

Phytoplankton-zooplankton coupling in a cascade of hypertrophic fishponds

Marija Radojičić,^{1*} Michal Šorf,^{1,2} Barbora Müllerová,¹ Radovan Kopp¹

¹Faculty of AgriSciences, Mendel University in Brno, Brno; ²Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic

ABSTRACT

In Central Europe, a significant proportion of shallow lake ecosystems are represented by artificial fishponds, where phytoplankton and zooplankton are an essential part of the food web. Owing to their high fish stocks and intensive fishery management, most fishponds are now eutrophic or hypertrophic, which has had clear impacts on plankton assemblages. To obtain a better understanding of phytoplankton-zooplankton coupling in fishponds, this study examines their ecological relationships in a cascade of three small fishponds over two subsequent years. In all, 133 phytoplankton taxa were recorded, mostly chlorophytes, diatoms and euglenophytes, and 60 zooplankton taxa. Multivariate analysis revealed dissolved oxygen content, dissolved inorganic nitrogen and biochemical oxygen demand as the main factors influencing phytoplankton biomass, while the most significant environment variables affecting zooplankton composition were dissolved oxygen, phosphate concentrations and conductivity. Co-correspondence analysis revealed a significant relationship between phytoplankton and zooplankton density, with cross-correlations from 76% to 87% within the first four axes. Such a high cross-correlation denotes a strong relationship between phytoplankton and zooplankton, even within the hypertrophic conditions determining fishpond ecosystems.

INTRODUCTION

Artificial fishponds represent a significant proportion of aquatic ecosystems in Central Europe. Phytoplankton and zooplankton represent fundamental components of the fishpond food web, with plankton assemblages, and particularly zooplankton, playing an essential role in aquaculture as their high-quality proteins and lipids

represent a nutritious natural feed source for farmed fish (Anton-Pardo and Adámek, 2015). This is particularly important for juvenile freshwater fish (Kloskowski, 2011; Declerck and de Senerpont Domis, 2023), including common carp (*Cyprinus carpio* Linnaeus, 1758), which is the main farmed fish in the Czech Republic (Adámek *et al.*, 2012). Overall, the nutritional quality of zooplankton as a food source for farmed fish will be largely influenced by the composition of the phytoplankton community (McBeain and Halsey, 2019; Trommer *et al.*, 2019).

Over the 20th century, fishponds in the Czech Republic underwent major changes because of intensification in fisheries management and other external influences, including notable changes in land use and increasing runoff from nearby agricultural areas. Subsequent eutrophication of most fishponds has led to changes in plankton communities, resulting in cyanobacterial blooms and altered zooplankton structure (Pechar, 2000). Furthermore, Williams and Moss (2003) were able to show that increasing fish biomass has had a strong effect on zooplankton size and abundance, changing the zooplankton structure from large-sized to smaller-sized taxa that are generally less efficient in controlling phytoplankton (Ger *et al.*, 2016; Erdoĝan *et al.*, 2021). Similar ‘trophic cascade’ effects have been observed in fishponds in the Czech Republic (Potuĝák *et al.*, 2007) and in other water bodies in Poland (Kozak and Goldyn, 2004) and Germany (Auer *et al.*, 2004). According to Sipaúba-Tavares *et al.* (2011), a similar pattern has also been noted in the tropics, where high rotifer biomass was associated with high cyanobacteria biomass. In this case, the known effects of cyanobacteria on zooplankton, *e.g.*, copepod filtration rate, egg production and mortality

Corresponding author: radojicic.marija88@gmail.com

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(Tillmans *et al.*, 2008; Ger *et al.*, 2016), were amplified by their increased growth rate at higher temperatures, leading to an over-representation of rotifers. Such impacts could be especially important in the face of ongoing climate change, with increasing air and water temperatures already affecting fish farming in temperate areas (Orság *et al.*, 2023).

Numerous studies have shown that a variety of factors can influence mutual relationships between phytoplankton and zooplankton, though nutrient availability is often among the most significant variables affecting the growth of both groups. Total phosphorus has long been considered a limiting nutrient in freshwater ecosystems; however, recent findings have shown that nitrogen limitation also increases with increasing trophic state (Scott *et al.*, 2019). The stability of phytoplankton-zooplankton trophic interactions can also vary with nutrient status, with Pan *et al.* (2014), for example, showing that both low and high nutrient loads can destabilise phytoplankton-zooplankton systems due to nutrient-dependent morphology in phytoplankton.

