

3.1. Chemical Parameters

The averages and ranges of physical and water parameters (TP, SRP, NO_3-N, NH_4-N, TN, Chl-*a*), and biomass of phytoplankton in nine fishponds are presented in Table 1. The 9 ponds were grouped into three clusters according to the similarity in the variables of water parameters (Figure 2).

PCA clustered the hypertrophic ponds (RZ, RT, HH) into a closely related group. The concentration of TP gradually increased from April to August and ranged from 0.04 mg L^{-1} to 0.7 mg L^{-1}, with an average concentration 0.2 mg L^{-1} (Supplementary Figure S1a). The concentration of SRP ranged from 0.01 to 0.2 mg L^{-1} and reached maximum values in the early summer, with the average concentration of 0.03 mg L^{-1} (Supplementary Figure S1b). The mean Chl-*a* concentration was 0.1 mg L^{-1}, and the maximum and minimum concentrations were found in RZ in April and August 0.5 mg L^{-1} and 0.004 mg L^{-1}, respectively

(Supplementary Figure S1c). The transparency of these ponds had lowest values in summer (0.1 m) and highest in April (2 m), averaging at 0.5 m (Supplementary Figure S1d).

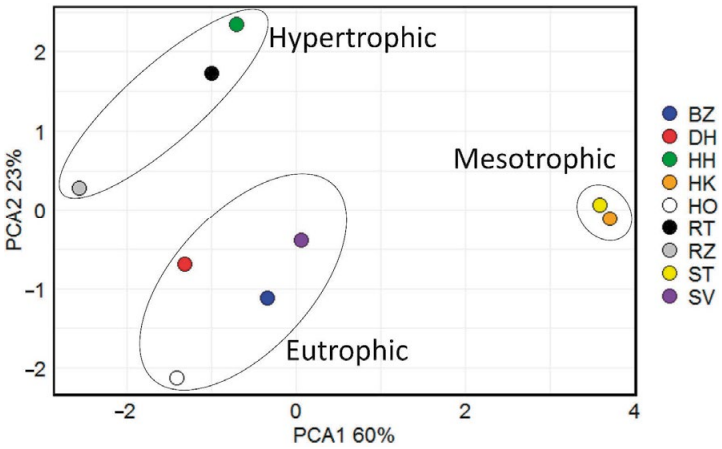

Figure 2. Biplot of the principal component analysis (PCA) according to the similarity in the variables of concentrations of total phosphorus (TP), nitrate (NO_3-N), ammonium (NH_4-N), total nitrogen (TN), soluble reactive phosphorus (SRP), Chlorophyll-*a* (Chl-*a*) Colored dots represent studied ponds. Percentages of total variance are explained by coordinates 1 and 2, accounting for 60 % and 23 %, respectively.

Maximum values of TN and NO_3-N in RZ were recorded in August and April (3.4 mg L^{-1} and 1.7 mg L^{-1}, respectively; Supplementary Figure S2a,b). The mean concentration of NH_4-N ranged from 0.1 mg L^{-1} to 0.2 mg L^{-1} with a maximum value in the beginning of October (0.68 mg L^{-1}; Supplementary Figure S2c).

Eutrophic ponds (SV, HO, DH, and BZ) were also grouped by PCA analysis (Figure 2). The average concentration of TP was 0.1 mg L^{-1} and, except for one case (DH 0.6 mg L^{-1} in May), it remained below 0.4 mg L^{-1} (Supplementary Figure S1a). The concentration of SRP ranged from 0.02 mg L^{-1} to 0.06 mg L^{-1}, peaking in the period between June and July (Supplementary Figure S1b).

The mean concentration of Chl-*a* was 0.06 mg L^{-1} and ranged from 0.001 mg L^{-1} to 2.2 mg L^{-1} (Supplementary Figure S1c). The average transparency was 0.63 m. The maximum transparency was found in SV (3.5 m in May; Supplementary Figure S1d).

The average concentration of TN ranged from 1.54 mg L^{-1} to 2.01 mg L^{-1} and peaked twice, namely in late spring and September (Supplementary Figure S2a). The concentration of NO_3-N ranged from 0.1 mg L^{-1} to 2.7 mg L^{-1} and peaked in April (Supplementary Figure S2b). The average concentration of NH_4-N was 0.22 mg L^{-1} and peaked in early summer and (Supplementary Figure S2c).

Mesotrophic ponds ST and HK formed the third cluster in the PCA, with the lowest concentration of nutrients (Figure 2). The mean concentration of TP was 0.04 mg L^{-1}, with a maximum 0.06 mg L^{-1} in HK during the autumn (Supplementary Figure S1a). The concentration of SRP in this group was lower than 0.3 mg L^{-1} throughout all seasons (Supplementary Figure S1b). The mean concentration of Chl-*a* was 0.027 and ranged from 0.002 mg L^{-1} to 0.21 mg L^{-1} (Supplementary Figure S1c). The mean transparency was the highest among all ponds, with 1.38 m and its maximal value was measured in May in ST 2.5 m (Supplementary Figure S1d).

Maximum of 2.1 mg L^{-1} TN within mesotrophic ponds was found in ST in April (Supplementary Figure S2a). The average concentration of TN was 1.1 mg L^{-1}, NH_4-N

0.09 mg L^{-1}, and NO_3-N 0.4 mg L^{-1}. Maximum of 1.6 mg L^{-1} NO_3-N was found in ST during the spring (Supplementary Figure S2b). The peak of NH_4-N in this group was found in October 0.4 mg L^{-1} (Supplementary Figure S2c).

3.2. Phytoplankton

The total biomass of phytoplankton (Phy TB) of the hypertrophic ponds ranged from 534 mg L^{-1} (RT) to 1943 mg $^{-1}$ (RZ). In these ponds, the biomass of cyanobacteria was twice as large as the biomass of algae, except for the RT pond, where algae biomass was 256 mg L^{-1} and cyanobacteria 278 mg L^{-1}.

The Phy TB in eutrophic ponds ranged from 390 mg L^{-1} (HO) to 778 mg L^{-1} (SV). The TB of algae was bigger than the TB of cyanobacteria except for SV, where the concentration of algae was 357 mg L^{-1} and for cyanobacteria 421mg L^{-1}.

In mesotrophic ponds, the Phy TB was in 158 mg L^{-1} (HK) and 284 mg L^{-1} (ST). The TB of algae was 109 mg L^{-1} and 172 mg L^{-1} in HK and ST, respectively. The TB of cyanobacteria was 49 mg L^{-1} and 113 mg L^{-1} in HK and ST, respectively.

The Phy TB continuously increased until August and September in hypertrophic ponds. In eutrophic ponds, Phy TB peaked in the beginning of autumn, and in mesotrophic ponds maximum values were found in July.

Phytoplankton communities in all 9 ponds consisted mainly of Cyanophyceae (on average 52% of total biomass), Bacillariophyceae (18%), and Chlorococcales (16%), with smaller contributions of Cryptophyceae (7%), Dinophyceae (2%), and Euglenophyceae, Volvocales and Chysophyceae (1%). Phytoplankton community structure varied among ponds (Figure 3). Biomasses of the phytoplankton are listed in Table 3.

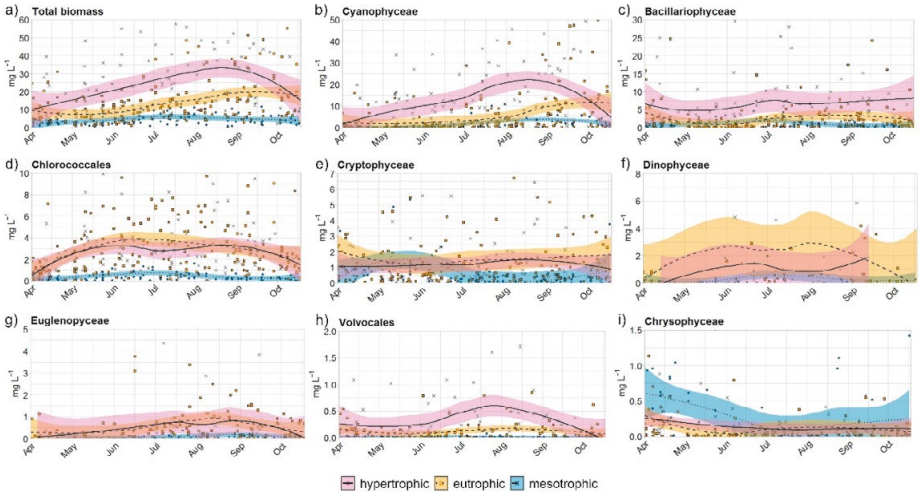

Figure 3. Seasonal course of (**a**) Total biomass (**b**) Cyanophyceae, (**c**) Bacillariophyceae (**d**) Chlorococcales, (**e**) Cryptophyceae (**f**) Dinophyceae, (**g**) Euglenophyceae, (**h**) Volvocales, (**i**) Chysophyceae in hypertrophic, eutrophic and mesotrophic ponds. The black lines are based on locally weighted smoothing (LOESS) function. The grey area represents 95% confidence interval. Each symbol represents an individual measurement.

Table 3. Minimum, average, and maximum in mg L^{-1} of Phy TB and groups of algae and cyanobacteria in 3 groups of ponds.

Phytoplankton	Hypertrophic			Eutrophic			Mesotrophic		
	min	Mean	max	min	Mean	max	min	Mean	max
Phy TB	0.7 (RZ)	24.5	130.3 (RZ)	1.1 (BZ)	13.3	74 (SV)	0.1	4.8	32.5
Cyanobacteria	0.003 (HH)	13.8	77.7 (RZ)	0.0005 (DH)	5.6	50.1 (HO)	0.005(HK)	1.8	10.9(HK)
Bacillariophyceae	0.02 (HH)	6.2	54.5 (RZ)	0.001 (DH)	2.5	45.3 (DH)	0.002(HK)	1.3	28.1(ST)
Chlorococcales	0.03 (HH)	2.7	9.9 (HH)	0.005 (SV)	2.9	16.2 (DH)	0.0044 (ST)	0.4	3.9 (ST)
Cryptophyceae	0.02 (RZ)	1.2	6.4 (RT)	0.01 (DH)	1.6	14.4 (HO)	0.01 (ST)	0.7	13.0 (HK)
Dinophyceae	0.005 (RZ)	1.0	5.8 (RZ)	0.01 (SV)	1.8	14.7 (SV)	0.0014 (ST)	0.3	2.0 (ST)
Euglenophyceae	0.004 (RZ)	0.6	4.3 (RZ)	0.002 (SV)	0.6	6.7 (SV)	0.0044 (ST)	0.1	0.8 (HK)
Volvocales	0.001 (RZ)	0.3	2.0 (RZ)	0.002 (BZ)	0.1	0.8 (DH)	0.0012 (ST)	0.02	0. 1(ST)
Chysophyceae	0.001 (RZ)	0.2	0.9 (HH)	0.001 (BZ)	0.1	2.3 (DH)	0.001 (ST)	0.3	2.9 (HK)

From June onwards, bloom-forming Cyanobacteria began to dominate the community of phytoplankton (Figure 3b). The dominant species of cyanobacteria in hypertrophic ponds were representatives of Nostocales, such as *Anabaena flos-aquae* and *Aphanizomenon gracile*. The peak of these species was from July to September. Species such as *Planktothrix agardhii* (RZ), *Microcystis aeruginosa* (HH), and *Pseudanabaena limnetica* (RT) were present in lower abundances.

Species of Nostocales (mostly *Anabaena flos-aquae* and *Aphanizomenon gracile*) were the dominant species in some eutrophic ponds (SV and DH) in the late summer period. However, in BZ and HO, *Planktothrix agardhii* represented bigger biomass than *Anabaena* spp., with a maximum in September in BZ (37.1 mg L^{-1}, compared to the biomass of Nostocales species of only 11.1 mg L^{-1}).

In the mesotrophic ponds, cyanobacteria were the dominant species from August to October, ranging from 69.7 mg L^{-1} in HK to 222 mg L^{-1} in ST (Figure 3b). The dominant cyanobacteria species were *Anabaena viguieri* and *Aphanizomenon yezoense*, with maximum values in August (28 mg L^{-1} and 58 mg L^{-1}, respectively). The biomass of colonial cyanobacteria such as *Woronichinia naegeliana*, *Coelomoron pusillum*, and species of the genera *Chroococcus* and *Synechococcus* were most common in ponds HK (13.1 mg L^{-1}) and ST (39.4 mg L^{-1}). Their populations peaked in summer and their maximum was observed in July (2.81 mg L^{-1}), mainly caused by *Woronichinia naegeliana.*

Bacillariophyceae a had a peak of growth in the early spring in hypertrophic and from mid-summer to the end of the growing season in eutrophic ponds (Figure 3c). The maximum biomass of Bacillariophyceae in the hypertrophic ponds was 54.5 mg L^{-1} in RZ during summer and was mainly composed of *Aulacoseira* species (*A. granulate*, *A. ambigua*), *Nitzschia acicularis*, and *Synedra acus.* In eutrophic ponds, the highest peak of Bacillariophyceae was in DH in July (45.3 mg L^{-1}), caused by *Aulacoseira* and *Synedra* species. In mesotrophic ponds, the maximum of Bacillariophyceae was observed in ST throughout June (28.1 mg L^{-1}) and was mainly caused by *Fragilaria crotonensis* and *Asterionella Formosa* (Figure 3c).

The development of Chlorococcales occurred from the beginning of spring till autumn (Figure 3d). In hypertrophic ponds, maximum values were found in May in RT and HH (8.2 mg L^{-1} and 9.93 mg L^{-1}, respectively) and September in RZ (9.5 mg L^{-1}), the biomass constituting mainly of *Scenedesmus quadricauda* and *Pediastrum boryanum*. In eutrophic ponds, the maximum concentration was found in DH in May (16.2 mg L^{-1}), in June in BZ (10.8 mg L^{-1}), in August in SV (9.7 mg L^{-1}), and in October in HO (9.1 mg L^{-1}). The maximum values were mostly caused by the genera *Pediastrum*, *Coelastrum*, and *Oocystis*. In mesotrophic ponds, the maximum values were found in June in HK and ST (1.7 mg L^{-1} and 3.9 mg L^{-1}, respectively). In these ponds, the bloom of Chlorophyta was caused by the genera *Planktosphaeria* and *Botryococcus*.

Species of Cryptophyceae such as *Cryptomonas reflexa*, *C. marssonii*, and *Rhodomonas minuta* did not have a clear peak of biomass in hypertrophic and eutrophic ponds, and were

evenly distributed throughout the year (Figure 3e). The Cryprophyceae biomass maximum in hypertrophic ponds was found in RT in August (6.5 mg L^{-1}); in eutrophic ponds the peak was shifted to the end of October (HO; 14.4 mg L^{-1}). Cryprophyceae in mesothrophic ponds peaked from April to July, with maximum biomass (13 mg L^{-1}, HK) in June, and minimum during late summer (0.01 mg L^{-1}, ST; Figure 3e).

The biomass of Dinophyceae, Euglenophyceae, Volvocales and Chrysophyceae was small compared to the previous groups (Figure 3).

Phy TB was positively correlated with TP in all three groups ($p < 0.0001$, $R^2 = 0.3$) (Figure 4a). Pairwise relationship between TN and CPhy was marginally significant ($p = 0.0205$) although quite weak ($R^2 = 0.02$) (Figure 4b).

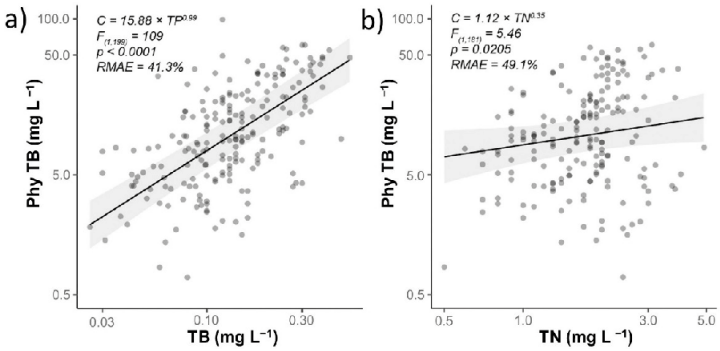

Figure 4. Pairwise relationship of Phy TB with (**a**) TP, (**b**) TN. LMM estimates and 95% confidence bands are displayed as lines and grey polygons. Summary of each model includes test statistics (F(df1,df2)), probability (p), and measure of predictive accuracy (RMAE).

