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THE EFFECT OF FISH PRODUCTION AND ENVIRONMENTAL FACTORS ON PHYTOPLANKTON IN HYPERTROPHIC FISHPONDS

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Abstract

Fishponds form substantial part of standing water ecosystems in the landscape of the Central Europe. We studied the effects of fish production and environmental parameters on phytoplankton in fifteen fishponds of various size, fish production and situated at different altitudes. Water and plankton samples were collected from April to October 2018 and 2019. Phytoplankton abundance, zooplankton biovolume, total phosphorus, total nitrogen, ammonia-nitrogen, nitrites, nitrates, phosphates, and total iron concentration were determined. Based on average values of total nitrogen (8.53 $mg.l^{-1}$), total phosphorus (0.399 $mg.l^{-1}$), and chl-a (180 $\mu g.l^{-1}$) all fishponds were classified as hypertrophic. Fish production was significantly correlated only with altitude. With increasing altitude, fishponds have a lower nutrient content, lower temperature, and hence lower production. The direct effect of fish production on phytoplankton was not observed. Two environmental parameters significantly explained the variability in phytoplankton – altitude and total iron concentration. Our results indicate that besides traditionally monitored parameters like nitrogen and phosphorus concentration, the attention should also be focused on other factors potentially affecting studied ecosystems, hypertrophic fishponds.

Keywords: phytoplankton, altitude, iron concentration, fishpond, fish production

INTRODUCTION

Fishponds represent the most numerous types of stagnant water in the Czech Republic. Even though their primary function used to be fish production, fishponds have different functions, such as recreation, nature protection, water retention and water cycle (Popp *et al.*, 2019).

Intensified management of fish production in the second half of the 20th century, including fertilization, manuring, liming and supplementary feeding, followed by increased stocking density, has led not only to the increase in fish production but also to significant eutrophication of fishponds in the Czech Republic (Pechar, 2000). Fishery management itself is just one of the sources of nutrient input to fishponds. Inadequate melioration measures of arable land and erosion in the watershed also affected the water quality of fishponds and significantly contribute to eutrophication (Moss, 2008). In recent years, wastewater treatment plants (WWTP) have brought additional problems. Even though a lot of WWTPs in small villages in the Czech Republic were constructed within the last decades, the level of organic substances and nutrients in the recipient catchments is still very high (Langhammer and Rödlová, 2013). With prolonged dry periods, especially during the summer season, and a small capability of dilution, inflow into the fishpond can consist exclusively of WWTP effluents in some cases.

Due to fishpond eutrophication, the seasonal dynamic of plankton communities has changed, and cyanobacterial blooms have become a regular phenomenon in the summer months. The phytoplankton development depends on different abiotic and biotic factors. Most of the studies emphasize limiting nutrients (phosphorus and nitrogen) as the main factor affecting the phytoplankton assemblage. For a long time, phosphorus was considered a limiting factor for phytoplankton growth, especially for the occurrence of cyanobacterial water blooms (O'Neil *et al.*, 2012). However, recent studies have shown that different forms of nitrogen determine the development of phytoplankton, especially in shallow water bodies. The denitrification process in eutrophic and hypertrophic ponds results in low nitrogen concentrations and favours the appearance of nitrogen-fixing cyanobacteria (Ivanova *et al.*, 2022). There are probably other important factors influencing phytoplankton development. Different environmental parameters can have different impacts in shaping phytoplankton at different temporal and spatial scales (Özkan *et al.*, 2013). This is especially pronounced in nutrient-rich ecosystems.