Interactions between phytoplankton and zooplankton can be highly complex, with zooplankton tending to affect phytoplankton both directly and indirectly. For example, zooplankton feed on phytoplankton, and thus influence phytoplankton composition and size structure directly. The main representatives of crustacean zooplankton have different feeding strategies, with cladocerans being unselective filtrators, though usually consuming smaller prey, whereas calanoid copepods are selective feeders, mainly consuming medium to large phytoplankton. Zooplankton also affect phytoplankton indirectly by recycling nutrients that support phytoplankton growth, a process that is particularly important in oligotrophic ecosystems. A recent study by Butts *et al.* (2022), however, has pointed out that the same process can also be important in driving spring phytoplankton dynamics in hypertrophic reservoirs. Thus, zooplankton-phytoplankton coupling may differ in waters of different trophic status, with coupling often weaker in lakes where productivity is either extremely low or extremely high (Elser *et al.*, 1990), though Yuan and Pollard (2018) have also shown that the ratio of zooplankton to phytoplankton biomass tends to decrease with increasing eutrophication.

In this study, we aimed to i) reveal the main abiotic factors explaining the development of phytoplankton and zooplankton assemblages in a cascade of three small fishponds in Central Europe, and ii) measure the strength of associations between phytoplankton and zooplankton using co-correspondence analysis (CoCA). We hypothesise that coupling between phytoplankton and zooplankton will be weaker under hypertrophic conditions with higher fish stock.

METHODS

Study area

The study site comprised a cascade of three shallow connected fish-farming ponds fed by a stream (Šumice) located near the village of Bohuslavice (Olomouc Region, Czech Republic; 49.6209642N, 16.9737492E), called, for the purposes of this study, Bohuslavice 1 (upstream; area 0.97 ha, average depth 0.9 m; hereafter B1), Bohuslavice 2 (middle; 1.1 ha, 1 m depth; hereafter B2) and Bohuslavice 3 (downstream; 1.2 ha, 1 m depth; hereafter B3) (Fig. 1). In each pond, maximum depth was at the outlet, this being ca. 1.5 m in B1 and 1.8 m in B2 and B3.

All three fishponds are mainly used for breeding juvenile carp, along with smaller numbers of tench (*Tinca tinca* Linnaeus, 1758) and grass carp (*Ctenopharyngodon idella* Valenciennes, 1844), and are stocked in spring and harvested in the autumn (October/November) each year. Unfortunately, data on fish biomass could not be included in this study due to a lack of relevant fish data kept by the fishpond managers.

Free floating duckweeds (*Lemna* spp. and *Spirodela polyrrhiza* (L.) Schleid.) were recorded at the ponds from April to August 2017, along with submerged macrophytes (*Ceratophyllum* sp. and *Myriophyllum* sp.) in B1 and B3 from June to August 2017.

Sampling and analytical methods

Sampling was conducted at the outlet of each fishpond five times in 2016 and eight times in 2017 from April to September. At the same time, water temperature, dissolved

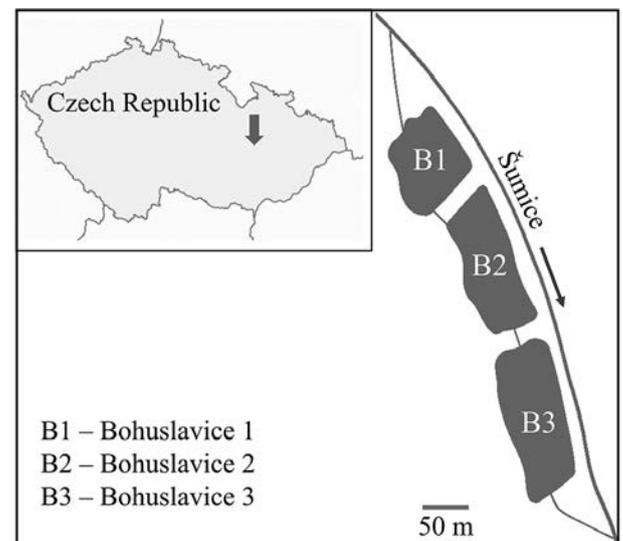


Fig. 1. Map and schematic layout of the fishpond cascade sampled in this study (B1 fishpond first in the cascade).

oxygen concentration (DO) and pH were measured using a HACH HQ40d multimeter (Hach Lange, USA), conductivity using a Hanna Combo HI98130 meter (Hanna Instruments, USA) and transparency using a Secchi disc.