3.3. Zooplankton

The total biomass of zooplankton (Zoo TB) ranged from 6.0 mg L^{-1} (HH)to 25.8 mg L^{-1} (RZ) in hypertrophic ponds. Zoo TB in eutrophic ponds ranged from 25.6 mg L^{-1} (HO) to 45.1 mg L^{-1} (DH). In mesotrophic ponds Zoo TB was 2.9 mg L^{-1} (HK) and 8.5 mg L^{-1} (ST). Zoo TB in all ponds had maximum values during the late spring (Figure 5). Biomass of large *Daphnia* species such as *D. cucullata, D. galeata,* and *D. magna* had diverse dynamics in the different ponds (Figure 5b). In hypertrophic ponds maximum of the dry biomass of these species was found in May in RZ (1.5 mg L^{-1}), in June in RT (2.4 mg L^{-1}), and in HH in August (1 mg L^{-1})**.** The average concentration of biomass ranged from 0.2 mg L^{-1} (RT) to 1.7 mg L^{-1} (RZ). In eutrophic ponds, DH and SV, the biomass maximum of large *Daphnia* species was detected at the end of the spring (5.4 mg L^{-1} and 7.9 mg L^{-1}, respectively), and in HO in July (3.75 mg L^{-1}). The average concentration of biomass ranged from 0.6 mg L^{-1} (SV) to 1.5 mg L^{-1} (HO) In mesotrophic ponds, *Dapnia* peaked in April (0.4 C mg L^{-1}, HK) and in May (1.3 mg L^{-1}, ST) and the average concentration was 0.08 mg L^{-1} in both ponds. The biomass of Other Cladocera, adult and copepodid Copepoda, and Rotifers was small compared to the previous group (Figure 5). Biomasses of the zooplankton are listed in Table 4 and Figure 5.

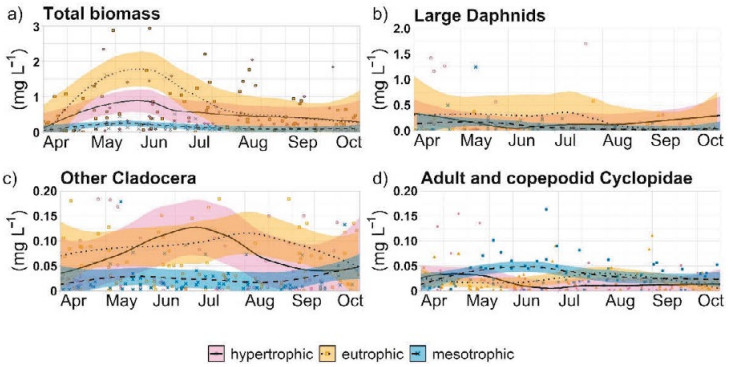

Figure 5. Seasonal course of (**a**) Total biomass of zooplankton (**b**) large *Daphnia*, (**c**) other Cladocera, (**d**) adult and copepodid Copepodae in hypertrophic, eutrophic and mesotrophic ponds. The black lines are based on locally weighted smoothing (LOESS) function. The grey area represents 95% confidence interval. Each symbol represents an individual measurement.

Table 4. Minimum, mean, and maximum in mg L^{-1} of Zoo TB and groups of algae and cyanobacteria in 3 groups of ponds. *—concentration < 0.001 mg L^{-1}.

Zooplankton	Hypertrophic			Eutrophic			Mesotrophic		
	min	Mean	max	min	Mean	max	min	Mean	max
Zoo TB	0.008 (RZ)	0.5	4.4 (RZ)	0.005 (SV)	0.8	8 (SV)	0.002 (HK)	0.1	1.6 (ST)
large *Daphnia*	0.001 (RT)	0.3	2.4 (RT)	0.002 (BZ)	0.2	7.9 (SV)	0.007 (ST)	0.08	1.2 (ST)
small *Daphnia*	*	*	*	0.001 (BZ)	0.1	0.4 (DH)	*	*	*
Other Cladocera	* (RZ)	0.07	2.8(RZ)	* (SV)	0.09	0.8 (HO)	0.002 (HK)	0.03	0.3 (ST)
adult Copepoda	* (RZ)	0.02	0.8 (RZ)	* (BZ)	0.02	0.4 (SV.HO, DH)	0.001 (HK)	0.03	0.2 (ST)
copepodid Copepoda	* (HH)	0.01	0.4 (RT)	* (SV)	0.02	0.1 (DH)	* (HK)	0.008	0.04 (ST, HK)
Rotifers	* (RZ)	0.01	0.04 (RZ)	* (BZ)	0.006	0.08(SV)	* (ST)	0.006	0.02 (ST)

3.4. Fish and Feed Supplement

At the start of the season, fish biomass in hypertrophic ponds ranged between 86 kg ha^{-1} and 383 kg ha^{-1}. The fish biomass at harvest was ranging between 307 kg ha^{-1} and 811 kg ha^{-1} (Figure 6a). Fish biomass in eutrophic ponds in the beginning of the season was approximately 391 kg ha^{-1}, and at harvest ranged between 649 kg ha^{-1} and 891 kg ha^{-1}.

The quantity of feed supplied to hypertrophic ponds annually was 403 ± 194 kg ha^{-1} in RT and RZ, and 1095 ± 431 kg ha^{-1} in HH. In eutrophic ponds, it was 885 ± 392 kg ha^{-1} in SV and HO, and 1095 ± 431 kg ha^{-1} in DH. The average monthly P supplied in feed to hypertrophic ponds was 81 ± 39 g ha^{-1} at RT and RZ, and 219 ± 86 g ha^{-1} at HH. In eutrophic ponds it was 177 ± 78 g ha^{-1} at SV and HO, and 219 ± 86 g ha^{-1} at HH.

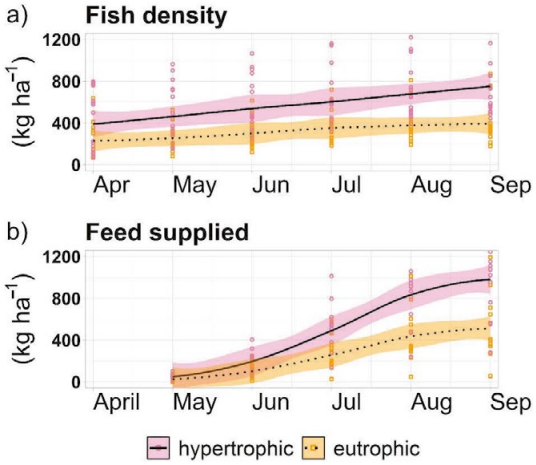

Figure 6. Fish density (**a**) and weekly cumulative feed (**b**) during the season in all studied ponds. The black lines are based on the locally weighted smoothing (LOESS) function. The colored area represents 95% confidence interval.

4. Discussion

The considerable differences in trophic state between ponds were caused by the fisheries management regime that entirely changed the metabolic pathways in hypertrophic and eutrophic fishponds compared to mesotrophic (Figure 7). Studied hypertrophic and eutrophic fishponds (with a mean TP of 200 μg L^{-1} and 130 μg L^{-1}, respectively) were used for commercial fish farming. The high concentration of TP was caused by weekly auxiliary feeding by cereals, which added more than 50% of the overall nutrient supply to the pond ecosystem [32,33]. On the other hand, mesotrophic ponds, with a mean TP concentration of 40 μg L^{-1}, were used only for recreational fishing. Their P input came mainly from the inflows, and less than 5% came from fishery management [32]. These different trophic levels significantly influenced phytoplankton communities and overall seasonal plankton development.

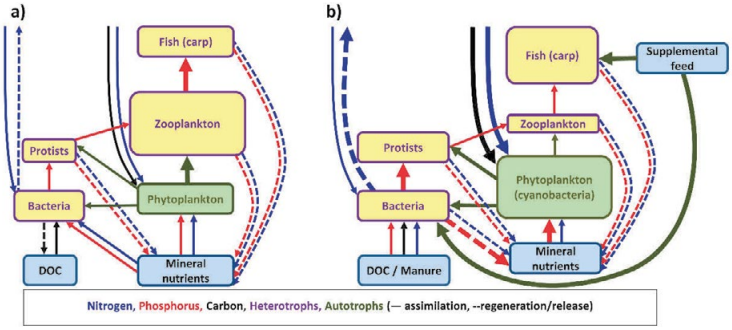

Figure 7. Schematic diagram of plankton food webs and nutrient cycling in fishponds. (**a**) Mesotrophic conditions and (**b**) eutrophic and hypertrophic conditions with high fish stocking density. The coloured lines represent nutrient pathways. Solid lines show assimilation of nutrients; dashed lines nutrient release or regeneration.

Hypothesis 2. (H2). *In line with a high fish predation scenario of the revised PEG model (Figure 2 in [33]), neither the spring peak of phytoplankton nor the clear-water phase were observed in the studied fishponds.*

In hypertrophic and eutrophic ponds, Phy TB rose continuously from spring to autumn, with cyanobacteria dominating throughout the whole season (Figure 3a,b). Interestingly, in mesotrophic ponds with a stable total Phy TB throughout the season (Figures 3a and 8c), the algae significantly dominated over cyanobacteria until the end of July, when algal biomass declined, and cyanobacterial biomass grew, reaching an identical Phy TB. Hypertrophic ponds contained a similar ratio of nostocalean N-fixing cyanobacteria and other cyanobacterial biomass throughout the season. It is generally agreed that many species without heterocysts fix nitrogen even less efficiently than Nostocales. These were present in phytoplankton of hypertrophic fishponds as e.g., *Pseudanabaena*, *Oscillatoria* or Chroococcalean species. However, further studies should resolve their role. In eutrophic ponds, biomass of nostocalean N-fixing cyanobacteria such as *Anabaena*, *Aphanizomenon* generally dominated the cyanobacterial community until September (Figure 8a,b).

Bacillariophyceae were the most abundant algae in all ponds, representing 18% of PhyTB. Bacillariophyceae generally have a lower thermal optimum than cyanobacteria, giving them the advantage of being dominant in early spring [34].

The grazing pressure of zooplankton is considered as a major factor regulating phytoplankton biomass in shallow eutrophic lakes [35]. The zooplankton biomass was low in all studied ponds; while being surprisingly highest in eutrophic ponds (Figure 5a). The major part of zooplankton biomass consisted of small cladocerans that were not able to effectively suppress phytoplankton. In addition, visually-oriented predators such as weed fish consume preferentially large *Daphnia* with multiple offsprings [36]. The lack of decline in Phy TB reflected a low density of zooplankton that was effectively suppressed by fish (Figure 6a).

The well-established key role of P in phytoplankton growth [37] (as proposed in hypothesis 1) was confirmed by our data and corresponded with the summer peak of TP concentration (Figures 3 and 4a; Supplementary Figure S1a).

Nitrogen input from the watershed [33] was not denitrified during winter and early spring, resulting in high NO_3-N concentration during spring (Supplementary Figure S2b). Rising temperatures in spring triggered the denitrification that effectively released N from fishponds, including that from auxiliary feed (Figure 7). The reported rate of denitrification in eutrophic lakes ranges from ~150 to 700 mg N m^{-2} d^{-1} [38–40]. At this rate, all N would be effectively released from the studied ponds in 3–21 days. Despite rapid denitrification, the TN levels remained high in the studied hypertrophic ponds and eutrophic ponds.

A long-standing debate exists over whether N effects on phytoplankton growth are dependent on the type of water body, trophic status, or season [41,42]. Data obtained during this study indicate that eutrophication of the water bodies could not be controlled by reducing N input. Soluble mineral N was almost absent in studied fishponds (Supplementary Figure S2). Proliferation of heterocystous as N-fixing cyanobacteria indicates low concentration of mineral N in water. As cyanobacteria fix inorganic C as well as molecular N dissolved in water, the concentration of NH_4-N and NO_3-N in water is not critical to their proliferation (Figure 8). Such result is inconsistent with hypothesis 1 and it is not plausible for mineral N.

The amount of phytoplankton steadily increased throughout the season, following the increase in P concentration (supplied by fish feed) in hypertrophic and eutrophic ponds, or remained stable in mesotrophic ponds. Rising phytoplankton biomass can be attributed to biomass of N-fixing cyanobacteria, which similarly increased throughout the growing season and declined at its end (Figure 8). Zooplankton biomass moderately increased in spring until temperatures over 17 °C allowed fish feeding, suppressing zooplankton throughout the rest of the growing season and efficiently disconnecting the entire food web. Hypothesis 3 proposed in the introduction is thus entirely invalid. In consequence, zooplankton was

not able to suppress the proliferation of phytoplankton. Therefore, the seasonal plankton dynamics in hypertrophic and eutrophic fishponds is constrained by the concentration of P and fish stock density, whereas in the mesotrophic ponds by P only. Cyanobacterial prevalence in nutrient-rich water reflects high N turnover rate by bacteria using high levels of organic carbon connected with rapid denitrification. Counterintuitively, the limitation of algae was caused by low levels of inorganic nitrogen during summer and autumn (Figure 8). Fixation of N by cyanobacteria is then assumed to explain their dominance in fishponds under a low mineral N state during summer in hypertrophic and eutrophic ponds and during autumn in hypertrophic, eutrophic, and mesotrophic fishponds.

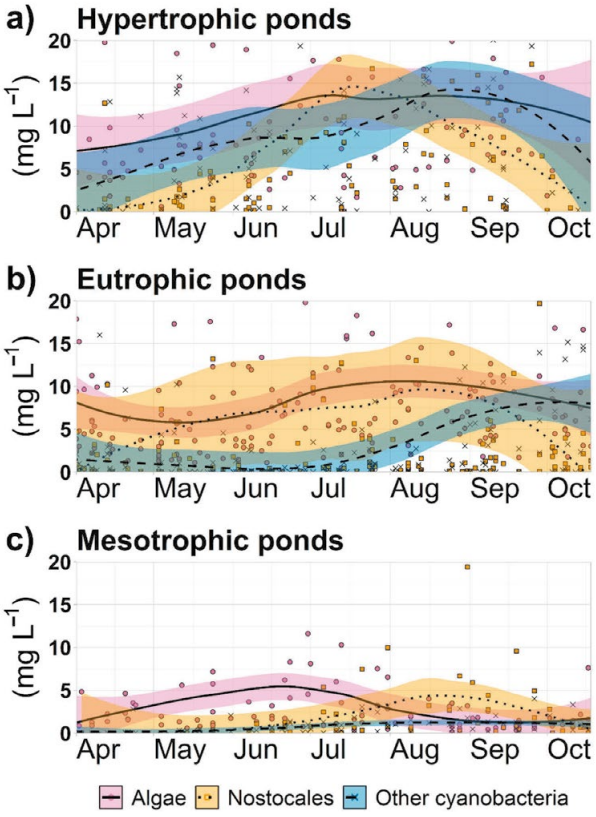

Figure 8. Seasonal changes in the total biomass of algae, Nostocales, and other cyanobacteria in (**a**) hypertrophic ponds, (**b**) eutrophic ponds, (**c**) mesotrophic ponds. The black lines are based on locally weighted smoothing (LOESS) function. The coloured area represents 95% confidence interval. Each symbol represents an individual measurement.