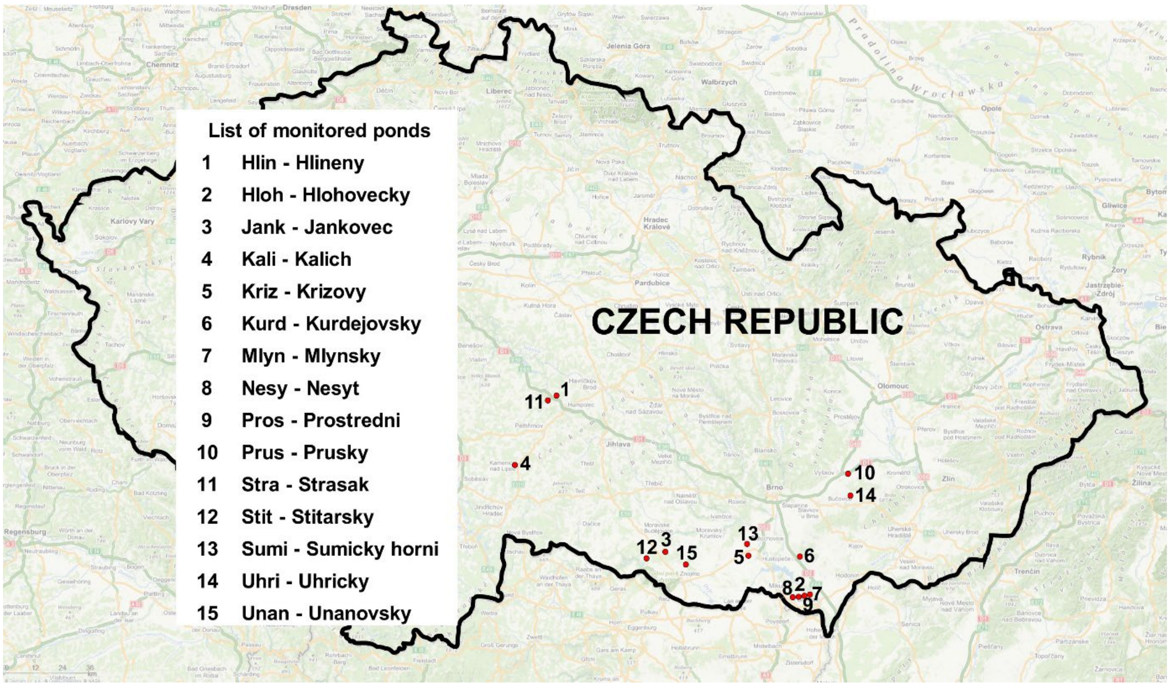
As mentioned before, one of the causes of fishpond eutrophication and deterioration of water quality is also the high density of stocked fish. Fish with different feeding strategies can have different effects on the phytoplankton: direct (herbivorous and omnivorous) and indirect (regulation of phytoplankton by zooplankton consumption) (Komárková, 1998). Dominant fish species in fishponds in the Czech Republic is usually the common carp (*Cyprinus carpio*, L.) (Adámek *et al.*, 2012; Eurostat, 2022) which is able to alter both the abiotic and biotic factors (Rahman, 2015a; Adámek *et al.*, 2016). It is generally accepted that carp is omnivorous fish. With its forage activities, it can have a positive impact on the development of phytoplankton by releasing nutrients from sediment through bioturbation (bottom-up effect). On the other hand, carp also can have strong grazing pressure on zooplankton (top-down effect). Juvenile and small-size carp favour feed on zooplankton (Rahman *et al.*, 2009). The low availability of benthic invertebrates can shift preferences in feed of large carp to zooplankton (Adámek *et al.*, 2016). Moreover, excessive carp growth can lead to an imbalance of the entire ecosystem (Rahman, 2015b).

The main goal of this study was to reveal (i) whether the fish production can directly affect phytoplankton, and (ii) what are the main factors explaining the phytoplankton development in hypertrophic fishponds.

MATERIALS AND METHODS

Studied Fishponds

In total, 15 different shallow fishponds of various sizes (area 1.8–250 ha) used mainly for carp production (yearly production 28–1271 kg.ha^{-1}) were selected for the study (Fig. 1). Fishponds are situated at altitudes from 163 to 557 m (Tab. I). The fishponds were also distinguished based on their geology and the presence of wastewater treatment plants above the studied ponds. Fishpond geological information was described using map server of the Czech Geological Survey (https://mapy.geology.cz/geocr50/) where the main representation of rocks was estimated.



1: *Location of the studied fishponds within the Czech Republic*

I: *Basic parameters of fishponds*

Fishpond code	Altitude (m)	Area (ha)	Geology	Fish production 2018 ($kg.ha^{-1}$)	Fish production 2019 ($kg.ha^{-1}$)	Zooplankton biovolume 2018 ($mm^3.dm^{-3}$)	Zooplankton biovolume 2019 ($mm^3.dm^{-3}$)
Hlin	557	2.8	gneiss	350	200	6.4 ± 4.53	17.72 ± 8.1
Hloh	168	94	calcareous clay and mudstone	445	28	5.55 ± 3.32	21.13 ± 11.17
Jank	362	4.54	migmatite	330	66	6.93 ± 3.9	17.14 ± 16.33
Kali	538	10.6	gneiss	477	477	9.2 ± 7.79	11.95 ± 9.1
Kriz	185	23.85	calcareous clay and mudstone	920	899	1.9 ± 1.04	115.81 ± 248.31
Kurd	199	6.58	calcareous clay and mudstone	745	398	5.53 ± 3.46	82.76 ± 132.35
Mlyn	163	100	clays and sands	565	445	4.97 ± 1.27	19.26 ± 29.88
Nesy	172	250	calcareous clay and mudstone	142	268	9.6 ± 5.59	126.35 ± 194.44
Pros	165	45	clays and sands	625	648	2.43 ± 1.42	36.72 ± 36.69
Prus	237	11.25	calcareous clay and mudstone	284	1271	3.2 ± 3.54	28.02 ± 23.52
Stra	438	1.8	gneiss	200	179	9.4 ± 5.43	29.3 ± 18.97
Stit	393	6.27	migmatite	288	160	6.37 ± 2.85	17.7 ± 16.84
Sumi	193	18.34	calcareous clay and mudstone	390	700	4.4 ± 0.85	47.72 ± 48.2
Uhri	255	13.11	calcareous clay and mudstone	57	214	19.1 ± 11.03	7.36 ± 4.38
Unan	275	3.07	granite and granodiorite	625	121	7.25 ± 0.21	19.63 ± 12.69