Samples for water chemistry analysis were obtained from 20 cm below the surface using a clean 1 L plastic bottle and transported directly to the laboratory. All chemical analyses were performed according to APHA (1998), with ammonium ions (N-NH₄) determined using the indophenol blue method, nitrite nitrogen (N-NO₂) using the N-(1-Naphthyl)ethylenediamine method and nitrate nitrogen (N-NO₃) using the sodium salicylate method. Dissolved inorganic nitrogen (DIN) was calculated as the sum of N-NH₄, N-NO₂ and N-NO₃, while total nitrogen (TN) was measured using dimethylphenol after transformation of all nitrogen compounds into nitrate using Koroleff's method. Total phosphorus (TP) and orthophosphate (P-PO₄) were determined using ascorbic acid and ammonium molybdate, and biochemical oxygen demand (BOD) using the dilution method. Finally, chlorophyll a concentrations were assessed spectrophotometrically after heated ethanol extraction, according to Lorenzen (1967).

Samples for the quantitative analysis of phytoplankton were collected from a depth of 10 cm using clean 50 mL plastic bottles and preserved using Lugol's solution. The samples were then concentrated using 12 mm diameter and 0.85 µm pore size ultrafiltration membrane filters (Marvan, 1957). Cells were counted in a Bürker chamber and then recalculated to the number of cells per 1 mL. After counting, the wet weight biomass of phytoplankton was calculated according to Hillebrand *et al.* (1999). All phytoplankton were then identified to taxa under an optical microscope and classified into eight taxonomic groups, *i.e.*, Cyanobacteria, Dinophyta, Cryptophyta, Chrysophyceae, Xantophyceae, Bacillariophyceae, Euglenophyta and Chlorophyta, for further analysis.

Zooplankton were collected by horizontal tow using a plankton net with a mesh size of 40 µm and a diameter of 30 cm. The zooplankton thus obtained were preserved with formaldehyde to a final concentration of 4%. In the laboratory, the zooplankton were identified to species level after having been subsampled into an open Sedgwick-Rafter counting chamber and recalculated as number of individuals per 1 L. Except for the genus *Daphnia*, which were divided into juveniles, males and adult females, male and female cladocerans were counted together as males were generally rare. Cyclopoid copepods were separated into adult females (determined to species level), and nauplii (together with calanoid nauplii), copepodites (of all stages together) and males (not determined to species level). Similarly, the only recorded calanoid copepod *Eudiaptomus gracilis* (G. O. Sars, 1862) was categorised as adult male, adult females and copepodites.

Data analysis

Phytoplankton-zooplankton coupling was tested using multivariate analysis, first by determining relationships between phytoplankton and environmental variables, then using constrained ordination methods to assess relationships between zooplankton and environmental variables, and finally by co-correspondence analysis. The environmental variables used were preselected based on Spearman rank order correlations in Statistica 14 (TIBCO Software Inc., 2020), with variables having significant correlations >0.5 excluded (*Tab. S1*). Final selection of environmental variables, transformed by log (x+1), was undertaken using the forward selection procedure. A hierarchical permutation test was then run with the sampling dates at each fishpond (forming a split plot) ordered in time series. All samplings belonging to one of three fishponds (whole plots) were freely permuted among each other. Variation partitioning was then used to reveal the amount of variation attributable to water properties (*i.e.*, DO, conductivity, TP, TN, BOD, P-PO₄, DIN) on the one hand, or zooplankton (total abundance of Cladocera, Copepoda and Rotifera) on the other.

Next, symmetric CoCA was applied to reveal possible associations within phytoplankton and zooplankton species matrices, with phytoplankton species with less than two occurrences excluded. The permutation design used was the same as for canonical correspondence analysis (CCA) and redundancy analysis (RDA). Cross-correlations between four CoCA axes were used as a measure of association between species matrices. Shared case weights were defined as an average of table weights. A dual diagram was constructed to summarise CoCA results with the 20 best-fitting taxa of both phytoplankton and zooplankton. All multivariate analyses were undertaken using Canoco v.5.15 (ter Braak and Šmilauer, 2018).