5. Conclusions

Nutrient concentration, phytoplankton, zooplankton composition, and fish stock densities were evaluated to study the seasonal succession of plankton in nine fishponds of different trophic status in the Czech Republic. High fish densities reduced the zooplankton biomass that did not grow fast enough to even marginally influence the phytoplankton. The

all-over growth of phytoplankton was constrained by the concentration of P according to its trophic levels. Algae were limited by low levels of inorganic nitrogen. The cyanobacteria formed a significant part of the phytoplankton community. Phytoplankton composition was driven by available mineral N that was reflected in the dominance of N-fixing cyanobacteria.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w14131979/s1. Figure S1: Concentration of (a) TP, (b) SRP, (c) Chl-*a*, and (d) transparency during the studied period in hypertrophic, eutrophic, and mesotrophic ponds. The black line is based on the locally weighted smoothing (LOESS) function. The coloured area represents the 95% confidence interval. Each symbol represents an individual measurement; Figure S2: Concentration of (a) TN, (b) NO_3-N, (c) NH_4-N throughout the monitored period in hypertrophic, eutrophic, and mesotrophic ponds. The black line is based on locally weighted smoothing (LOESS) function. The coloured area represents 95% confidence interval for the fitted curve. Symbols represent individual measurements.

Author Contributions: A.P.I. and O.S. contributed substantially to the study's conception, data acquisition and analysis; J.P. gatherd and analysed data for the study; A.P.I., O.S. and J.V. contributed substantially to drafting the manuscript.; A.P.I. performed statistical analysis.; A.P.I., O.S. and J.V. designed the figures; J.R. analyzed the fish data. All authors have read and agreed to the published version of the manuscript.

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CHAPTER 4

DIVERSITY OF CYANOBACTERIA AT THE ALASKA NORTH SLOPE WITH DESCRIPTION OF TWO NEW GENERA: *GIBLINIELLA* AND *SHACKLETONIELLA*

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My share on this work was about 10%.

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Research Article

RESEARCH ARTICLE

Diversity of cyanobacteria at the Alaska North Slope with description of two new genera: *Gibliniella* and *Shackletoniella*

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One sentence summary: Novel cyanobacteria from the Alaskan North Slope.

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ABSTRACT

The diversity of cyanobacteria along the Alaskan North Slope was investigated. We isolated and cultivated 57 strains of cyanobacteria and sequenced a section of their rRNA operon containing a fragment of the 16S rRNA gene. Here, we describe 17 found species belonging mainly to families Coleofasciculaceae, Microcoleaceae, Oculatellaceae, Leptolyngbyaceae and to the order Synechococcales. In pursuing a conservative polyphasic approach, we utilized suggested thresholds in 16S rRNA gene differences in parallel with morphological differences between new and already described taxa for the description of new species and genera. Based on a combination of morphological, molecular and ecological analysis of collected and cultured strains we describe two genera *Gibliniella* and *Shackletoniella* as well as six cyanobacterial species; *Cephalothrix alaskaensis*, *Tildeniella alaskaensis*, *Pseudophormidium americanum*, *Leptodesmis alaskaensis*, *Albertania alaskaensis* and *Nodosilinea alaskaensis*. Here, a polyphasic approach was used to identify eight novel and nine established cyanobacterial taxa from a previously non–investigated region that uncovered a high degree of biodiversity in extreme polar environments.

Keywords: Alaska; cyanobacteria; *Gibliniella*; morphology; new taxa; *Shackletoniella*

INTRODUCTION

Due to high ecological valence cyanobacteria thrive in many extreme habitats (Whitton 2012). Cyanobacteria are well adapted to freezing and desiccation while being capable of restoring an active metabolism in minutes following their unfreezing or rehydration (Rajeev *et al.* 2013). Cyanobacteria are often regarded as dominant primary producers in polar regions. This is especially true in polar deserts (Vincent 2002) and glaciers

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(Anesio et al. 2017). Cyanobacteria play an important role in carbon and nitrogen economy in polar soils (Vincent 2002) and they are the main nitrogen fixers both as free-living cells or as a mutualistic part of lichen or in moss–cyanobacterial associations (Chapin and Bledsoe 1992).

The diversity of cyanobacteria is still highly underestimated as can be deduced from recent studies in previously less-explored environments (Powell et al. 2005; Sciuto, Moschin and Moro 2017; Mai et al. 2018). More recently, next generation sequencing (NGS) has been used extensively with the aim to accurately describe cyanobacterial diversity in particular ecosystems. NGS is undoubtedly an essential tool to uncover the biological diversity, history and processes of natural habitats (e.g. Monchamp et al. 2016). However, NGS results often provide a high number of abstract organizational taxonomic units (OTUs), higher taxonomical groups or a pure description of alpha diversity (e.g. Staley et al. 2013). A combination of NGS methods with classic phytological determination approaches (including, but not limited only to, detailed light microscopy observations of various life stages) results in the most accurate estimation of real numbers of species residing on site (Strunecky et al. 2019). This approach however requires employing extensive and lengthy observations and often does not provide answers about metabolomic properties of the studied taxa.

Classic phycology approaches based on microscopic evaluation of microalgal morphologies and cultivation of strains that are complemented by their basal sequencing hold strong potential for bringing new knowledge about community structure and diversity (Bohunická et al. 2015; Strunecky et al. 2017). New discoveries of species diversity is important for many previously non-investigated environments, particularly in habitats where climatic changes pose a risk to future sustainability. Here, we isolated and cultivated eight novel and nine previously identified cyanobacterial species from the Alaskan North Slope. This research provides a foundation for further detailed ecological, biogeographical and genomic studies.

MATERIAL AND METHODS

Sampling

A total of 111 samples were taken at the Alaska North Slope. The sampling area was limited by the Atigun Pass to the south and Prudhoe Bay to the north spanning 240 km and was ∼10 km eastwards or westwards away from Dalton Highway (Fig. 1). Samples were taken from freshwater environments (i.e. rivers, streams, lakes, pools), soil and bare rock. The geographic position and biotopes of cultivated strains are indicated in Supplementary Table S1, see online supplementary material. Cyanobacterial mats were sampled to Nalgene 1.8 mL cryogenic tubes in duplicates. For several hours after sampling one tube was put on ice and kept there at low light until cultivation. A second tube was frozen at –20°C for transportation and storage. The majority of samples were determined from first subsamples on site using a Zeiss Axioskop Upright Fluorescence Microscope at the Toolik Field Station (Institute of Arctic Biology, University of Alaska, Fairbanks, AK, USA, 68° 37' 39.11'' N, 149° 35' 51.93'' W).

Cultivation

A total of 66 samples with cyanobacteria previously confirmed by optical microscopy were cultivated on Petri dishes and in test tubes in Z medium (Zender in Staub 1961) solidified by 2% agar and in the mineral nutrient medium BG11 (Rippka et al. 1979) solidified by 1.5% agar. Cultures were maintained in a growth chamber with a 12:12 hour light:dark photoperiod provided by cool-white fluorescent illumination (4400 lx, PAR 12 Wm^{-2}, 48.6 μmol (photon) m^{-2} s^{-1}) at 15°C. Thirty-six samples yielded vitally growing cyanobacteria and altogether contained 57 individual cyanobacterial strains.

PCR amplification and sequencing

Total genomic DNA was isolated using NucleoSpin® Soil (Machery–Nagel) using a standard protocol. A section of the rRNA operon containing a fragment of the 16S rRNA gene and the 16S–23S internal transcribed spacer (ITS) was amplified using the primers 359F (5′–GGGGAATYTTCCGCAATGGG–3′) (Nübel, Garcia–Pichel and Muyzer 1997) and 23S30R (5′–CTTCGCCTCTGTGTGCCTAGGT–3′) (Taton et al. 2003) yielding a fragment of expected size 450 bp. The template DNA was mixed with 5 pmol of each primer in 50 μL of commercial PCR mix with Taq polymerase (Plain PP Master Mix, Top Bio, Czech Republic). Amplification was performed using the following settings: a starting denaturation step at 94°C for 5 min; 40 cycles of 30 s at 94°C, 30 s at 53°C and 3 min at 72°C; final extension for 7 min at 72°C and cooling to 4°C. The PCR was verified by running a sub-sample on a 1.5% agarose gel stained with ethidium bromide. Sequencing was performed at Macrogen (Amsterdam, The Netherlands) using the primers 23S30R (5'–CTTCGCCTC TGTGTGCCTAGGT–3'), 1492R (5'–TACGGYTACCTTGTTACGACTT–3') and 810R (5'–GTTATGGTCCAGCAAAGCGCCTTCGCCA–3') (Strunecky, Komarek and Smarda 2014).

Sequences were aligned in MAFFT (www.mafft.cbrc.jp) (Katoh and Toh 2010). A fragment of ∼1081 nt of the 16S rRNA gene was used for the phylogenetic analysis (starting at *Escherichia coli* ATCC 11 775 residue 302).

Phylogenetic analysis

The sequences of cyanobacterial strains outside of Alaskan clades were added as outgroup taxa for inferring congruent phylogeny as used elsewhere (Bohunická et al. 2015; Mareš et al. 2019b). The phylogenetic tree of the 16S rRNA gene was inferred in MrBayes 3.2.6 (Ronquist and Huelsenbeck 2003). For the Bayesian analysis, four runs of four Markov chains were calculated for 30 million generations, each one with one cold and three heated chains, sampled every 2000 generations. Stop early if the convergence diagnostics falls below 0.01 was set to yes. An initial 25% of the generated trees were discarded as burn-in. The ML analysis in RAxML v. 8 (Stamatakis 2006) was via the CIPRES supercomputing facility (Miller et al. 2012). The most suitable substitution evolutionary model was identified using jModelTest 2 based on both the Akaike and Bayesian criteria employed. The general time-reversible + invariant + gamma (GTR+I+G) substitution model, with 1000 bootstrap pseudo–replications was used.

RESULTS

Phylogenetic results based on 16S rRNA phylogeny

Altogether 57 cultivated and sequenced cyanobacterial strains belonged to 20 OTUs (Figs 2, 3 and 4). Description of taxa follows according to their position in the phylogenetic tree inferred from the 16S rRNA gene (Figs 2, 3 and 4).

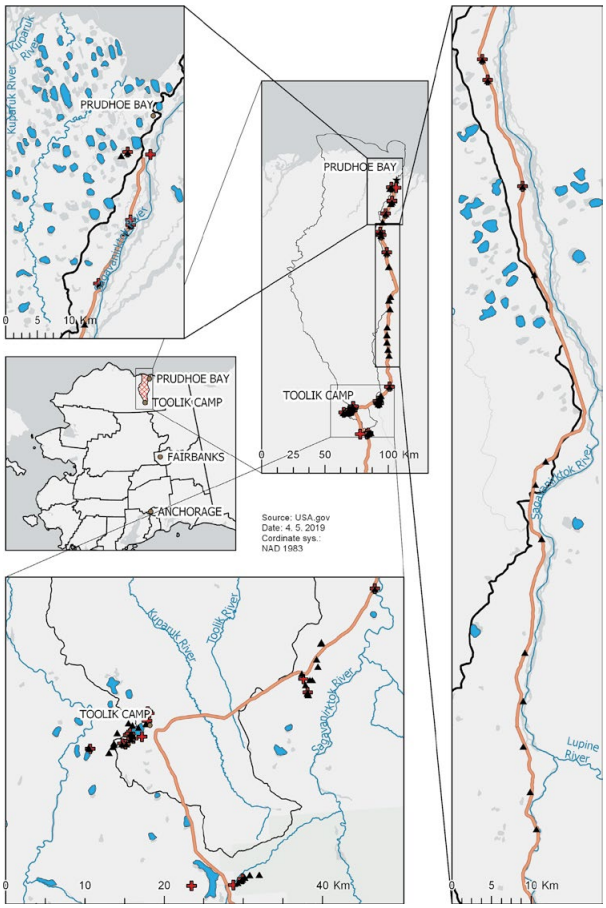

Figure 1. Location of sampling sites at Alaska, USA. Red crosses indicate sites with cultivated cyanobacteria. Triangles indicate the remaining sampling sites.

In Coleofasciculaceae, three strains belonged to *Cephalothrix* Malone (Fig. 2) that formed a distinct clade standing out among the other described species within this genus. Delimitation of a new species *Cephalothrix alaskaensis* described below is sufficiently supported by both their 2.4% nucleotide difference in the 16S rRNA gene and their distinct morphology that will be described later. Nucleotide sequence in the 16S rRNA gene of three strains placed into genus *Anagnostidinema*, Strunecky, Bohunicka, and Johansen et Komarek differed from the other sequences always less than 1.2%.

Three strains KT20, T28 and KL29 are placed within the Microcoleaceae (Fig. 2). Their 99.9% 16S rRNA gene similarity with the adjacent strains qualify them as representative members of the ubiquitous *Microcoleus vaginatus*. Similarly, the 16S rRNA gene sequence of the strains V9 and L24 (Fig. 2) corresponded well with the genus *Kamptonema* Strunecky, Komarek et Smarda with <1.1% difference in the 16S rRNA gene in comparison with a typical strain of the genus *K. animale* CCALA 139.

Four isolated strains belonged to order Chroococcales (Fig. 3). Three strains of *Gloeothece* KL21, L26 and L33 (Fig. 3) were isolated from a single locality. The sequence of their 16S rRNA gene reveals 98.3% similarity with *Gloeothece tepidariorum* CCALA 1112 (A. Braun in Rabenh.) Lagerheim 1883, considered as a valid member of this cyanobacterial lineage. A strain of *Geminocystis* sp. KT25 was isolated and sequenced but perished before thorough microscopic documentation.

The 16S rRNA gene of 12 isolated strains clustered into Oculatellaceae (Fig. 3). Six strains L1, L11, KL12, KV23, L27 and T27 formed a distinct clade. Their 16S rRNA gene nucleotide similarity was 97.36% with *Albertania skiophyla* Zammit. Three strains of Alaskan cyanobacteria T16, KL28, KL16 fell close to a genus *Tildeniella* Mai, Johansen et Bohunická with 16S rRNA gene nucleotide

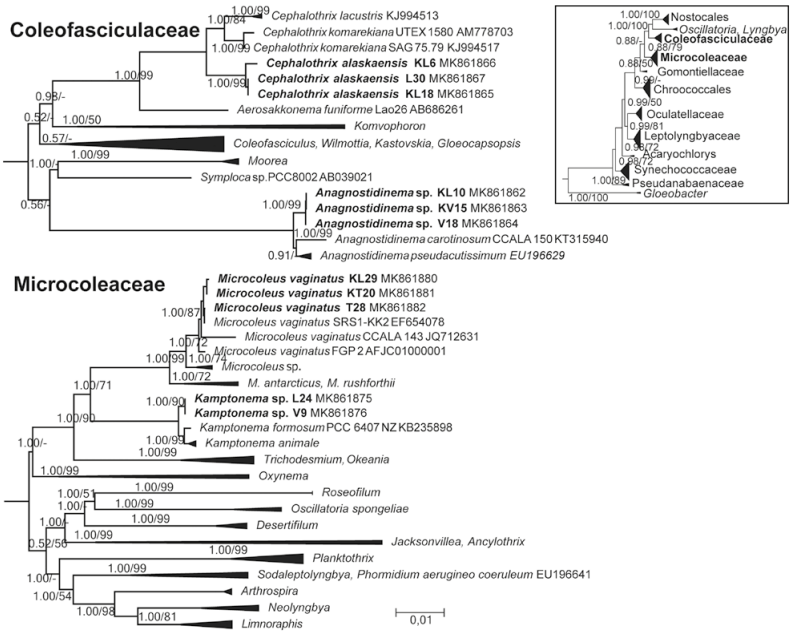

Figure 2. Phylogenetic relationships of the cultivated cyanobacteria from families of Coleofasciculaceae and Microcoleaceae from Alaska. The topology of the complete phylogenetic tree is shown in the box. This was done using Bayesian analysis in MrBayes 3.2.6 and ML in RAxML v. 8. Values indicate Bayesian posterior probabilities and bootstrap pseudo-replications; values <0.5/50% are not shown. A fragment of ~1081 nt was used for phylogenetic analysis of the 16S rRNA gene.

similarity between 98.1 and 95.1% with *Tildeniella torsiva* and only 91.3% identity with *Tildeniella nuda*. The other two cultivated strains KL8 and KL15 were identified as being phylogenetically closely related to *Leptolyngbya antarctica* (West and West 1911) Anagnostidis et Komárek.