Laboratory Analyses

Water and plankton parameters were collected from April to October 2018 and 2019. All samples were collected in the outlet area. Water temperature, oxygen content, pH (all measured using Hach HQ 40D, Hach–Lange, USA), conductivity (Conmet 1, Hanna Instruments) and transparency (Secchi disk) were measured in situ. Water for chemical analyses was collected using a plastic bottle (volume of 1 l), 10 cm below the surface. Total phosphorus (TP), total nitrogen (TN), ammonia-nitrogen ($N\text{-}NH_4$), nitrite-nitrogen ($N\text{-}NO_2$), nitrate-nitrogen ($N\text{-}NO_3$), phosphate-phosphorus ($P\text{-}PO_4$), and total iron (TFe) were analysed according to Horáková (2007). Chlorophyll-a (chl-a) was determined spectrophotometrically following the ethanol extraction, according to Lorenzen (1967).

Samples for phytoplankton determination were collected using a plankton net (mesh size 20 µm) and analysed in their native state. Samples for the analyses of phytoplankton abundance were collected using plastic containers from a depth 10 cm below the surface and fixed with Lugol's solution. Samples were concentrated by ultrafiltration equipment (Marvan, 1957). Enumeration of cyanobacteria and algae cells was done using a Bürker chamber under the optical microscope Olympus BX51.

Samples for zooplankton analyses were collected by horizontal tows, using a plankton net mesh size 40 µm and preserved with formaldehyde to the final concentration of 4% in the sample. The sedimentation method for biovolume determination was used to evaluate the quantity of zooplankton. The collected zooplankton sample was poured over a 20 µm sieve to remove formaldehyde and quantitatively transferred to the graduated cylinder of the appropriate volume (typically 10 or 25 ml according to plankton biomass in the sample). The biovolume was recorded after 24 hours and recalculated to $mm^3.dm^{-3}$.

Statistical Analyses

Spearman rank order correlations of annual averages were used to determine univariate relationships among all the studied parameters. Correlations were also used for preselection of explanatory variables for multivariate analysis. The redundancy analysis (RDA) was used to determine relationships between phytoplankton taxonomical groups and environmental parameters. Explanatory variables were transformed by log (x + 1). The final selection of used explanatory variables was done using the forward selection procedure. The RDA was done in hierarchical design with the particular fishpond forming the whole plot. The annual averages of both the studied years were used as split-plots. Whole plots were freely permuted between each other while split plots were not allowed to permute. The RDA analysis was done using Canoco 5.15 (ter Braak and Šmilauer, 2018), Spearman correlations using Statistica 13 (TIBCO Software Inc. 2020).

RESULTS

Environmental Parameters

The summary of measured environmental variables indicates a large variability among the fishponds, and between both the studied years (Tab. II). Based on the very high average values of TN (8.53 $mg.l^{-1}$), TP (0.399 $mg.l^{-1}$), and chl-a (180 $\mu g.l^{-1}$) fishponds can be characterized as hypertrophic. Values of all parameters varied throughout the season. Chl-a peaked in the July–September period, while other factors varied without a clear pattern throughout the vegetation season. The average nutrient concentrations in 2018 (TN – 6.32 $mg.l^{-1}$, TP – 0.370 $mg.l^{-1}$, N-NH_4 – 0.19 $mg.l^{-1}$, N-NO_2 – 0.013 $mg.l^{-1}$, N-NO_3 – 0.14 $mg.l^{-1}$ and P-PO_4 – 0.079 $mg.l^{-1}$) were lower than in 2019 (TN – 10.75 $mg.l^{-1}$, TP – 0.427 $mg.l^{-1}$, N-NH_4 – 0.27 $mg.l^{-1}$, N-NO_2 – 0.022 $mg.l^{-1}$, N-NO_3 – 0.91 $mg.l^{-1}$ and P-PO_4 – 0.173 $mg.l^{-1}$), while chl-a was higher in 2018 (219.3 $\mu g.l^{-1}$) compared to 2019 (140.7 $\mu g.l^{-1}$).

Chl-a as an indicator of primary production was strongly negatively correlated with altitude and positively correlated with conductivity and TN. Altitude was negatively correlated with most of the analysed parameters. On the other hand, fish production correlated only with altitude (Tab. III).