RESULTS

Environmental parameters

All three hypertrophic fishponds were characterised by a high nutrient content (overall mean TP: 0.25 mg L⁻¹; overall mean TN: 1.77 mg L⁻¹; *Tab. 1*). Both nutrients (TP, TN) and conductivity were generally higher, and DIN lower, in all fishponds in the second year of the study (2017; *Tab. 1*). In B1 and B3, chlorophyll-a was higher in 2016 (mean 81.4 µg L⁻¹; 111 µg L⁻¹, respectively) than 2017 (26.5 µg L⁻¹ and 46.8 µg L⁻¹), but slightly higher in 2017 (48.3 µg L⁻¹) than in 2016 (45.9 µg L⁻¹) in B2. Very low oxygen saturation was recorded in all three ponds in both sampling years, reaching levels below 6% in B3 and B2 from July to September in 2016, and varying between 13 and 37% over the same period in B1. While average DO values in 2017 were higher than those in 2016 (*Tab. 1*),

values below 10% were recorded in all three ponds in June and July. For an overall correlation matrix (Fig. S1).

Phytoplankton

A total of 133 phytoplankton taxa were determined, the most diverse phytoplankton groups being chlorophytes with 59 taxa, followed by diatoms with 22 taxa and Euglenophyta with 18 taxa (Tab. 2). All three ponds showed highest biomass between July and September in both years, when Dinophyta and Euglenophyta dominated. Dinophyta, with *Peridinium* sp. as the main representative, dominated phytoplankton in July 2016 in B3 at 749.7 mg L⁻¹, and August 2017 in B1 at 12.4 mg L⁻¹. Highest phytoplankton biomass was recorded in July 2016 in B1 (52.2 mg L⁻¹), July 2017 in B2 (13.68 mg L⁻¹) and in September 2017 in B3 (23.67 mg L⁻¹), with Euglenophyta (mainly *Trachelomonas* spp.) dominant. The chlorophytes

Crucigeniella spp., *Monoraphidium* spp., *Desmodesmus* spp., *Didymocystis* spp., *Pediastrum* spp., *Planktosphaeria gelatinosa* G.M.Smith 1918, and *Scenedesmus* spp., along with diatom taxa, were present in all three ponds throughout both years at varying densities (Tab. 2). Peaks in representatives of other groups only occurred exceptionally, e.g., Cyanobacteria mainly in September 2016 and April 2017, Chrysophyceae in August and September 2016 in B1 and all three fishponds in the same months of 2017, and Cryptophyta in September 2016 and April and May 2017 in all three ponds.

Zooplankton

A total of 60 zooplankton taxa were recorded (Tab. 3), comprising 40 rotifer, 14 cladoceran and six copepod taxa. The most common cladoceran species was the small-bodied *Bosmina longirostris* O. F. Müller, 1776, with densities up to 2130 ind L⁻¹ in B2. *Ceriodaphnia pulchella*

Tab. 1. Median and range of selected physical and chemical parameters of fishpond water.

Year (n)	B1		B2		B3	
	2016 (5)	2017 (8)	2016 (5)	2017 (8)	2016 (5)	2017 (8)
Water temperature (°C)	19.2 (9.8-21.5)	17.2 (12.9-20.1)	18.3 (10-22.3)	17.7 (12.8-21.4)	18.2 (11.1-22)	17.6 (13.9-21.9)
Dissolved oxygen (%)	27.2 (13-102)	25.3 (5.2-143)	6.1 (4.8-128)	18.9 (5.5-132)	5 (3.6-92)	35.7 (3.2-122)
pH	7.37 (7.19-8.32)	7.59 (6.78-8.85)	7.42 (7.11-8.87)	7.25 (6.48-8.69)	7.42 (7.33-8.28)	7.00 (6.35-8.25)
Conductivity (mS m ⁻¹)	33.4 (28.3-39.9)	39.4 (35.4-42.2)	35.6 (32.9-40.0)	43.5 (42.7-45.8)	35.5 (32.2-44.7)	44.5 (34.6-50.8)
Transparency (cm)	55 (40-140)	100 (45-125)	70 (50-100)	70 (25-100)	80 (30-110)	100 (15-110)
Total nitrogen (mg L ⁻¹)	1.26 (0.8-3.19)	1.75 (1.4-3.2)	0.97 (0.76-1.98)	1.92 (1.28-2.61)	1.3 (0.59-2.13)	2.27 (1.18-2.58)
Dissolved inorganic nitrogen (mg L ⁻¹)	0.001 (0-2.13)	0.121 (0.023-1.09)	0.278 (0.027-1.66)	0.142 (0-0.827)	0.292 (0.001-1.96)	0.207 (0.01-1)
Total phosphorus (mg L ⁻¹)	0.19 (0.08-0.37)	0.22 (0.04-0.65)	0.16 (0.07-0.32)	0.24 (0.04-0.75)	0.23 (0.09-0.39)	0.22 (0.04-0.77)
Phosphate (mg L ⁻¹)	0.028 (0.019-0.037)	0.051 (0.016-0.148)	0.029 (0.023-0.086)	0.035 (0.006-0.093)	0.051 (0.044-0.12)	0.041 (0.005-0.118)
Chlorophyll-a (µg L ⁻¹)	45.9 (0-252)	21.5 (2.96-68.1)	45.9 (0-112)	42.9 (2.96-121)	90.3 (10.4-268)	14.1 (2.96-191)
Biochemical oxygen demand (mg L ⁻¹)	4.7 (3.78-16.3)	4.4 (1.83-6.08)	4.3 (2.31-8.49)	5.16 (3.58-10.6)	9.29 (3.75-16.9)	4.54 (1.47-11.6)