A distinct part of the phylogenetic tree of Leptolyngbyaceae contained eight strains (Fig. 4). The strain L25 clustered with *Stenomitos* Miscoe *et al.* 2016 Johansen with 97.8% similarity. Strains KT1 and KT18 come together with the strains designated as *Pseudophormidium* sp. from Atacama Desert with high similarity of 98.4% in 16S rRNA gene sequence. The taxonomic designation of these strains however does not fully correspond with its current taxonomic designation to *Microcoleaceae*. Resolving this taxonomic issue lies outside the scope of this study due to the lack of the 16S rRNA gene sequence of the type of species *Pseudophormidium*, the *P. phormidioides*. Strain V20 was a second cultivated strain within the clade of *Leptodemis* Raabová, Kovacik et Strunecký with 97.1% 16S rRNA gene sequence similarity with strain *Leptodemis paradoxa* LK021. Strain KT5 collected in Sagavanirhtok river had 99.0% nucleotide identity in 16S rRNA gene with *Phormidesmis communis* Raabová, Kovacik, Elster et Strunecký.

A total of 23 strains were cultivated that belong to the core Synechococcales (Fig. 4). Two strains L5 and L31 formed a separate clade with maximal 92.6% 16S rRNA gene sequence identity to the closest taxa *Haloleptolyngbya alcalis*. Strains T21, T29 and L32 belong to *Nodosilinea* Perkerson *et al.* according to their 98.1% nucleotide similarity in 16S rRNA gene to the closest strain *N. nodulosa* UTEX B2910. The rest of *Nodosilinea* strains from Alaska were found within previously published strains of *Nodosilinea* with various species descriptions (Fig. 4).

All obtained sequences were submitted to Genbank under accession numbers MK861856-MK861912 (Supplementary Table S1, see online supplementary material).

Strain morphology

Light microscopy examination of the freshly collected samples at the Toolik Field Station led to the determination of several species that were not isolated later in the laboratory. They belonged mainly to the Chroococcales, Nostocales and Oscillatoriales and their morphological appearance is shown in Fig. 5A–W.

Two species were found in Coleofasciculaceae. The morphology of strains within *Cephalothrix* differs from *C. komarekiana* and *C. lacustris* from South America by the morphology of apical cells with absent conical calyptra (Figs 7A–G and 9A, Table 1). Trichomes are characteristically twisted unlike the straight trichomes of South American strains, additionally, the aerotopes were not found by optical microscopy. The morphology of *Anagnostidinema* strains KV15, KL10 and V18, including the presence of typically bent 'hockeystick'-like ends with rather thin trichome exhibiting distinctive rotational motility, corresponded well with typical traits of *A. pseudacutissimum* Strunecký, Bohunická, Johansen et Komárek.

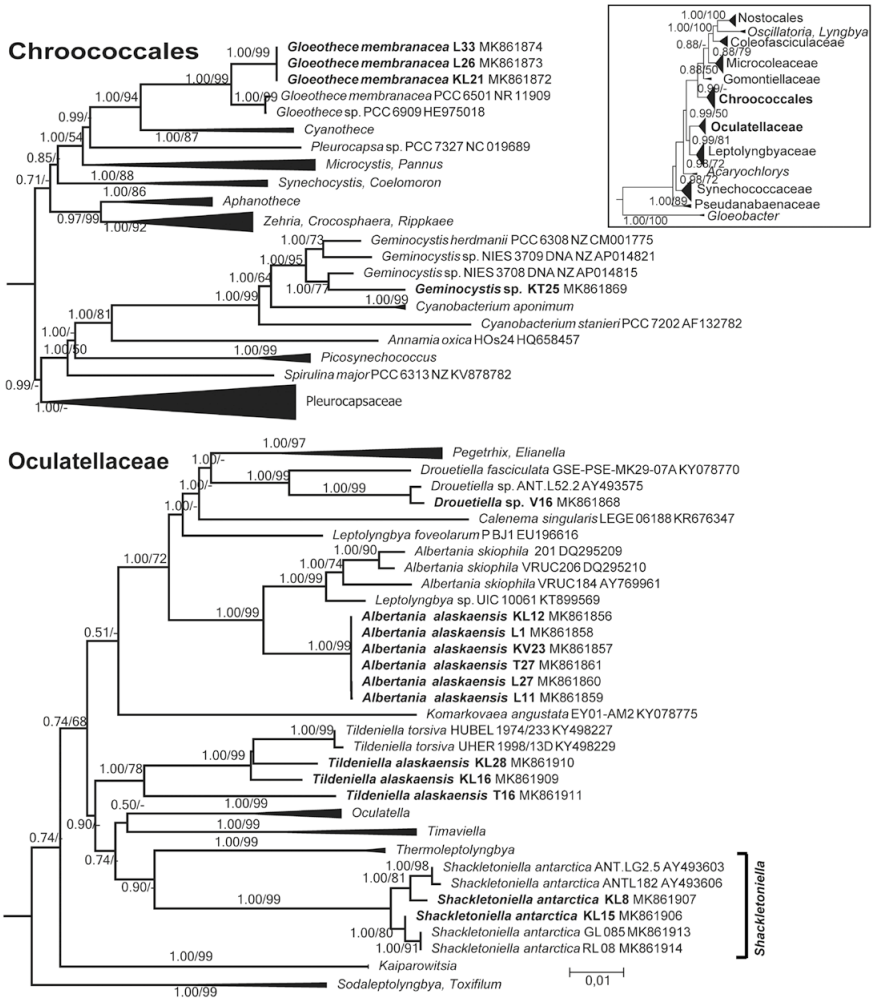

Figure 3. Phylogenetic relationships of the cultivated cyanobacteria from order of Chroococales and family Oculatellaceae from Alaska. The topology of the complete phylogenetic tree is shown in the box. This was done using Bayesian analysis in MrBayes 3.2.6 and ML in RAxML v. 8. Values indicate Bayesian posterior probabilities and bootstrap pseudo-replications; values <0.5/50% are not shown. A fragment of ~1081 nt was used for phylogenetic analysis of the 16S rRNA gene.

In Microcoleaceae the strains of *Microcoleus* possess typically attenuated trichomes with calyptra occasionally present (Fig 5K). The morphology of *Kamptonema* strains KV15, KL10 and V18, including the presence of a typically bent terminal cell and the trichome exhibiting a typical S bend (Fig. 5L) followed by rapid release of tension causing movement, corresponded well with typical traits of *Kamptonema*. The only observable difference found was the presence of a blunt terminal cell that was not as long and attenuated in natural populations as in a typical *K. animale*. Altogether, the morphology of strains collected at Alaska is very similar to known *Kamptonema* strains collected around the world.

The taxonomic designation of *Gloeothece membranacea* (Rabenhorst) Bornet 1886 was supported by morphological traits of studied strains, explicitly the formation of cell clusters joined by common mucilage into gelatinous or membranous

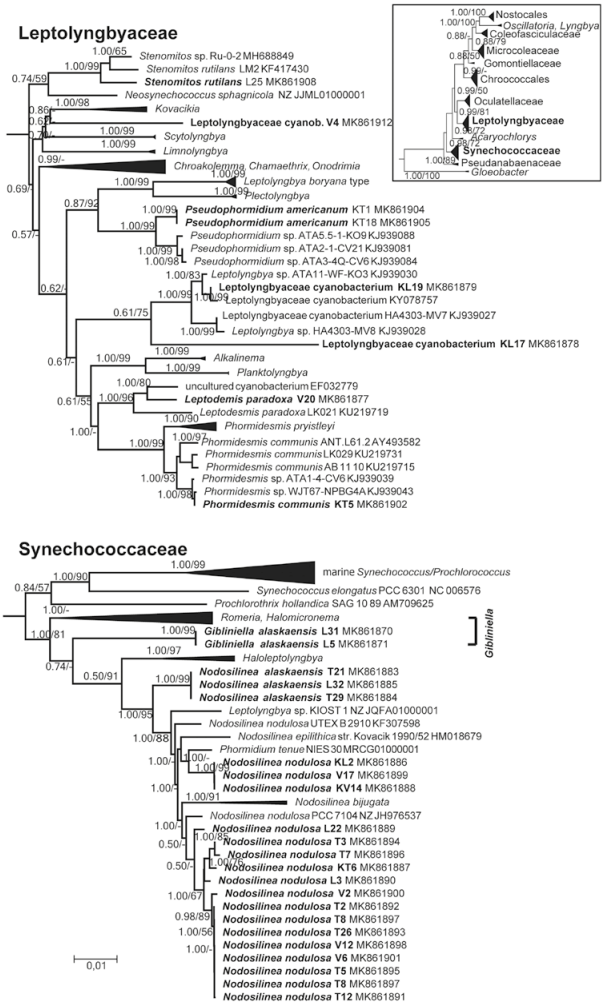

Figure 4. Phylogenetic relationships of the cultivated cyanobacteria from Leptolyngbyaceae and Synechococcales. The topology of the complete phylogenetic tree is shown in the box. This was done using Bayesian analysis in MrBayes 3.2.6 and ML in RAxML v. 8. Values indicate Bayesian posterior probabilities and bootstrap pseudo-replications; values <0.5/50% are not shown. A fragment of ~1081 nt was used for phylogenetic analysis of the 16S rRNA gene.

colonies, formation of colorless but lamellated envelopes surrounding the cells, cell size of 6–8 × 4–6 μm, along with their presence in a freshwater biotope (Fig. 5F).

Strain V16 with Oculatellaceae-like morphology was isolated from stream periphyton. It belonged to a clade with *Drouetiella* sp. ANT.L52, however, no specific morphologic features were observed in this strain. The solitary, non-fasciculated trichomes 3 μm wide are slightly constricted at septal cell walls with green, isodiametric or longer than wide cells with no distinct morphological features (Fig. 5M–O). The overall appearance resembled well the *Drouetiella lurida* (Gomont 1892) Mai, J.R. Johansen et Pietrasiak with the exception of a liver-brown filament coloration.

Six cultivated strains were grouped within Ocullateaceae showing typical morphology of the family with thin cells that are longer than they are wide. These strains were isolated from

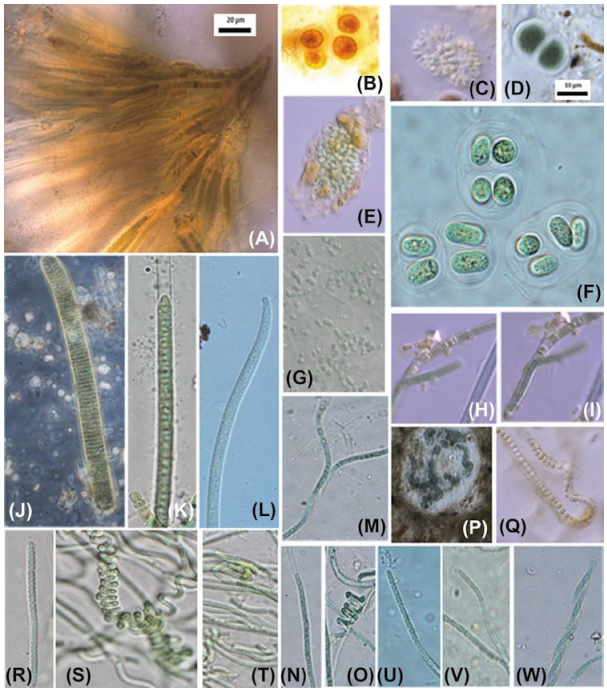

Figure 5. Morphological diversity of cyanobacteria found at site and within the cultivated strains. **(A)** *Rivularia* sp., **(B)** *Gloeocapsa* sp., **(C)** *Snowella* sp., **(D)** *Chroococcus* sp., **(E)** *Aphanothece* sp., **(F)** *Gloeothece membranacea* (strain L33), **(G)** *Aphanocapsa* sp., **(H, I)** *Scytonema* sp., **(J)** *Oscillatoria* sp., **(K)** *Microcoleus vaginatus* (strain T20), **(L)** *Kamptonema* sp. (strain L24), **(M–O)** *Drouetiella* sp. (strain V16), **(P, Q)** *Nostoc* sp., **(R, T)** *Nodosilinea nodulosa* strain L22, **(S)** *Nodosilinea nodulosa* strain V2, **(U–V)** *Stenomitos sp.* (strain L25), **(W)** *Phormidesmis communis* (strain KT5). Scale bar for microphotographs (A) and (J–I) is 20 μm in 1000X magnification and for (B–I) and (M–W) is 10 μm in 1000X magnification.

freshwater and one was found on a permanent snow field. During the preparation of the manuscript the description of the genus *Albertania* Zammit was validly published. Species of *Albertania skiophila* grew in aerophytic biofilms in Malta and were phylogenetically close to this group. The Alaskan strains exhibited a distinct morphology with coiled or undulating trichomes with occasionally present perpendicular cells (Fig. 6A–L). In addition, their distinct ecological traits provide further support for the formation of a new species *Albertania alaskaensis* (Figs 3, 6A–L and 9F, Table 1). Strains of *Tildeniella* (Figs 7M–P, 9C, Table 1) possessed morphological features similar to Oculatellaceae members with very thin (1–2.2 mm) cells that were partially longer than they were wide. Strains KL28 and KL16 differ morphologically from *Tildeniella torsiva* in their distinct arctuation both in nature and in cultures. We therefore included them in a new species, *T. alaskanesis* (Fig. 7M–P).

The last two cultivated strains in Oculatellaceae, KL8 and KL15, were identified as being closely related to previously described Antarctic species *Leptolyngbya antarctica* (West and West 1911) Anagnostidis et Komárek. They had very thin trichomes 1–1.5 (2) µm wide with predominately cells longer than they were wide up to 7 (10) μm. The formerly established species *L. antarctica* however did not comply either phylogenetically or morphologically with the genus *Leptolyngbya*. We therefore combined the strains of *L. antarctica* collected at the Antarctic and at Alaska and collectively described them in a newly formed genus *Shackletoniella*. (Figs 7U–Z, 9B, Table 1).

Unlike Oculatellaceae which had essentially cells longer than they were wide, the main diagnostic markers in the family Leptolyngbyaceae are very thin trichomes up to 4 µm combined generally with cells shorter than their length after division. Additionally, a visible circular structure of unknown function in the middle of the cell was recognisable by optical microscopy (Figs 6 and 7, Table 1). Strain L25 with short untapered trichomes, unconstricted at the crosswalls, occasionally coiled, 2 µm wide with cells growing to two times longer size before division, with parietal thylakoids and colorless sheaths, tightly adhering to trichomes, visible only when empty (Fig. 5U–V), corresponded well to the original description of *Stenomitos* Miscoe *et al.* 2016 Johansen. Strain V4 was lost before proper morphological analysis, but its micrographs obtained during cultivation showed morphological similarity with *Scytolyngbya* Song et Li except for missing false branching and incrustation of sheaths present in *Scytolyngbya timoleontis*. The width of filaments was ~3 µm, cells were isodiametric, up to 5 times longer than wide before division, with distinct, long and rounded-end cells.

Table 1. Comparison of morphological features of new species with morphological features of type species.