Phytoplankton Community

As observed for the chemical parameters, phytoplankton abundance also varied among fishponds and between years. In majority of the fishponds the cyanobacterial water bloom occurred at least in one of the studied years. The cyanobacterial bloom was not observed only in the Unan fishpond. The abundance of cyanobacteria during water bloom varied from 1×10^5 up to 11×10^6 $cells.ml^{-1}$ (Tab. IV). The peak of Cyanobacteria occurred from July to September in both years. The dominant species of Cyanobacteria were filamentous taxa *Aphanizomenon, Dolichospermum, Cylindrospermopsis raciborskii, Raphidiopsis mediterranea, Sphaerospermopsis aphanizomenoides, Cuspidothrix issatschenkoi, Anabaenopsis, Pseudanabaena limnetica, Planktothrix aghardii,* and *Limnothrix redekei.* In some of the fishponds, coccal Cyanobacteria (mainly *Microcystis*) were dominant during the summer months. In fishponds Hloh, Pros and Mlyn, cyanobacterial water bloom was also recorded in April 2018, at the beginning of the growing season, with *Pseudanabaena limnetica* as the dominant species (8.5×10^6, 2.7×10^6 and 9.1×10^6 $cells.ml^{-1}$) alongside abundant *Limnothrix redekei*.

Chlorophyta were present in all fishponds throughout the growing season in different quantities. The highest abundance was recorded in Kurd in October 2018 (8.73×10^5 $cells.ml^{-1}$). In some fishponds, Chlorophyta were the most abundant group at the peak of phytoplankton development in May (Stit 2018, Sumi 2018 and 2019), July (Unan 2018), September (Hlin 2019) or October (Kurd 2018). The main representatives of Chlorophyta were *Scenedesmus, Desmodesmus, Kirchneriella, Crucigeniella, Dyctiosphaerium, Tetrastrum,* and *Monoraphidium*. Representatives of other groups were present in different abundances. During the June–September period their presence was negligible, when compared with Cyanobacteria. They were more abundant only at the beginning and the end of the season, and in the fishponds with no algal blooms.

The RDA analysis of taxonomic groups revealed two environmental factors explaining the variability of phytoplankton, altitude, and concentration of total iron (TFe). Altitude explained 13.8% of variability (pseudo-F = 4.5, P = 0.001) and was positively correlated with the presence of Chrysophyceae, Dinophyta and Euglenophyta, and negatively with Chlorophyta and filamentous Cyanobacteria (Fig. 2A). The TFe explaining 11.6% of variability (pseudo-F = 4.2, P = 0.002) was positively correlated with Bacillariophyceae, Xantophyceae, coccal Cyanobacteria and Euglenophyta (Fig. 2A).

DISCUSSION

Water quality, plankton communities and fish production in fishponds are interrelated and affected by interactions of both the abiotic and biotic factors, and fishery management.

Our results show that fish production was significantly correlated with the only one parameter, altitude. Fishponds at higher altitudes have a lower nutrient content, lower temperature, and hence lower fish production. The most abundant phytoplankton taxa in all the studied fishponds were cyanobacteria and chlorophytes, which are commonly found in eutrophic and hypertrophic waterbodies (Borics *et al.*, 2000; Dembowska *et al.*, 2018).

Surprisingly, our study did not reveal relation effects of fish production on primary producers. Chumchal and Drenner (2004) observed a high phytoplankton abundance in a mesocosm experiment with stocked carp and high phosphorus concentration compared to a carp-free mesocosm. Tátrai *et al.* (1997) studied Hungarian fishponds stocked with carp, bream, white bream and roach with production higher than 500 $kg.ha^{-1}$. They found that fish biomass enhanced the phytoplankton growth, which consequently lead to a change in algal species composition towards larger-sized cyanobacteria. In addition to carp, which is a dominant species in the studied fishponds, the influence of other fish (invasive fish, white fish, II: *Average values (± SD) of selected parameters. N – number of samplings in a given year*