n, number of samplings in a given year; B1, Bohuslavice 1; B2, Bohuslavice 2; B3, Bohuslavice 3.

Tab. 2. Median and range of phytoplankton biomass (wet weight in mg L⁻¹) in fishponds over the 2016 and 2017 growing seasons.

Year (n)	B1		B2		B3	
	2016 (5)	2017 (8)	2016 (5)	2017 (8)	2016 (5)	2017 (8)
Cyanobacteria	0.021 (0-1.57)	0 (0-0.89)	0 (0-0.73)	0 (0-1.02)	0.012 (0-0.69)	0.009 (0-3.73)
Dinophyta	0 (0-0.953)	0 (0-194)	0.476 (0-9.55)	0 (0-16.62)	8.592 (0-750)	0.119 (0-12.4)
Cryptophyta	0.391 (0.1-2.13)	0.531 (0-4.08)	0.228 (0-2.33)	1.45 (0.434-6.33)	1.86 (1.183-3.13)	1.47 (0.049-7.75)
Chrysophyceae	0 (0-9.22)	0.002 (0-2.81)	0.068 (0-0.268)	0.042 (0-6.34)	0 (0-0.25)	0.002 (0-9.63)
Xantophyceae	0.062 (0.011-0.17)	0.002 (0-0.062)	0.031 (0-0.134)	0.01 (0-0.352)	0.005 (0-0.179)	0.002 (0-0.153)
Bacillariophyceae	0.071 (0-0.196)	1.465 (0.246-6.56)	0.012 (0-0.076)	0.708 (0.084-8.06)	0.355 (0-1.847)	1.35 (0.286-3.59)
Euglenophyta	16.32 (0.253-52.2)	3.008 (0.358-14.6)	5.81 (0.143-13.7)	13.35 (0.398-63.6)	23.7 (0.143-64.7)	2.78 (0-27.6)
Chlorophyta	1.382 (0.167-7.16)	0.656 (0.136-2.92)	0.687 (0.002-3.28)	3.003 (0.059-11.9)	1.16 (0.058-3.83)	0.521 (0-3.8)

n, number of samplings in a given year; B1, Bohuslavice 1; B2, Bohuslavice 2; B3, Bohuslavice 3.

Sars, 1862 regularly occurred in June and July in all fishponds, but more abundantly in B3 and B2 in 2017. Four species from the genus *Daphnia* were recorded, with *Daphnia* spp. juveniles, together with adult females of *D. gr. longispina*, the most commonly recorded, with highest occurrence in spring. Copepods were dominated by juvenile stages, which represented a significant proportion of all zooplankton in B2 in both years of the study. The most diverse zooplankton groups were rotifers, with *Anuraeopsis fissa* (Gosse, 1851) (B1), *Brachionus angularis* Gosse, 1851 (B2), *Keratella cochlearis* (Gosse, 1851) (B2 and B1) and *Polyarthra dolichoptera* Idelson, 1925 (B2 and B1) tending to dominate. Colonial rotifers of the family Conochilidae were mainly observed in the spring of 2016.