	Thallus	Filaments	False branching	Motility	Filament width (μm)	Sheaths	Color of trichomes
Cephalothrix alaskaensis	Fasciculate blue-green yellowish in 4–6 weeks older culture	Cylindrical, straight or twisted sometimes bent and coiled not attenuated	None	None	6.4–7.1 (7.8)	Hyaline, firm, sometimes loosening at the end of filaments	Colorless bright green to pale green in young cultures, yellowish-green in old cultures
Cephalothrix komarekiana	Fasciculate blue-green	Cylindrical, straight trichomes bent at the end slightly attenuated	None	None	Not available	Facultative hyaline, firm, narrow or wide	Not available
Albertania alaskaensis	Characteristic tufted, isolated colonies penetrating a few mm into agar, hardly removable bright green to yellow-brown when old	Solitary or fasciculate, narrow or coiled, wavy, heterogeneous filaments, width front of filament wider than end, with shorter cells, end markedly narrower with longer cells (1–12) forming hair	None in young culture, often in the old culture	None	0.8–1.3 end cells 1.8–3 cells	Lamellate, thin, firm, closed at the ends of the filament, sometimes widened colorless	Bright yellow-green to olive green
Albertania skiophila	Compact subaerophytic biofilms on solid substrata green, green-brown and black	Single trichome per sheath, trichomes occasionally slightly attenuated toward their ends, trichome fragmentation via random occurrence of necridic cells	Occasionally	None	Not available	Firm, thin, often open at the ends, colorless	Colorless
Tildeniella alaskaensis	Compact mats blue-green to dark green	Solitary, flexuous, irregularly curved and coiled, untampered, cylindrical, isopolar, uniseriate, one per sheath	None	None	1–2.2	Thin, firm, colorless	Bright green
Tildeniella torsiva	Colonies fasciculate, spreading irregularly, irregular clumps, filaments penetrating the agar bright blue-green, olive green (old)	Straight, flexuous or spirally coiled, often entangled	Rare false branching in older cultures	None	1.7–2.5	Thin, up to 0.7 μm wide, often not evident, colorless	
Shackletoniella antarctica	Compact biofilms greenish or reddish	Fine, short to long, arctuated, waved or intensely coiled, ensheathed and unbranched without necridic cells	None	None	1–1.5 (2)	Thin, firm, enveloping one trichome, usually firmly attached, colorless	Pale blue-green, brownish, olive green, yellowish or reddish

Table 1. Continued

	Thallus	Filaments	False branching	Motility	Filament width (μm)	Sheaths	Color of trichomes
Pseudophormidium americanum	Filamentous, joined into microscopic mats, bright green	Solitary, cylindrical, isopolar, uniseriate, irregularly curved an coiled, without necridia, not attenuated at the ends	Intensive and irregular	None	2.9–3.6 (4)	Thick, firm, tube like, colorless	Green
Pseudophormidium phormidioides	Thin, membranaceous, somewhat lubricous dark or blackish blue green, expanded	Rarely solitaire, densely coiled	Present	None	5.6–7	Narrow, colorless	Blue-green, greyish, blue-green, olive green, dirty brownish-violet
Leptodesmis alaskaensis	Entangled in mats intensely green surface layer	Straight, curved, flexuous or wavy, solitary, one filament per sheath pale-blue green to dark green	None	Motile	1.8–2.6	Thick, colorless	Pale blue-green to green
Leptodesmis paradoxa	Compact mats, filamentous green to dark green	Straight, curved, flexuous or wavy, solitary, one filament per sheath pale blue-green to dark green	None	None	2.5–3.5 (4)	Thick, colorless	Not available
Nodosilinea alaskaensis	Compact mats bright green	Single trichome, sometimes presenting circular growth, long or short, straight or bent	None	Motile in young cultures	2–2.5 (3)	Usually present, thin, soft, colorless	Green to pale green
Nodosilinea nodulosa	Not available	Filaments typically with a single trichome, but sometimes becoming multiseriate, with nodules forming under low light conditions	None	None	Not available	Usually present, thin, soft, inflated, colorless	Not available
Gibliniella alaskaensis	Thin yellow-green to green	Cylindrical filaments without necridic cells, solitary or densely entangled to mats	None	Intensely motile	1.2–1.7	Thin, firm clear	Yellow-green, green or yellow-brown
Cell width (μm)	**Crosswalls**	**Cell form**	**Apical cell**	**Cell length (μm)**	**Hormogonia**	**Special features**	**Ecology**
(4.7) 5–6.5 (7.1) narrow end (3) 4–5	Constricted	Always wider than long, sometimes longer than wide, without aerotopes prominent parietal thylakoid pattern with orange granules and visible stacked thylakoids no aerotopes	Rounded, not capitate, without calyptra	(1.4) 1.8–2 (2.9)	Formation by characteristic biconcave necridic cells	Not observed	Periphyton of the water soil shoreline

Table 1. Continued

	Thallus	Filaments	False branching	Motility	Filament width (μm)	Sheaths	Color of trichomes
4.8–7.3	Constricted or not constricted	Wider than long cell content with facultative aerotopes	Slightly or strongly capitate	2.0–3.5	Hormogonia formation by biconcave necridic cells	Not observed	Grows in an alkaline lake in the Brazilian Pantanal wetlands
2.1–3.2	Slightly constricted	Isodiametric or longer than wide before division, parietal thylakoids, end cells markedly narrowed, longer	Tapered, rounded, sometimes form mushroom like calyptra	1.4–4	Not observed	Characteristic 90 degrees turned cell within the trichome	Freshwater, periphytic on roots or in snow
2–3	Not constricted	Variable shape, mostly isodiametric, sometimes slightly shorter or longer than , cell content homogenous, occasionally grainy necridic cells sometimes present	Rounded	2–4	Straight, made up of 2–8 cells	Filaments grow preferentially in dimly lit areas	Subaerophytic biofilms attached to calcareous substrata
1.1–1.6	Not constricted	Isodiametric or slightly longer than wide	Rounded without calyptra	1.6–3	Not observed	Arctuation of majority of filaments, but they are never spirally coiled	Meltwater, periphyton on roots
1.4–1.9	Slightly constricted	Rarely isodiametric, mostly longer than wide, contents usually homogeneous, without granulation	Rounded	1.5–2.7	None	Cell division occurring throughout the trichome	Limestone wall
1.7–7 (10)	Constricted	Longer than wide, without aerotopes, prominent parietal thylakoid pattern	Rounded, long with prominent granules at the apex	1.7–7 (10)	Not observed	End cells without thickened cell walls or calyptras, cells divide by a symmetrical crosswise binary fission, cells grow longer before next division	Freshwater, flowing in the water, or form mats in pelagial
(1.9) 2.2–2.7 (2.9)	Constricted	Barrel shaped, isodiametric or slightly shorter than wide, no aerotopes	Conically-rounded without calyptra	(1) 1.2–2.5 (3.4)	Not observed	Cell division crosswise, perpendicular to the long axis of the trichome, daughter cells grow to the original size before the next division	Soils

Table 1. Continued

	Thallus	Filaments	False branching	Motility	Filament width (μm)	Sheaths	Color of trichomes
5.6–7	Constricted	1/2–1/3 longer than wide	Rounded	Not available	None	Cell division crosswise	Freshwater, stones in mountain clear stream
1–1.5	Slightly constricted	Barrel shape or shorter than wide, occasionally longer than wide, central granules, uniseriate, length of cells differs at opposite trichome ends	Rounded occasionally slightly conical without calyptra	1.4–4.3	Not observed	Two types of cells in filaments, beginning of filaments have shorter, wider cells, end of filaments have longer cells	Green mat in dry channels on side of lake
2.5–3.5 (4)	Slightly constricted	Barrel shaped or shorter than wide, occasionally longer than wide, central granules	Rounded without calyptra	1–2	Not observed	Filament morphology changes during the life cycle, young filaments similar to *Leptolyngbya cortícola* or *L. boryana*, older ones similar to *Phormidesmis*	Mud of pool littoral
(1) 1.4–1.8	Not constricted	More or less longer than wide, lacking aerotopes, peripheral thylakoids	Rounded or conical	(1.5) 2–4.1 (4.8)	Not observed	Tolerates growth on nitrogen-free media	Crust on stones, freshwater
1–1.5	Distinct	Isodiametric, flattened	Rounded or bluntly rounded	1–2.4	Common	Capable of anaerobic N fixation	Marine phytoplankton euryhaline
0.7–1.2	Not constricted	Isodiametric, rarely longer than wide before division, parietal thylakoids	Rounded without calyptra	0.9–3.6	Not observed	Filaments coiled together like fishing-net filaments without necridia, intensely motile	Freshwater, crust on stones

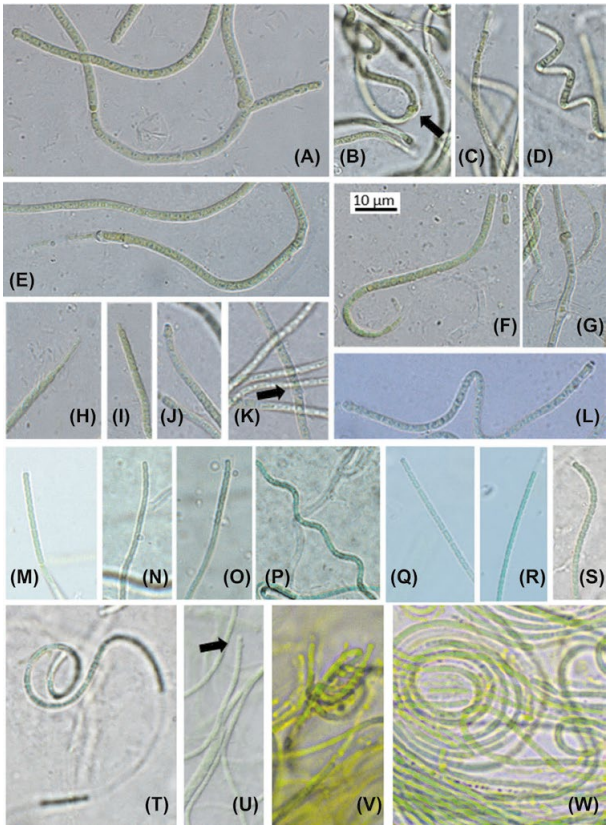

Figure 6. Morphological diversity of cultivated cyanobacteria. **(A–L)** *Albertania alaskaensis* [(A–D) strain KL12, (E–J) L1, (K–L) L11], **(M–P)** *Tildeniella alaskaensis* (strain KL16), **(Q–W)** *Nodosilinea alaskaensis* [strains (Q–S), (W) L32, (T–U) V2, (V) L22]. Scale bar for all microphotographs is 10 μm in 1000X magnification.

The morphology of strains KT1 and KT18 from Alaska (Figs 7H–M, 9G, Table 1) closely resembles *Pseudophormidium* (Hansgirg et Forti 1907) Anagnostidis et Komárek with its characteristic false branching that is a strikingly evident morphologic feature. These strains were thicker than the other species within Leptolyngbyaceae reaching 4 µm in width. The cells were however slightly thinner than typical cells of *P. phormidioides* that should be 5.6–7 µm. The other ubiquitous group of *Phormidesmis* included strain KT5 collected in Sagavanirhtok river. This strain was morphologically identical with *Phormidesmis communis* Raabová, Kovacik, Elster et Strunecký. It had thin filaments (1–3 µm), barrel-shaped isodiametric cells with prominent centroplasma with a central granule and parietal thylakoids (Fig. 5W). Morphologically similar strain V20 (Figs 7N–T and 9D) exhibited changes in its morphology throughout its life cycle. Namely, changing the cell ratios, from shorter to longer than their width throughout aging of its culture, suggested that it belonged to genus *Leptodemis* Raabová, Kovacik et Strunecký (Fig. 7N–T).

A total of 23 cultivated strains belonged to the core Synechococcales. Two strains were quite morphologically different from all others found in Alaska as they did not show any characteristic morphological features of their filaments due to their restricted size (Figs 8A–G and 9H, Table 1) and absence of heterocysts. Both strains exhibited sleek, yellow-green, intensely motile and exceptionally thin filaments with 0.7–1.2 µm wide cells, with thin and firm sheaths. The filaments were bundled altogether in one common sheath forming a characteristic fish-net like structure (Figs 8E–G, 9H). The morphological traits observed during microscopic examination, including the formation of string-like filaments, and altogether highly distinct habitus with respect to all other Syne-

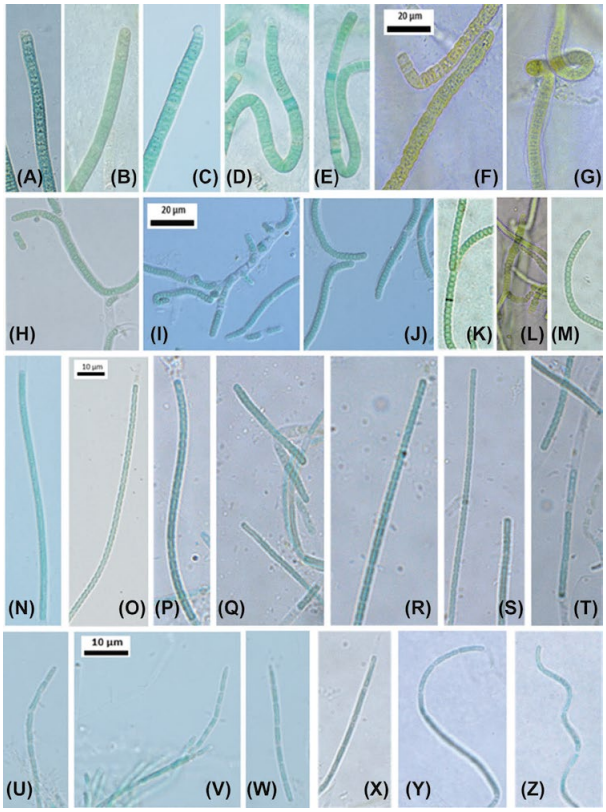

Figure 7. Morphological diversity of cultivated cyanobacteria. **(A–G)** *Cephalothrix alaskaensis* [(A) strain KL6, (B–E) KL18, (F–G) L30], **(H–M)** *Pseudophormidium americanum* [(H–K) KT1, (L–M) KT18], **(N–T)** *Leptodesmis alaskaensis* (strain V20), **(U–Z)** *Shackletoniella antarctica* (strain ULC037). Scale bar for microphotographs (A–M) is 20 μm in 1000X magnification and for (N–Z) 10 μm in 1000X magnification.

chococcales, provide support for the creation of a new genus *Gibliniella*.

The rest of the cultivated strains belonged to *Nodosilinea*. Strains T21, T29 and L32 showed similar knot-like traits (Figs 6Q–W, 9E) originating from cell division in more than one plane. They were motile during approximately the first month of cultivation in culture unlike previously reported strains of genus *Nodosilinea*. Barrel-shaped cells of slightly variable cell length with thicker sheaths retain morphologic features of straight filamentous forms similar to phylogenetically close *Halolebtolyngbya* Dadheech *et al.* 2012, contrasting with arctuated filaments with almost invisible sheaths in the strains of *Nodosilinea nodulosa* (Fig. 5R–S, Table 1). We find the differences sufficient to place these strains into a new species *N. alaskaensis* (Fig. 6Q–W). On the other hand, none of our strains designated as *Nodosilinea* species showed morphological features typical of the genus with cells dividing throughout the trichome forming nodular regions. Eleven of the other strains that we isolated and designated as *Nodosilinea nodulosa* (Fig. 5R–S) showed the other typical features, such as cells dividing perpendicularly to the trichome length that converge with the typical morphology of *Nodosilinea*. The last isolated strain found in the Pseudanabaneales perished before proper morphologic analysis.

TAXONOMIC TREATMENT

Cephalothrix alaskaensis sp. nov.

Description: Thallus fasciculated, blue-green or yellow-green when old. Cylindrical, straight or twisted trichomes, sometimes bent and coiled, not attenuated, constricted at the cross-walls with variable cell size within trichomes; with pairs of cells having conical trim outside of common cell wall, hyaline and firm sheath. End cells rounded, not calyptrate. Cells yellowish-green to pale green, sometimes with orange granules and visible stacked thylakoids, always wider than long, (1.4) 1.8–2 (2.9) µm

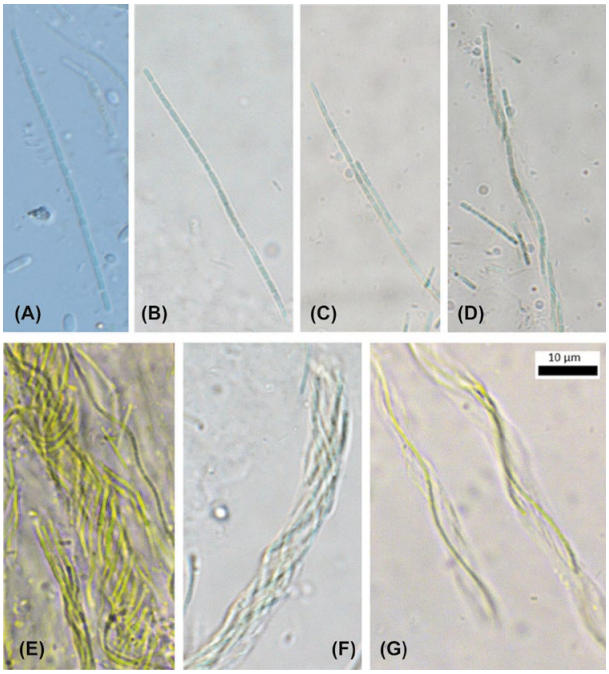

Figure 8. Morphological diversity of *Gibliniella* gen. nov. *Gibliniella alaskaensis*. (**A**) strain L5, (**B–G**) L31. Scale bar for all microphotographs is 10 μm in 1000X magnification.

long, (4.7) 5–6.5 (7.1) µm wide. Apical cell not capitate, cell content without aerotopes. Hormogonia formation by characteristic thin biconcave necridic cells. (Figs 7A–G, 9A)

Etymology: *alaskaensis* (L.): found at Alaska.