Fishpond	Year (N)	TN (mg.l⁻¹)	TP (mg.l⁻¹)	TFe (mg.l⁻¹)	Chlorophyll a (µg.l⁻¹)	$N\text{-}NH_4$ (mg.l⁻¹)	$N\text{-}NO_3$ (mg.l⁻¹)	$N\text{-}NO_2$ (mg.l⁻¹)	$P\text{-}PO_4$ (mg.l⁻¹)	Transparency (cm)	Conductivity (mS.m⁻¹)
Hlin	2018 (5)	4.17 ± 0.89	0.062 ± 0.047	1.07 ± 0.4	55.89 ± 30.1	0.03 ± 0.06	0.26 ± 0.58	0.014 ± 0.031	0.01 ± 0.007	77 ± 9.75	49.4 ± 1.4
	2019 (6)	7.32 ± 4.99	0.064 ± 0.028	0.43 ± 0.31	40.7 ± 33.34	0.04 ± 0.02	3.11 ± 4.67	0.023 ± 0.028	0.014 ± 0.018	129.17 ± 47.16	51.0 ± 1.3
Hloh	2018 (7)	8.97 ± 1.66	0.237 ± 0.088	0.31 ± 0.17	243.57 ± 110.5	0.06 ± 0.09	0.01 ± 0.03	< 0.001	0.04 ± 0.05	30 ± 4.63	129.5 ± 5.0
	2019 (6)	14.87 ± 5.45	0.793 ± 0.297	0.45 ± 0.36	211.39 ± 234.15	0.5 ± 0.61	0.09 ± 0.12	0.02 ± 0.033	0.319 ± 0.425	64.17 ± 57.05	145.7 ± 16.4
Jank	2018 (7)	3.02 ± 1.69	0.286 ± 0.188	1.5 ± 0.7	80.55 ± 72.88	0.42 ± 0.37	0.04 ± 0.11	0.009 ± 0.02	0.152 ± 0.135	93.57 ± 85.28	47.9 ± 3.7
	2019 (5)	6.81 ± 2.98	0.173 ± 0.087	0.47 ± 0.16	62.16 ± 25.4	0.04 ± 0.02	0.24 ± 0.2	0.003 ± 0.002	0.043 ± 0.037	59 ± 17.1	48.0 ± 2.1
Kali	2018 (6)	2.08 ± 0.43	0.062 ± 0.068	0.49 ± 0.2	36.96 ± 35.08	0.04 ± 0.04	0.38 ± 0.49	0.018 ± 0.028	0.032 ± 0.051	106.67 ± 26.58	17.7 ± 0.9
	2019 (5)	4.52 ± 2.22	0.096 ± 0.05	0.21 ± 0.09	52.39 ± 37.71	0.04 ± 0.01	2.31 ± 3.15	0.016 ± 0.014	0.013 ± 0.01	86 ± 39.75	16.4 ± 0.4
Kriz	2018 (7)	4.48 ± 2.15	0.284 ± 0.131	0.83 ± 0.46	229.4 ± 186.9	0.03 ± 0.02	0.08 ± 0.13	0.001 ± 0.002	0.028 ± 0.025	39.29 ± 19.24	110.8 ± 6.7
	2019 (6)	11.91 ± 5.9	0.357 ± 0.148	0.26 ± 0.11	131.97 ± 129.21	0.83 ± 1.8	0.13 ± 0.16	0.004 ± 0.005	0.111 ± 0.193	72.5 ± 56.1	103.7 ± 3.0
Kurd	2018 (8)	7.34 ± 2.47	0.181 ± 0.094	0.49 ± 0.19	113.04 ± 97.96	0.19 ± 0.22	< 0.01	0.011 ± 0.028	0.059 ± 0.052	61.25 ± 43.32	257.0 ± 7.3
	2019 (5)	10.51 ± 5.38	0.146 ± 0.084	0.21 ± 0.14	51.21 ± 47.75	0.23 ± 0.22	0.13 ± 0.08	0.021 ± 0.032	0.036 ± 0.052	114 ± 79.87	253.6 ± 13.0
Mlyn	2018 (6)	9.27 ± 2.62	0.325 ± 0.168	0.71 ± 0.33	340.89 ± 243.43	0.05 ± 0.03	0.01 ± 0.02	0.001 ± 0.002	0.018 ± 0.013	23.33 ± 10.8	136.1 ± 9.8
	2019 (5)	15.67 ± 7.29	0.187 ± 0.043	0.1 ± 0.05	88.8 ± 35.57	0.11 ± 0.1	0.08 ± 0.12	0.004 ± 0.006	0.007 ± 0.003	39 ± 15.17	149.1 ± 6.0
Nesy	2018 (7)	9.36 ± 2.48	0.427 ± 0.38	0.5 ± 0.22	381.63 ± 439.63	0.97 ± 2.04	0.06 ± 0.16	0.011 ± 0.026	0.06 ± 0.083	51.43 ± 47.67	139.1 ± 16.0
	2019 (6)	14.89 ± 5.69	0.481 ± 0.256	0.31 ± 0.29	273.8 ± 225.35	0.33 ± 0.46	0.07 ± 0.07	0.01 ± 0.014	0.213 ± 0.346	55 ± 37.42	147.6 ± 8.8
Pros	2018 (7)	8.96 ± 2.36	0.153 ± 0.075	0.26 ± 0.13	203.61 ± 104.26	0.02 ± 0.01	0.01 ± 0.02	0.001 ± 0.002	0.012 ± 0.008	32.86 ± 8.09	132.7 ± 6.9
	2019 (6)	15.22 ± 6.29	0.458 ± 0.222	0.38 ± 0.35	249.38 ± 209.14	1.02 ± 1.14	0.16 ± 0.16	0.03 ± 0.041	0.116 ± 0.17	40.83 ± 28	127.9 ± 8.4
Prus	2018 (6)	6.31 ± 2.76	0.211 ± 0.107	1.42 ± 1.25	224.07 ± 198.74	0.69 ± 1.62	0.04 ± 0.06	0.019 ± 0.047	0.02 ± 0.008	40.83 ± 22.45	716.17 ± 2.3
	2019 (6)	10.37 ± 5.72	0.702 ± 0.415	1.26 ± 1.09	427.65 ± 234.52	0.31 ± 0.36	0.1 ± 0.12	0.017 ± 0.02	0.202 ± 0.422	20.83 ± 6.65	686.33 ± 5.3
Stra	2018 (6)	4.63 ± 1.96	0.371 ± 0.36	1.26 ± 0.83	324.61 ± 288.45	0.02 ± 0.04	0.57 ± 1.36	0.028 ± 0.067	0.016 ± 0.004	37.5 ± 26.41	22.7 ± 2.7
	2019 (6)	10.36 ± 7.62	0.153 ± 0.097	0.23 ± 0.17	101.21 ± 69.08	0.04 ± 0.02	4.84 ± 6.19	0.068 ± 0.08	0.018 ± 0.021	56.67 ± 28.75	29.4 ± 3.6
Stít	2018 (7)	4.66 ± 1.68	0.31 ± 0.148	2.86 ± 1.27	146.94 ± 25.5	0.01 ± 0.02	0.03 ± 0.08	< 0.001	0.027 ± 0.019	22.86 ± 6.99	76.1 ± 5.1
	2019 (6)	9.37 ± 4.23	0.424 ± 0.2	1.57 ± 0.85	194.79 ± 83.34	0.04 ± 0.01	0.08 ± 0.06	0.002 ± 0.001	0.074 ± 0.112	25 ± 5.48	73.9 ± 6.5
Sumi	2018 (6)	5.46 ± 1.09	0.578 ± 0.503	0.59 ± 0.22	202.76 ± 60.98	0.01 ± 0.01	0.26 ± 0.4	0.026 ± 0.051	0.128 ± 0.107	36.67 ± 12.11	70. ± 3.8
	2019 (5)	10.26 ± 5.47	0.295 ± 0.09	0.3 ± 0.16	138.23 ± 53.86	0.1 ± 0.09	0.87 ± 1.12	0.02 ± 0.024	0.051 ± 0.059	38 ± 5.7	73.1 ± 3.3
Uhri	2018 (5)	10.59 ± 8.78	1.203 ± 0.953	1.33 ± 0.66	549.67 ± 575.86	0.03 ± 0.03	0 ± 0.01	0.002 ± 0.003	0.116 ± 0.087	27 ± 41.47	94.8 ± 15.1
	2019 (6)	4.22 ± 3.05	0.252 ± 0.2	0.63 ± 0.45	60.98 ± 65.55	0.1 ± 0.03	0.22 ± 0.37	0.002 ± 0.002	0.078 ± 0.109	65.83 ± 43.98	173.3 ± 30.2
Unan	2018 (7)	5.48 ± 1.52	0.862 ± 0.466	1.08 ± 0.41	156.25 ± 116.75	0.23 ± 0.15	0.32 ± 0.42	0.057 ± 0.047	0.467 ± 0.277	35 ± 8.66	96.4 ± 8.6
	2019 (5)	14.89 ± 8.33	1.828 ± 0.586	0.13 ± 0.05	25.46 ± 29.92	0.34 ± 0.56	1.27 ± 1.6	0.089 ± 0.13	1.301 ± 0.328	164 ± 85.83	93.0 ± 11.5