Holotype (here designated):–dried specimen of the strain L30 is deposited in the herbarium collection of the University of South Bohemia, under the number CBFS A–108–1.

Reference strain: L30, kept at culture collection of University of Ss. Cyril and Methodius, Trnava, Slovak Republic. Its 16S rRNA gene sequence has Genbank accession number MK861867.

Type locality: Toollik lake area, Lake S15, periphyton on the water soil shoreline.

Taxonomic notes: *C. alaskaensis* differs from *C. komarekiana* and *C. lacustris* from South America by the morphology of apical cells, i.e. conical calyptra is not present. Trichomes are characteristically twisted and bent unlike the straight trichomes of South American strains. Also, the aerotopes were not found by optical microscopy. The 2.4% difference in 16S rRNA gene sequence and rather big geographical distance provide additional means for the validity of the new species.

Albertania alaskaensis sp. nov.

Description: Thallus bright green to yellow-brown when old with characteristic growth pattern on agar making well isolated spots . Trichomes solitary, not fasciculated, bright yellow-green to olive green, with characteristic 90 degrees turned cell within the trichome (Fig. 6B and G), occasionally 1–12 thin cells (hair) at the end of filament (0.8–1.3 µm wide, 1.4–3 µm long) (Fig. 6E, F and H), occasionally false branching (Fig. 6A), not constricted at cross-walls, necridia not observed. Cells mostly isodiametric, or longer than wide before division, with parietal thylakoids, 1.8–3 µm wide, 1.9–4 µm long. End cells tapered, rounded, making characteristic mushroom like calyptra. Sheath clear, thin, and firm, occasionally widened. Freshwater, periphytic on roots or in snow. (Fig. 6A–L, 9F)

Etymology: *alaskaensis* (L.): found at Alaska.

Holotype (here designated): dried specimen of the strain KL12 is deposited in the herbarium collection of the University of South Bohemia, under the number A–109–1.

Reference strain: KL12, kept at culture collection of University of Ss. Cyril and Methodius, Trnava, Slovak Republic. Its 16S rRNA gene sequence has Genbank accession number MK861856-MK861912.

Type locality: Meltwater brook close to Poplar lake, periphyton on willow roots.

Tildeniella alaskaensis sp. nov.

Description: Trichomes flexuous, irregularly curved and coiled, untapered, cylindrical, isopolar, uniseriate, not constricted

at the visible cross-walls, always with thin sheath, without necridia and hormogonia. Cells 1.1–1.6 µm wide, isodiametric or slightly longer 1.6–3 µm than wide. End terminal cells rounded. The main diacritical feature is the arctuation of the majority of

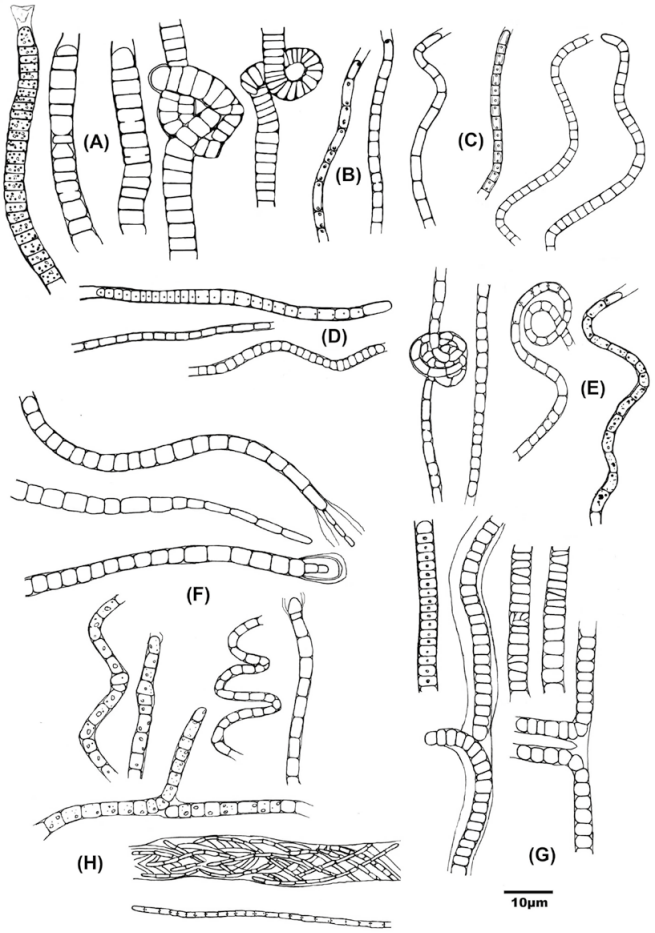

Figure 9. Illustrations of newly described taxa. **(A)** *Cephalothrix alaskaensis*, **(B)** *Shackletoniella antarctica*, **(C)** *Tildeniella alaskaensis*, **(D)** *Leptodesmis alaskaensis*, **(E)** *Nodosilinea alaskaensis*, **(F)** *Albertania alaskaensis*, **(G)** *Pseudophormidium americanum*, **(H)** *Gibliniella alaskaensis*. Scale bar for all illustrations is 10 µm.

filaments but they are never spirally coiled nor false branched. (Figs 6M–P, 9C Table 1)

Etymology: *alaskaensis* (L.): found at Alaska.

Holotype (here designated):–dried specimen of the strain KL16 is deposited in the herbarium collection of the University of South Bohemia, under the number CBFS A–116–1.

Reference strain: KL16, kept at culture collection of University of Ss. Cyril and Methodius, Trnava, Slovak Republic. Its 16S rRNA gene sequence has Genbank accession number MK861909.

Type locality: Meltwater brook close to Poplar lake, periphyton on willow roots.

Taxonomic notes: Different from *T. torsiva* and *T. nuda* by the absence of constriction between cells in fillaments, in their arctuation, and in the occasional presence of false branching. Molecularly distinguished by from T. torsiva by et least 3%.

Pseudophormidium americanum sp. nov. Strunecky et Raabova

Description: Filamentous; filaments solitary, joined into microscopic mats, firm, tube-like, colorless multilayered sheaths, commonly and intensely irregularly falsely branched. Trichomes, cylindrical, isopolar, uniseriate, irregularly curved and coiled, (1.9)2.2–2.7(4) µm wide, composed of barrel-shaped cells constricted at cross-walls, not attenuated to the ends, non-motile. Cells isodiametric or slightly shorter than wide with cell

length (1)1.2–2.5(3.4) μm, without aerotopes, without necridia and hormogonia, green. End cells conically-rounded. Cell division crosswise, perpendicular to the long axis of a trichome, daughter cells grow to the original size before the next division. Isolated from soil. (Figs 7H–M, 9G, Table 1)

Etymology: *americanum* (L.): from the Americas, to date all sequenced strains were found at North or South America.

Holotype (here designated):–dried specimen of the strain KT1 is deposited in the herbarium collection of the University of South Bohemia, under the number A–115–1.

Reference strain: KT1, kept at culture collection of University of Ss. Cyril and Methodius, Trnava, Slovak Republic. Its 16S rRNA gene sequence has Genbank accession number MK861904.

Type locality: Dry soil at top of Jade Mountain.

Taxonomic notes: The original morphological designation of all described species within *Pseudophormidium* (Forti 1907) (Anagnostidis and Komarek 1988) with typical short non-constricted cells, clearly visible multilayered sheaths and intense false branching was identical to our strains designated as *Pseudophormidium*. Independent taxonomic designation of morphologically identical and phylogenetically closest strains from South America, designated as *Pseudophormidium* by Miscoe *et al.* 2016 supported our definition of new species according the rules of the botanical code as *Pseudophormidium americanum*.

Leptodesmis alaskaensis sp. nov.

Description: Thallus entangled in mats forming an intensely green surface layer. Filaments straight, curved, flexuose or wavy, solitary, one filament per sheath. Motile. Filaments pale blue-green to dark green, slightly constricted at cross-walls, may become slightly wider on the apical part of filament. Sheaths thick, colorless. Filaments 1–1.5 (1.6) μm wide, without necridic cells. Cell length 1.4–4.3 μm. Cells barrel-shaped longer than wide, isodiametric or shorter than wide, with central granules in cells. Uniseriate, length of cells differs at opposite trichome ends. Apical cell is rounded without calyptra. In young cultures cells are longer than wide whereas with central granules, in old cultures (~4–6 weeks) cells isodiametric or shorter than wide. (Figs 7N–T and 9D, Table 1)

Etymology: *alaskaensis* (L.): found at Alaska.

Holotype (here designated): dried specimen of the strain V20 is deposited in the herbarium collection of the University of South Bohemia, under the number CBFS A–111–1.

Reference strain: V20, kept at culture collection of University of Ss. Cyril and Methodius, Trnava, Slovak Republic. Its 16S rRNA gene sequence has Genbank accession number MK861877.

Type locality: Green mat in dry channels on east side of Lake NE2 in Toolik lake area.

Gibliniella gen. nov. Strunecky et Raabova

Thallus thin, yellow-green, green or yellow-brown. Cylindrical filaments without necridic cells solitary or densely entangled to mats. Trichomes long, wavy, typically spirally coiled, rarely straight, untapered, isopolar with rounded-end cells, 0.6–1 μm wide, intensely motile. Sheets thin and firmly attached to filaments. Cells not or slightly constricted at the translucent cross-walls. Phylogenetically distinct from all other genera in the Synechococcales.

Gibliniella alaskaensis sp. nov. Strunecky et Raabova

Description: Thallus yellow-green to green. Trichomes solitary or fasciculated, wavy, spirally coiled, rarely straight, bright yellow-green to bright green, without false branching, not constricted at cross-walls, necridia not observed, intensely motile. Cells mostly longer than wide, with parietal thylakoids, 0.7–1 μm wide. End cells rounded. Sheath clear, thin and firm. Freshwater in lake. (Figs 8A–G, 9H, Table 1)

Etymology: *Gibliniella* named in honor of Anne E. Giblin, a well–known North American ecologist who studies anthropogenic inputs to ecosystems.

Holotype (here designated): dried specimen of the strain L31 is deposited in the herbarium collection of the University of South Bohemia, under the number A–114–1.

Reference strain: L31, kept at culture collection of University of Ss. Cyril and Methodius, Trnava, Slovak Republic. Its 16S rRNA gene sequence has Genbank accession number MK861870.

Type locality: Research lake S7 in Toolik lake area, crust on stones.

Shackletoniella gen. nov. (West et G.S.West), Strunecky, Raabova et Bernardova

Filamentous cyanobacteria, forming greenish or reddish compact biofilms. Filaments fine, long, arctuated, waved or intensely coiled, ensheathed and unbranched without necridic cells. Sheaths colorless, thin, firm, enveloping one trichome and usually firmly attached. Trichomes fine 1–3 μm wide, cylindrical, slightly constricted at cross-walls. Up to date found in the Antarctic and north of North America.

Shackletoniella antarctica (West et G.S.West) Strunecky, Raabova et Bernardova

Description: Filaments short to long, curved, wavy or coiled, with colorless, thin sheaths. Filaments 1–1.5 (2) μm wide, slightly constricted on cross-walls, without necridic cells, cells longer than wide 1.7–7 (10) μm long, without aerotopes, with prominent parietal thylakoid pattern, pale blue-green, brownish, olive green, yellowish or reddish; end cells without thickened cell walls or calyptras. Cells divide by a symmetrical crosswise binary fission; cells grow long before next division. Heterocytes and akinetes absent. Apical cell rounded, long with prominent granules at the apex. Freshwater in flowing water or forming thick mats at lake bottom. (Figs 7U–Z, 9B, Table 1)

Etymology: the generic name '*Shackletoniella*' was derived from the name of Sir Ernest Shackleton, a polar explorer who led British expeditions to the Antarctic including scientific investigations.

Basionym: *Phormidium antarcticum* West and West 1911: 292, Syn.: *Leptolyngbya antarctica* (West and West 1911) Anagnostidis and Komárek 1988: 390

Type species: *Shackletoniella antarctica*

Holotype (here designated): dried specimen of the strain is deposited in the herbarium collection of the University of South Bohemia, under the number CBFS A–155.

Reference strain: *Shackletoniella* ULC037 (ANT.LH18.2) kept at BCCM/ULC Cyanobacteria Collection at University of Liege, Belgium. Its 16S rRNA gene sequence has Genbank accession number AY493606.

Type locality: Prydz Bay at East Antarctica, Larsemann Hills, Lake 18, microbial mat.

Nodosilinea alaskaensis sp. nov.

Description: Thallus bright green. Filaments with a single trichome, sometimes presenting circular growth. Trichomes long or short, straight or bent, immotile or motile. Cells more or less longer than wide (1)1.4 × 1.8 wide, 2–4.1(8) μm long, lacking aerotopes, with peripheral thylakoids not constricted at the cross walls. Sheath usually present, thin, soft, colorless. Can grow on nitrogen free media. Freshwater. (Figs 6Q–W, 9E, Table 1)

Holotype (here designated): dried specimen of the strain L32 is deposited in the herbarium collection of the University of South Bohemia, under the number CBFS A–113–1.

Reference strain: L32, kept at culture collection of University of Ss. Cyril and Methodius, Trnava, Slovak Republic. Its 16S rRNA gene sequence has Genbank accession number MK861885.

Type locality: Research lake S5 in Toolik lake area, crust on stones.

DISCUSSION

To date there have been limited studies into cyanobacterial diversity in Alaska, providing little understanding into the biodiversity of their life in extreme climates. Here, we present evidence that cyanobacteria exhibit high levels of species richness in a relatively small sample area. We found 17 unique cyanobacterial genera despite the limitations of our methodical approach being focused on cultivable species. As expected, all the cultivated strains belonged to faster growing species of cyanobacteria. Attempts to cultivate species belonging to Nostocales failed despite the fact they were frequently identified in our samples upon collection. These slower growing species were probably overgrown by the faster growing Oculalteaceae and Lepotolynbyaceae. A total of 36 strains were lost after their isolation from the initial sample to single species culture prompting speculation about the supposed need for a particular chemical compound(s) produced by a co–symbiont within the cyanobacterial consortium.

NGS is the most efficient method in describing natural diversity, however, this technique faces complications in the presence of a high amount of cyanobacterial extrapolysacharides such as is the case in biofilms at the majority of sampling sites in Alaska. These chemical agents disproportionally lower the yields of DNA isolated from environmental samples as well as primer mismatch during amplification processes in NGS (Strunecky *et al.* 2019). Following our previously successful approach we employed here a combination of methods comprising the cultivation of cyanobacteria, optical microscopy and 16S rRNA gene sequencing (Raabova *et al.* 2019). Observed cyanobacterial diversity supported by the sequencing efforts underestimate the true biodiversity at the sample collection sites. The results of our cultivation trials suggest that diversity at the North Slope is much higher. Our discovery of new species demonstrates that further research using NGS is needed to uncover the true biodiversity of the Alaskan region.

Our investigation resulted in identification of members of many ubiquitous cyanobacterial genera such as *Nodosilinea* (Fig. 5R–T and 6Q–W), *Phormidesmis* (Fig. 5W) and *Microcoleus* (Fig. 5K). We also found genera that were described only recently and their known species have few recorded incidences such as *Cephalothrix* (da Silva Malone *et al.* 2015), *Albertania* (Zammit 2018) and *Stenomitos* (Miscoe *et al.* 2016). Their exact phytogeography is expected to emerge in the near future due to the growing number of sequenced specimens.

We described here *Cephalothrix alaskaensis* gen. nov. belonging to Coleofasciculaceae according the original description by Malone *et al.* 2015 and our current phylogenetic analysis. Morphology of *Cephalothrix* resembles the phylogenetically close *Coleofasciculus* (Siegesmund *et al.* 2008) and *Wilmottia* (Strunecky *et al.* 2011). Their soft pale green constricted >5 μm wide trichomes and simple rounded-end cell helps identify this family. A meticulous observation under the microscope is imperative for distinguishing constrictions between the adjacent cells and the characteristic cell granulation.

Two ubiquitous species in Chroococcales were evaluated. Chroococcales in general have similar cellular morphologies, and typically lose their mucilage and colony characteristics in culture (Mareš *et al.* 2019a), often contributing to their misidentification. *Gloeothece membranacea* found in our study however showed its typical morphology as was described in recent revision by Mareš *et al.* (2019a) as well as *Geminocystis* (Korelusova, Kastovský and Komarek 2009). Both species currently have a small number of confirmed observations in polar environments, in particular the Alaskan region.

As the largest family in the Synechococcales the Leptolyngbyaceae have received significantly more attention in recent years (Mai *et al.* 2018). The species within the family have been repeatedly shown to be highly divergent in their 16S rRNA gene sequence, which led to the split into several families. The recently recognized daughter family of Oculateaceae (Mai *et al.* 2018) acquired many species during the latest taxonomical reevaluations. Species within this family have cells about 3 μm wide, generally longer than wide, with visibly parietal thylakoids. Cells are soft and it is easy to crush them during manipulation. Filaments often have a pigmented eye (oculus) in the terminal cell during their growing phase concomitant with the filament motility. Oculatellaceae contained four taxa in our set of cultivated cyanobacteria. Two of them were identified on the species level. *Albertania alaskaensis* exhibits several morphological peculiarities such as perpendicularly oriented cells within trichomes.

The last clade contained strains found to date only in the Arctic and Antarctic. These Alaskan strains clearly do not belong to Leptolyngbyaceae due to their long cells and the arctuated character of the trichomes. Similar strains were previously reported only from mats at the bottom of Antarctic lakes (Taton *et al.* 2006). Our samples were collected in the periphyton of the flowing Alaskan rivers. The members of our newly established genus *Shackletoniella* are strictly aquatic according to current knowledge. The long cells within the trichomes observed in optical microscopy display rounded structures at both ends of cells (Figs 7U–Z, 9B, Table 1). The origin of these structures is unknown, reflecting possibly a special thylakoid pattern (Mareš *et al.* 2019b). Due to recent recognition of many species within Oculatellaceae we expect even more species and genera described within this family that were previously determined as *Leptolyngbya* (Figs 6 and 7).

Unexpectedly, we cultivated a small amount of strains belonging to the Leptolyngbyaceae family. Surprisingly, three strains were not able to grow for an extended time under laboratory conditions. The members of the *Leptolyngbya* group are generally considered versatile, undemanding species growing under any conditions. Our research demonstrates that bacterial dark matter (Lloyd *et al.* 2018) may be more common among cyanobacteria than expected. On the other hand, we found several rarely found species within Leptolyngbyaceae. One of them was *Pseudophormidium* that has a clearly distinguishable overall appearance with typical short non-constricted cells, visi-

bly multilayered sheaths and intense false branching filaments. The phylogenetic placement of *Pseudophormidium* is still unclear according to Komárek *et al.* (2014) because all sequenced representatives are still assigned to Leptolyngbyaceae. According to the older morphology based studies *Pseudophormidium* was assigned to *Phormidium* (Komárek and Anagnostidis 2005) which was later transferred to *Microcoleus* (Komárek *et al.* 2014). Strains within the same cluster as Alaskan strains were previously recognized as *Pseudophormidium* sp. by Osorio-Santos *et al.* (2014) during their study of cyanobacteria from the Atacama Desert. All morphological features of *Pseudophormidium americanum* sp. nov. agree well with the original description of its type *Pseudophormidium phormidioides* (Hansgirg ex Forti 1907). To resolve the question about the proper taxonomic designation of *Pseudophormidium*, the type material shall be obtained, and more sequences compared in further studies. Until then, we follow the morphology based determination which is moreover in congruence with previously sequenced strains. Because the species designation was not figured out previously, we follow the geographic origin of strains found to date in both the South and North Americas and selected the name for this species as *Pseudophormidium americanum*.

Microscopic determination of *Leptodesmis alaskaensis* sp. nov. is highly problematic due to the changes in cell and trichome morphology. Originally, it was described as having long cells without constrictions in young trichomes while older trichomes exhibit short constricted barrel-shaped cells. We found *L. alaskaensis* to be motile. This characteristic was not observed in any other previously described species belonging to the Leptolyngbyaceae.

The largest group of strains that were cultivated belonged to Synechococcales. *Gibliniella alaskaensis* sp. nov. fell phylogenetically between th moderately halophilic *Halomicronema* (Abed, Garcia-Pichel and Hernandez-Marine 2002) and halophilic *Haloleptolyngbya* indicating possible halophily of this species (Fig. 4). Our strains were isolated from a lake undergoing a long-term nutrient enrichment experiment. We cannot rule out either the possibility of their transport to the site along with the fertilizer (Daniels, Kling and Giblin 2015) or their genuine preference for this type of environment. The *Gibliniella* species are extremely thin and intensely motile. Their morphological identification is possible under an optical microscope equipped with precise size measurement suitable for single filament width measurements or localization of characteristic fish-net like fascicles (Fig. 8E–G, 9H). The other cultivated strains in Synechococcales were classified as members of *Nodosilinea* genus despite lacking some of the morphological features typical of *Nodosilinea* such as formation of specific nodules in trichomes (Perkerson *et al.* 2011).

Description of cyanobacterial taxa is based on three pillars: morphology, ecology and similarity of a few selected genes. Morphological determination poses many challenges, especially in cyanobacteria exhibiting simple morphology such as thin trichomes or small coccoid cells. Strains that prosper under laboratory conditions may lose features such as mucilage layers, oculus or end cell shape, rendering morphology based identification difficult or close to impossible. On the other hand, cells exposed to stress or suboptimal conditions can form peculiar structures in nature as we witnessed many times in the samples from Alaska. Correct determination to family or generic level therefore requires inspection of the studied natural material as well as cultured strains.

Ecology and biogeography play a very important role in modern classification of cyanobacteria (Komárek and Anagnostidis 2005; Strunecky, Komarek and Elster 2012). Future discussions will shape the judgement of how much weight should be given to ecological factors in making taxonomic decisions. The assumption that two organisms cannot be the same species if they colonize significantly different habitats may seem intuitive, but increasingly available molecular data indicates that this is not always the case. Examples of this are evident in the fact that several ubiquitous species of Leptolyngbyaceae have been found in hot deserts as well as in freshwater of cold regions (Raabova *et al.* 2019).

The most used gene in bacterial taxonomy is still the 16S rRNA gene. Many of the current bacterial species with validly published names do not respect minimal 95% (for genus) and 98.7% (for species) sequence similarity thresholds that are currently recommended to classify bacterial isolates (Rossi-Tamisier *et al.* 2015). A similar situation can be found in the description of cyanobacterial taxa during the last few years, making recent cyanobacterial classification even more vague, although no detailed analysis has been published.

In conclusion, we followed an established approach to investigate the cyanobacterial diversity of a previously unexplored region of the Alaskan North Slope. This methodology led us to discover two new genera and six new species of cyanobacteria, suggesting the existence of high biodiversity in extreme polar environments. This research provides a foundation for further detailed ecological, biogeographical and genomic studies.

SUPPLEMENTARY DATA

Supplementary data are available at *FEMSEC* online.

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CHAPTER 5

General discussion

The main objective of this thesis was to contribute to a better knowledge of cyanobacteria. Cyanobacteria is the main consequence of eutrophication in water ecosystems. They are considered highly undesirable species in fish ponds. Many fishponds in the South Bohemian region suffer from cyanobacterial blooms (Pokorný and Květ, 2018). As a result, our study has been focused on their dynamics, species composition, and factors controlling their biomass. We tried to suggest management schemes to reduce their biomass. Also, we studied cyanobacterial diversity in North Slope, providing little understanding of the biodiversity of their life in extreme climates.

Relationship between phytoplankton and nutrients

The key role of P on the phytoplankton abundance was recognized a long time ago (Schindler, 1977) and was naturally seen in our data (Chapter II and III). There is a long-lasting debate, whereas the N limit the phytoplankton growth considering the type of lake, trophic status, and season (Downing and McCauley, 1992; Kolzau et al., 2014), and what is the stoichiometric ratio for the cyanobacterial dominance (Smith, 1983).

The situation with a low concentration of TN was almost entirely absent (Chapter II and III). In general, two scenarios might explain it. Either there is high N input from the watershed or cyanobacteria fix a sufficient amount of N. Higher winter NO_3-N and TN concentrations decrease as temperature rises due to denitrification. The reported rate of denitrification in eutrophic fishponds is in the range of ~ 150–700 mg N m^2 d^{-1} (Jensen et al., 1992; Olah et al., 1994; Piña-Ochoa and Álvarez-Cobelas, 2006); which means that all nitrogen could be effectively released from studied ponds within 3 to 21 days. Despite the fast denitrification, the TN levels remain high and N:P ratio bellow 10:1 can be rarely found in studied hypertrophic ponds (Chapter II). TN increases again during the high phytoplankton season. During this phase, the majority of both main nutrients are particulated, i.e. they are bound in phytoplankton.

Interestingly, both the above-mentioned scenarios are probably at play. Nitrogen input from the watershed and added manure (Rutegwa et al., 2019a) is slowly denitrified during winter and early spring resulting in higher TN concentration. Rising temperatures in spring trigger the denitrification that effectively releases N from fishponds along with N from added feed (Chapter II and III) and additional inflow. The presence of heterocystous cyanobacteria fixing nitrogen indicates low disposable N in water. A plausible explanation of cyanobacteria prevalence in nutrient-rich water should be a higher turnover N rate by bacteria using high levels of organic carbon connected with fast denitrification, and contra intuitively limitation of algae by a low amount of dissolved nitrogen during the summer and autumn.

The phytoplankton biomass, which was mostly produced by cyanobacteria, was significantly correlated with TP (Chapter II). The cyanobacterial biomass peaks also closely correspond to the summer peak of P concentration (Chapter II). As the cyanobacteria might almost simultaneously fix atmospheric carbon and nitrogen dissolved in water, even with the species-specific rate (De Nobel et al., 1997), the concentration of NH_4^+ and NO_3-N in water is not critical for their development. Interpretation of obtained data thus implies the relatively low importance of C:N:P ratios for hypertrophic ponds dominated by cyanobacteria.

An environment with extreme nutrient input and simultaneously yearly or bi-yearly “restart” of initial conditions is well represented by a fishpond. Daily nutrients supply via fish feed is provided due to the focus on the highest fish production during the season. These environments are also under strong predation pressure from farmed and weed fish, which efficiently disconnect the ecosystem network by the elimination of zooplankton. The data

from this study provide a relatively simple explanation of the relationships between main nutrients and primary productivity of hypertrophic lakes due to the simplification of ecological networks caused by the conditions mentioned above.

Dynamic of phytoplankton in fish ponds

The phytoplankton that is considered the main marker of eutrophication flourished in the studied ponds with cyanobacterial blooms at the end of the season. The biomass of phytoplankton during winter was slightly under 1 mg C L^{-1}. Even it was low compared to summer values, such a level of biomass was still above the order of magnitude higher than maximal summer values in oligotrophic lakes (Nedbalova et al., 2006) or mesotrophic reservoirs. According to traditional models (Sommer et al., 2012), spring production of phytoplankton is followed by zooplankton feeding on phytoplankton and subsequently lowering its concentration. This mechanism has not been observed at our sites. The observed April concentrations of 1 to 2 mg C L^{-1} in CPhy rose continually to maximal values of (5) 10 mg L^{-1} during summer without any appearance of spring peak of phytoplankton. Such behavior can be easily explained by the low amount of zooplankton that was effectively suppressed by fish. Top-down control caused by high stock densities of planktivorous fish (Carpenter and Kitchell, 1988) effectively suppressed the development of big zooplankton species and opened space for uncontrolled phytoplankton growth.

The grazing pressure of zooplankton is a key factor in regulating phytoplankton biomass (Hrbáček et al., 1961). The zooplankton biomass was inhibited by fish predation in fishponds and especially large Cladocerans cannot grow big enough to prevent the cyanobacterial blooms. Such a process should more likely explain the declining zooplankton amount from its highest levels from May to July in most ponds. Fish density alone could not explain all over fluctuations in zooplankton biomass that varies in a frame of 1 mg C L^{-1} under comparable fish densities. Our results strongly suggest that fish concentration higher than 20 mg L^{-1} (~280 kg ha^{-1}) effectively suppresses the zooplankton growth from spring to autumn. On the contrary, the amount of fish below 5 mg L^{-1} (~70 kg ha^{-1}) had high zooplankton production resembling natural lakes. Small amount of available data on fish densities, around 10 mg L^{-1} (~150 kg ha^{-1}) hamper a complete understanding of top-down control by fish in this study. However, combined with zooplankton dynamics documented by (Korinek, 1967) during spring, fish densities below 150 kg ha^{-1} still enable the "clear water phase" followed by efficient transformation of energy through the ecosystem to fish production after temperature rise. If the ecosystem functions of ponds would be preferred over fish production the suggested fish stock densities should be lower than 150 kg ha^{-1}. Such densities still prevent ingrowth of water plants and sediment lake fill and simultaneously enable sustainable fish production.

Phytoplankton communities in mesotrophic, eutrophic, and hypertrophic ponds

The physicochemical parameters of monitored in our study fishponds fluctuated in a wide range. The significant variation in the phytoplankton community and different concentrations of nutrients could be caused by the fisheries management regime mainly liming and fertilizing. Ponds Hejtman-Hamerský, Ratmírovský, Rožmberk, Bezdrev, Dehtář, Horusický, Svět were actively used by fish farming. The high concentration of TP originated from wastewaters and feed and manure applications (Potužák et al., 2016, Rutegwa et al., 2019b). Ponds Stankovsky and Hejtman-Koštěnický are situated close to each other and used only for angling. The TP input was mainly from inflows and negligibly from fishery management. Water quality in these ponds was considerably better than in other investigated (Potužák et al., 2016).

The cyanobacterial specific feature, i.e. frequent formation of colonial assemblages is commonly explained as a defense to grazing by zooplankton However, it has been documented that Cladocera scramble, browse or graze cyanobacterial colonies as well as break down and digest filamentous species, effectively using them as a food source until the concentration of Chl-a reaches 100-fold that observed in our study (Vanni, 2009; Tonno et al., 2016). Colonies formed at hypertrophic fishponds typically consist of more than one species. Species as *Microcystis* and *Pseudanabaena* with aerotopes coalesced with nitrogen fixers as *Anabaena* and *Anabaenopsis.* The more likely explanation is that species in cyanobacterial colonies share virtues of particular ones. Moreover, they can migrate faster in the water column due to their higher buoyancy than a single cell (Kromkamp and Walsby, 1990). Aerotopes-bearing cells drive the movement of the cyanobacterial consortium to the light or bottom phosphorus-rich water layer according to its current requirements.