III: *Correlation matrix of fish production, plankton, altitude, and physico-chemical parameters of water (Spearman rank order correlations). Fish – fish production (kg.ha^{-1}), Zoopl. – zooplankton biovolume (mm^3.dm^{-3}), Temp – water temperature, DO – dissolved oxygen concentration, Cond – conductivity, TN – total nitrogen, TFe – total iron, Chl-a – chlorophyll-a concentration. Significant correlations are in bold.*

	Altitude	Fish	Zoopl.	Temp.	DO	pH	Cond.	TN	TFe	Chl-a	N-NH_4	N-NO_2	P-PO_4	DIN:TDP
Altitude	1.00													
Fish	**-0.36**	1.00												
Zoopl.	-0.04	-0.16	1.00											
Temp.	**-0.61**	0.06	0.01	1.00										
DO	**0.34**	-0.02	-0.12	**-0.39**	1.00									
pH	-0.30	-0.02	-0.03	0.02	**0.36**	1.00								
Cond.	**-0.72**	0.11	0.06	**0.64**	**-0.43**	0.23	1.00							
TN	**-0.52**	-0.01	**0.64**	**0.40**	-0.19	**0.37**	**0.61**	1.00						
TFe	0.29	-0.13	**-0.45**	0.11	**-0.38**	**-0.37**	-0.15	**-0.44**	1.00					
Chl-a	**-0.49**	0.07	-0.08	**0.38**	**-0.35**	0.28	**0.45**	**0.49**	0.27	1.00				
N-NH^4	**-0.36**	0.03	**0.39**	0.29	**-0.32**	-0.04	**0.34**	**0.34**	-0.18	0.02	1.00			
N-NO^2	0.23	-0.10	0.29	-0.06	0.01	-0.14	-0.18	0.17	-0.15	-0.10	0.23	1.00		
P-PO^4	-0.15	-0.12	0.24	0.28	**-0.67**	-0.17	**0.39**	**0.38**	0.22	**0.39**	**0.34**	0.27	1.00	
DIN:TDP	0.28	-0.03	0.32	**-0.42**	**0.45**	-0.09	**-0.39**	-0.05	**-0.34**	-0.29	0.15	**0.36**	**-0.49**	1.00

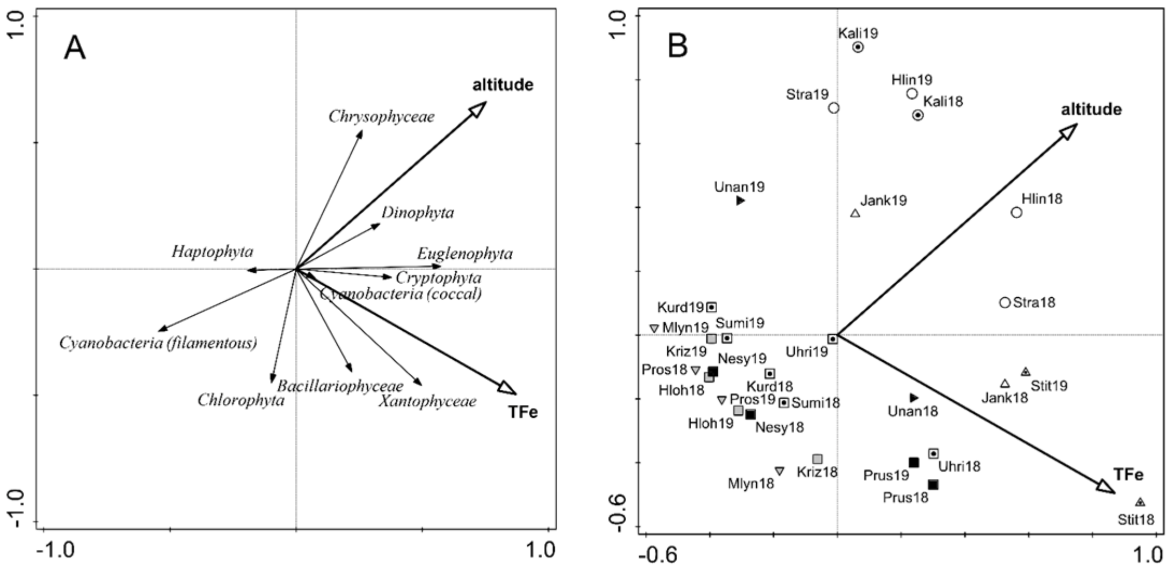
IV: *The average values of phytoplankton abundance in the fishponds during the growing season. The category "Others" comprises of Dinophyta, Chrysophyceae, Xantophyceae and Haptophyta. N – number of samplings in a given year.*

Fishpond	Year (N)	Cyanobacteria filamentous	Cyanobacteria coccal	Cryptophyta	Bacillariophyceae	Euglenophyta	Chlorophyta	Others
		Number of cells per ml						
Hlin	2018 (5)	36,696	24,321	125	2,000	4,643	14,250	4,750
	2019 (6)	250	924	1,500	278	510	26,816	6,333
Hloh	2018 (7)	1,904,003	13,065	0	4,911	446	21,920	0
	2019 (6)	597,656	25,000	0	11,002	807	11,125	1,302
Jank	2018 (7)	18,482	804	1,649	1,528	801	5,050	45
	2019 (5)	12,917	39,667	1,608	497	481	12,297	1,861
Kali	2018 (6)	41,752	36,782	365	2,313	813	3,004	104
	2019 (5)	83,768	70,991	2,009	5,232	1,188	10,661	688
Kriz	2018 (7)	241,598	150,830	2,589	4,619	900	21,387	618
	2019 (6)	571,626	0	3,137	1,134	153	19,225	116
Kurd	2018 (8)	244,807	5,964	2,005	327	156	113,642	0
	2019 (5)	111,131	750	351	150	238	20,259	119
Mlyn	2018 (6)	1,372,917	1,901	391	3,906	651	86,932	0
	2019 (5)	898,438	1,250	250	438	0	23,938	0
Nesy	2018 (7)	982,282	10,145	1,089	13,315	0	27,614	0
	2019 (6)	918,469	4,740	17	838	712	11,244	0
Pros	2018 (7)	2,714,851	62,470	893	2,738	0	12,202	0
	2019 (6)	39,271	163,727	347	4,063	608	60,891	0
Prus	2018 (6)	23,552	83,113	1,713	2,407	2,616	62,831	3,588
	2019 (6)	130,853	220,159	6,094	7,545	9,087	31,994	1,042
Stra	2018 (6)	173,061	19,485	266	961	58	5,000	52
	2019 (6)	235,685	54,688	2,418	3,564	2,500	17,649	3,609
Stit	2018 (7)	1,429	4,286	10,804	8,973	8,482	20,714	536
	2019 (6)	52,300	1,122	2,493	5,268	3,422	24,662	2,103
Sumi	2018 (6)	72,505	48,891	10,337	14,673	1,196	122,232	1,254
	2019 (5)	49,621	84,125	4,375	4,042	0	53,046	904
Uhri	2018 (5)	152,800	1,135,875	4,675	4,525	1,600	91,700	4,875
	2019 (6)	6,896	134	91	892	451	1,340	35
Unan	2018 (7)	786	0	8,929	4,946	241	33,598	89
	2019 (5)	5,313	292	885	156	448	2,458	0