Moreover, some species can produce extracellular phosphatases to increase furthermore P intake (Štrojsová et al., 2003; Xu et al., 2018). The biomass concentration of non-fixing cyanobacteria in eutrophic ponds generally increased from July till late autumn, while in hypertrophic ponds, it started to grow almost simultaneously with N-fixing cyanobacteria from April. It can be explained that The gas vesicles in non-fixing cyanobacteria helped them to move up and down in the water column, which increases access to nutrients in autumn when the concentration of TP starts to decrease. If such symbiosis does exist, it is a matter of further studies; however, we consider it the most likely explanation of frequently observed phenomena.

Colonial cyanobacteria such as *Woronichinia, Chroococcus, Coelomoron* developed widely in mesotrophic ponds. Cyanobacteria, such as *Woronichinia naegeliana*, were classified as characteristic of oligo-mesotrophic and meso-eutrophic water bodies (Brettum, 1989; Nowicka-Krawczyk and Żelazna-Wieczorek, 2017).

In all ponds, Bacillariophyta were the second abundant group of algae after cyanobacteria. Bacillariophyta has a lower thermal optimum than cyanobacteria which gives them the advantage of being dominant in early spring (Nalley et al., 2018). *Nitzscia, Aulacoseira, Synedra* were the most cosmopolitan and abundant species in eutrophic and hypertrophic. They are common in shallow, enriched turbid lakes (Reynolds et al., 2002). In mesotrophic ponds, the dominant diatom was *Asterionella formosa*. It can be abundant when P availability is very low and be an indicator of an oligo-mesotrophic state (Saros et al., 2005).

Representatives of Chlorococcales such as *Coelastrum, Pediastrum, Scenedesmus* are typical in shallow, enriched with nutrients eutrophic and hypertrophic water bodies (Reynolds et al., 2002; Napiórkowska-Krzebietke et al., 2011). Mass growth of these species was observed in eutrophic and hypertrophic ponds. Colonial Chlorophytes such as *Botryococcus, Planktosphaeria* are dominant in mesotrophic ponds. These species are most common in low nutrient water bodies with high turbidity (Reynolds et al., 2002).

Chrysophyeseae were abundant during the spring season. The maximum number of representatives of golden-brown algae were found in ponds mesotrophic ponds. Optimum conditions for the growth of chrysophytes are clear waters with low concentrations of nutrients (Poulíčková, 2011). The site was characterized by the occurrence of clear water species as *Dinobryon* (Kitner et al., 2003). Their spring maxima and low biomass in the summer were regulated by zooplankton grazing (Watson and McCauley, 1988). Chrysophyeseae rarely occur in highly eutrophic lakes (Watson et al., 1997). However, the presence of *Dinobryon* in eutrophic and hypertrophic ponds, might be explained due to temporal changes in the trophic status of water reservoirs in spring (Celewicz-Goldyn, 2005).

As a Chrysophyeseae, Cryptophyeseae are regulated by zooplankton grazing (Watson and McCauley, 1988). However, the biomass of Cryptophytes increased with the enrichment of nutrients due to the absence of natural predators in eutrophic and hypertrophic ponds.

Trachelomonas has been the most widespread genus in Euglenophyceae as being found in all studied ponds. The development of Euglenophyceae species is typical for shallow waterbodies enriched with organic matter (Poniewozik and Juráň, 2018). Trachelomonas species showed better growth in summer and autumn in eutrophic and hypertrophic ponds when the concentration of organic matter in water was higher in the ponds.

The development of unicellular Volvocales (*Chlamydomonas* spp.) occurred in the summer when the concentrations of nutrients were relatively high. Volvocales preferred highly eutrophic and organically polluted waters and were almost absent in oligo and mesotrophic water bodies.

Diversity of cyanobacteria at the Alaska North Slope

Cyanobacterial diversity at the North Slope of has not been systematically studied previously. We presented evidence that cyanobacteria exhibit high levels of species richness in a small sample area in extreme climates. We found representatives of 17 unique cyanobacterial genera (Chapter IX). All the cultivated strains belonged to faster-growing species of cyanobacteria. Our investigation identified members of many omnipresent cyanobacterial genera such as *Nodosilinea, Phormidesmis,* and *Microcoleus*. We also found genera that were described only recently and their known species have a few recorded incidences, such as *Cephalothrix* (da Silva Malone et al., 2015), *Albertania* (Zammit, 2018), and *Stenomitos* (Miscoe et al., 2016). Their exact phytogeography is expected to emerge in the near future due to the growing number of sequenced specimens.

Ecology and biogeography play a very important role in the modern classification of cyanobacteria (Komárek, 2005). Future discussions will shape the judgment of how much weight should be given to the ecological factor in taxonomic decisions. The assumption that two organisms cannot be the same species if they colonize significantly different habitats may seem intuitive. Still, the increasingly available molecular data indicates this is not always the case. Examples of this are evident in the fact that several ubiquitous species of Leptolyngbyaceae have been found in hot deserts and in cold freshwater regions (Raabová et al., 2019).

Conclusion

The main goal of this thesis is to enhance our comprehension of the cyanobacteria proliferation in Czech fishponds and their ability to thrive in extreme environments. This thesis aims to explore how fishery management practices and the amount and distribution of nutrients affect the prevalence of cyanobacteria in fish ponds, aiming to improve our understanding of this phenomenon. Czech fishponds are an important part of the landscape. However, their use for intensive fish breeding and economic benefits often harms the ecological situation in the fishponds. The only way to improve the situation is to intensively collect and evaluate pond data to gain general knowledge and propose strategies for improving water quality. The results of this thesis extended earlier studies on the dynamics of nutrients, plankton, and fish biomass and their relationship and also provided strategies for improving water quality and reducing the amount of phosphorus due to the reduction of fish stock.

The main conclusions from these studies were:

1. Nutrient concentration, phytoplankton, zooplankton composition, and fish stock densities were evaluated to study the seasonal succession of plankton in nine fishponds

of different trophic statuses in the Czech Republic. The cyanobacteria formed a significant part of the phytoplankton community in summer in ponds with a different trophy.

2. The phytoplankton of biomass correlated with the concentration of phosphorus. The low concentration of dissolved nitrogen was compensated by the high ratio of nitrogen-fixing cyanobacteria that formed a significant part of the phytoplankton community.
3. High fish densities reduced the zooplankton biomass, which did not influence the phytoplankton biomass. We suggest that the combined effects of lower fish densities can cut phosphorus concentrations in fishponds without a hardly achievable decrease in phosphorus input from the watershed.
4. We investigate the cyanobacterial diversity of a previously unexplored region of the North Slope. We discover two new genera and six new species of cyanobacteria, suggesting the existence of high biodiversity in extreme polar environments.

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English summary

The taxonomical and physiological diversity of cyanobacteria from water environment

The main objective of this thesis was to contribute to better knowledge of cyanobacteria from fishponds with great significance to taxonomy and ecology and polyphasic evaluation of cyanobacteria from Alaska North Slope.

The fishponds represent a highly modified environment that is different from natural lakes. Extreme nutrient load and intense predation pressure from farmed and weed fish alter the seasonal succession of plankton. Weekly feeding is provided to maximize fish production, and high fish density impairs the trophic food web by eliminating zooplankton. The results of this thesis is to describe plankton succession in fishponds and the high influence of top-down forcing.

Cyanobacteria dominated the total biomass of eutrophicated and hypertrophicated ponds and mesotrophic in summer. Their specific feature, i.e. frequent formation of colonial consortia is commonly explained as a defence against grazing by zooplankton. However, it has been documented that Cladocera scramble, browse or graze cyanobacterial colonies as well as break down and digest filamentous species, effectively using them as a food source until the concentration of Chl-a reaches 100-fold that observed in our study.

The zooplankton biomass was reduced by fish predation in the studied fishponds, and populations of large Cladocerans did not grow fast enough to prevent cyanobacterial blooms. Top-down control triggered by high density of planktivorous fish opened a niche for uncontrolled phytoplankton growth.

The role of phosphorus in phytoplankton growth was confirmed by our data. As cyanobacteria fix inorganic carbon as well as molecular nitrogen dissolved in water, the concentration of NH_4-N and NO_3-N in water is not critical to their proliferation. A high P concentration thus lowers the relevance of stoichiometric C:P and N:P ratios in a hypertrophic system dominated by cyanobacteria. Data obtained through this study indicate that eutrophication of water bodies could not be controlled by reducing nitrogen input. Soluble nitrogen was almost absent in studied fishponds.

The parameters controlling seasonal plankton dynamics in hypertrophic fishponds might be constrained by the concentration of phosporus and fish stock density. Maximization of fish production effectivity and phosphorus transfer captured by phytoplankton via zooplankton at fish densities below 300 kg ha^{-1} would be a simple measure to lower the amount of cyanobacteria and consequently improve water quality. Control of fish density can enhance the quality of fishponds and moderate cyanobacterial blooms without a hardly achievable decrease in phosphorus input.

We also investigated the cyanobacterial diversity of a previously unexplored region of the Alaskan North Slope. We described two new genera and six new species of cyanobacteria, suggesting the existence of high biodiversity in extreme polar environments. This research provides a foundation for further detailed ecological, biogeographical and genomic studies.

Czech summary

Taxonomická a fyziologická diverzita sinic z vodního prostředí

Hlavním cílem této práce bylo přispět k lepšímu poznání sinic z rybníků s velkým významem pro taxonomii a ekologii a polyfázové hodnocení sinic z aljašské oblasti zvané North Slope.

Rybníky představují prostředí, které se liší od přírodních jezer. Extrémní zatížení živinami a intenzivní predační tlak ze strany chovaných a plevelných ryb mění sezónní posloupnost planktonu. Přikrmování ryb se provádí za účelem maximalizace produkce ryb a obsádky ryb v rybnících narušují trofickou potravní síť tím, že je výrazně eliminován zooplankton. Výsledky této dizertace popisují změny ve složení a biomase planktonu v rybnících a vysoký vliv predačního tlaku ze shora dolů (top-down effect) na tyto změny.

Sinice dominovaly v celkové biomase mezotrofních, eutrofních a hypertrofních rybníků v létě. Jejich specifický rys, tedy častá tvorba koloniálních konsorcií, se běžně vysvětluje jako obrana proti pastvě zooplanktonem. Bylo však zdokumentováno, že perloočky spásají kolonie sinic a také rozkládají a tráví vláknité druhy a efektivně je využívají jako zdroj potravy, až dokud koncentrace chlorofylu-a nedosáhne zhruba 100násobku hodnot pozorovaných v této práci.

Biomasa zooplanktonu byla redukována predací ryb ve studovaných rybnících a populace velkých perlooček nerostly dostatečně rychle, aby zabránily rozkvětu sinic. Kontrola shora dolů vyvolaná vysokou hustotou planktonožravých ryb otevřela prostor pro nekontrolovaný růst fytoplanktonu.

Role fosforu v nárůstu fytoplanktonu byla našimi studiemi potvrzena. Jelikož sinice fixují anorganický uhlík i molekulární dusík rozpuštěný ve vodě, není koncentrace NH_4-N a NO_3-N ve vodě kritická pro jejich množení. Vysoká koncentrace fosforu tak snižuje relevanci stechiometrických poměrů C : P (uhlík : fosfor) a N:P (dusík : fosfor) v hypertrofním systému, kterému dominují sinice. Údaje získané při řešení této práce naznačují, že eutrofizaci vodních útvarů nelze kontrolovat snížením přísunu dusíku. Rozpustný dusík ve studovaných rybnících téměř chyběl.

Parametry řídící sezónní dynamiku planktonu v hypertrofních rybnících mohou být upraveny koncentrací fosforu a hustotou rybí obsádky. Maximalizace efektivity produkce ryb a přenosu fosforu zachyceného fytoplanktonem přes zooplankton při hustotě ryb pod 300 kg ha^{-1} by bylo jednoduchým opatřením ke snížení množství sinic a následně ke zlepšení kvality vody. Řízení hustoty obsádky ryb může zlepšit kvalitu rybníků a zmírnit květy sinic bez těžko dosažitelného snížení přísunu fosforu.

Zkoumali jsme také diverzitu sinic v dosud neprozkoumané aljašské oblasti zvané North Slope. Objevili jsme dva nové rody a šest nových druhů sinic, což naznačuje existenci vysoké biodiverzity v extrémních polárních prostředích. Tento výzkum poskytuje základ pro další podrobné ekologické, biogeografické a genomické studie.

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List of publications

Peer-reviewed journals with IF

Strunecký, O., **Ivanova, A.P.**, Mareš, J., 2023. An updated classification of cyanobacterial orders and families based on phylogenomic and polyphasic analysis. Journal of Phycology 59: 12–51. (IF 2021 = 3.173)

Ivanova, A.P., Vrba, J., Potužák, J., Regenda, J., Strunecký, O., 2022. Seasonal development of phytoplankton in South Bohemian fishponds (Czechia). Water 14: 1979. (IF 2021 = 3.53)

Ivanova, A.P., Vrba, J., Svitok, M., Potužák, J., Regenda, J., Strunecký, O., 2022. Mechanisms of plankton succession in hypertrophic ponds. Contemporary Problems of Ecology (IF 2021 = 0.732) (accepted)

Prokešová, M., Bušová, M., Zare, M., Tran Quang, H., Kučerová, E., **Ivanova, A.P.**, Gebauer, T., Stejskal, V., 2021. Effect of humic substances as feed additive on the growth performance, antioxidant status, and health condition of African catfish (*Clarias gariepinus*, Burchell 1822). Animals 11: 2266. (IF 2020 = 2.752)

Strunecký, O., Raabová, L., Bernardová, A., **Ivanova, A.P.**, Semanová, A., Crossley, J., Kaftan, D., 2020. Diversity of cyanobacteria at the Alaska North Slope with description of two new genera: *Gibliniella* and *Shackletoniella*. FEMS Microbiology Ecology 96: fiz189. (IF 2019 = 3.675)

Manuscripts

Dvořáková Prokešová, M., Korytář, T., Gebauer, T., Bušová, M., Pojezdal, L., Lieke, T., Tran Quang, H., Ferrocino, I., Franciosa, I., Zare, M., **Ivanova, A.P.,** Minářová, H., Reschová, S., Čížek, A., Stejskal, V., Performance and immunological response of rainbow trout, *Oncorhynchus mykiss* (Walbaum, 1792) juveniles fed with leonardite humic substances. Aquacuture (submitted)

Training and supervision plan during study

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Ph.D. courses	**Year**
Basic of scientific communication	2017
Pond aquaculture	2017
Applied hydrobiology	2017
English language	2018
Ichthyology and fish taxonomy	2020
Principles of experimental methods in biology	2020

Scientific seminars	**Year**
Seminar days of RIFCH and FFPW	2017 2018 2019 2020

International conferences	**Year**
Ivanova A.P., Vrba J., Svitok M., Regenda J., Strunecký O. Seasonal succession of plankton in hypertrophic ponds Is constrained by fish density and available phosphorus. Aquaculture Europe 2021, Funchal, Madeira	2021

Foreign stays during Ph.D. study at RIFCH and FFPW	**Year**
Centre for prorein Engineering, Institute of Chemistry, University of Liege, Liege, Belgium	2018

Pedagogical activities	**Year**
Consultant of master thesis of Bc. Martin Svoboda entitled "DIVERZITA AKVATICKÝCH A AEROFYTNÍCH SINICOVÝCH SPOLEČENSTEV".	2020–2022

Curriculum vitae

PERSONAL INFORMATION

Name: Anna
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EDUCATION

2009 – 2014 Master of Bioecology, M. Akmullah Bashkir State Pedagogical University, Ufa, Russia.

2016 – present Ph.D. student at the University of South Bohemia in České Budějovice, Faculty of Fisheries and Protection of Waters, Laboratory of Controlled Reproduction and Intensive Fish Culture, Czech Republic.

COMPLETED COURSES

Basic of scientific communication, Ichthyology and fish taxonomy, pond aquaculture, English language, Statistical course, Hydrobiology, Principles of experimental methods in biology

RESEARCH STAY AND COLLABORATIONS

10.11. – 9.12.2018 Centre for prorein Engineering, Institute of Chemistry, University of Liège, Belgium