predator fish) cannot be neglected. A change in fish stock can differently influence phytoplankton, often contrary to what was predicted (Komárková, 1998). In addition to the total fish production, detailed data about fish populations (present species, age and size structure) are necessary for a better understanding of fish–phytoplankton relations.

Altitude was highly negatively correlated with chl-a. In the standing waters of the Czech Republic, phytoplankton abundance is generally high at low altitudes characterised by higher average water temperature (Lepšová-Skácelová *et al.*, 2018). Altitude was not only correlated with most of the studied parameters, but significantly affected the phytoplankton assemblage (cf. Fig. 2). Our results also showed that Chrysophyceae and Dinophyta were positively correlated with altitude. Lepšová-Skácelová *et al.* (2018) noted that Chrysophyceae, and in some cases Dinophyta, Cryptophyta and Bacillariophyta were related to higher altitudes and colder seasons. One of the probable causes, in addition to temperature, can be generally lower nutrient content of fishponds located at higher altitudes. Such lower nutrient concentration determines lower phytoplankton abundance (Poulíčková *et al.*, 2003; Lepšová-Skácelová *et al.*, 2018).

We found total iron concentration significantly affecting phytoplankton in our study. Iron has an important function in photosynthetic activity, and the assimilation of nitrogen (Geider and La Roche, 1994). It is well-known that iron can be a limiting factor for phytoplankton growth in oceans (Zhang *et al.*, 2021), but data about freshwater systems are very scarce. Yuan *et al.* (2021) studied the interactive effect of iron and light on phytoplankton assemblage in a eutrophic lake. In their study, Bacillariophyta preferred low iron-light conditions, while Cyanobacteria and Chlorophyta dominated



2: *Results of the redundancy analysis showing correlations between the main phytoplankton taxonomical groups and environmental parameters selected by the forward selection procedure (A) and the position of fishponds within the ordination (B). Fishpond symbols indicate geology: circle – gneiss, square – calcareous clay and mudstone, diamond –calcareous or quartz sandstones, up triangle – migmatite, down triangle clays and sands, right triangle – granite and granodiorite. Fishpond symbol colour represents the absence of the water treatment plant (empty), the presence of the water treatment plant just above the studied fishpond (black) or the outflow from the water treatment plant flow through other fishpond(s) situated above the studied fishpond (grey); communal pollution (black dot inside the symbol).*

at higher iron-light conditions. Furthermore, Sharma *et al.* (2009) showed the negative effect of iron on phytoplankton growth. Even though our study showed a strong relationship between phytoplankton and iron, it should be emphasized that only the total iron concentration was determined. The analysis of the bioavailable iron would be needed for a better explanation of the phytoplankton–iron interaction. Differences in iron concentration between fishponds can depend on the geology of the surrounding area of the fishpond (cf. Fig. 2B). Generally, acidic soils with a higher iron content bind more organic matter, ammoniacal nitrogen and phosphorus to the sediment (Colombo *et al.*, 2014; Fink *et al.*, 2016) which contributes to more stable fishpond ecosystem. On the other hand, neutral and alkaline soils with lower iron concentration release nutrients (Ng *et al.*, 2022) from sediments and hence increases phytoplankton abundance. Iron and its bioavailability appear to be one of the key factors affecting the functioning of the entire fishpond ecosystem including development of phytoplankton.

Water chemistry, including iron concentration, can also be affected by the effluents from wastewater treatment plants and municipal pollution. Nevertheless, our study does not point to a clear difference between these factors.

CONCLUSION

All fifteen fishponds in our study were hypertrophic. The most abundant phytoplankton groups were cyanobacteria and chlorophytes. Cyanobacterial bloom regularly occurred in almost all fishponds. We found a significant correlation between phytoplankton taxonomical groups with both the altitude and the total iron concentrations. Altitude was very important factor in our study, significantly correlated with different parameters, not only with phytoplankton itself. Iron concentration is important factor in phytoplankton development. However, further studies are necessary for better explanation of the iron-phytoplankton relation. Direct effect of fish stocking on the phytoplankton of hypertrophic ponds with carp production has not been detected.

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