Relationships between plankton and environmental parameters

CCA analysis of phytoplankton biomass showed that three environmental factors explained 16.5% of total variance, with DO explaining 6.4% (pseudo-F=2.5, $p=0.002$), BOD explaining 5.4% (pseudo-F=2.2, $p=0.002$) and DIN explaining 4.7% (pseudo-F=2.0, $p=0.025$) (Fig. 2, Tab. 4). The chlorophytes *Lagerheimia genevensis* (Chodat) Chodat 1895 and *Chlamydomonas* sp., the diatom *Fragilaria acus* (Kützing) Lange-Bertalot 2000 and centric diatoms, and the cyanobacteria *Aphanizomenon* sp. and *Oscillatoria* sp. were all positively correlated with DO, while *Euglena texta* (Dujardin) Hübner 1886 and *Tetrademus obliquus* (Turpin) M.J.Wynne 2016 were strongly positively correlated with DIN, and *Euglena*, Chrysophyceae and *Crucigenia tetrapedia* (Kirchner) Kuntze 1898 were closely positively correlated with BOD (Fig. 2, Tab. 4). *Gomphonema* spp. and *Coelastrum astroideum* De Notaris 1867 were clearly separated on the biplot and were negatively correlated with BOD (Fig. 2, Tab. 4).

DO, phosphate (P-PO₄) and conductivity were all selected as factors influencing zooplankton abundance in the RDA model (Fig. 3, Tab. 4), with DO explaining 7.6% (pseudo-F=3.1, $p=0.014$), P-PO₄ explaining 6.4% (pseudo-F=2.7, $p=0.003$) and conductivity 4.6% (pseudo-F=2.0, $p=.043$). Taxa positively correlated with DO included the rotifers *Synchaeta oblonga/tremula*,

Brachionus variabilis Hempel, 1896, *Notholca squamula* Müller, 1786, and the daphnids *Daphnia pulicaria* Forbes, 1893 and *Daphnia* spp. males (Fig. 2). *Eudiaptomus gracilis* males and calanoid copepods were closely correlated with P-PO₄, while *Cyclops vicinus* Ulyanin, 1875 and male *Daphnia* spp. were closely correlated with conductivity. Variation partitioning revealed that water parameters explained 5.2% of phytoplankton variability, with 4.2% this attributable to zooplankton and 5% to the combination of zooplankton and water properties.

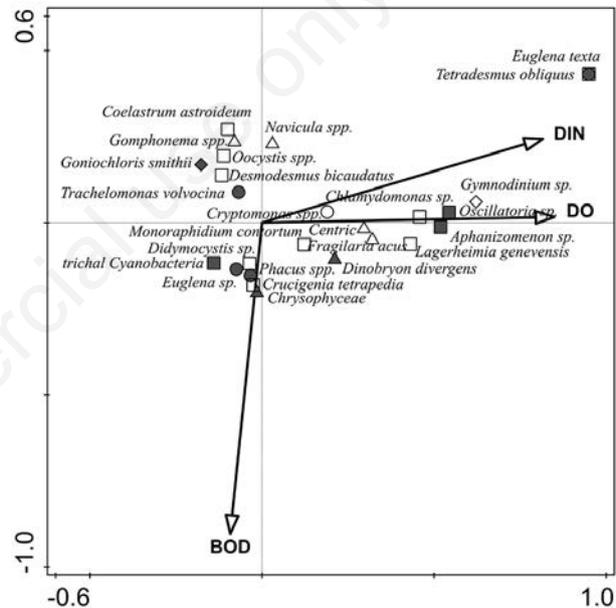


Fig. 2. Results of canonical correspondence analysis, showing relationships between phytoplankton biomass (wet weight) and environmental variables selected by the forward selection procedure. Dissolved oxygen concentration (DO), dissolved inorganic nitrogen (DIN) and biochemical oxygen demand (BOD) accounted for 7.5% of total variability. Empty square, Chlorophyta; full square, Cyanobacteria; empty circle, Cryptophyta; full circle, Euglenophyta; empty triangle, Bacillariophyceae; full triangle, Chrysophyceae; empty diamond, Dinophyta; full diamond, Xantophyceae.

Tab. 3. Median and range of zooplankton abundance (ind L⁻¹) in fishponds over the 2016 and 2017 growing seasons.

Year (n)	B1		B2		B3	
	2016 (5)	2017 (8)	2016 (5)	2017 (8)	2016 (5)	2017 (8)
Rotifera	380 (10-3513)	87 (10-2339)	324 (72-1714)	117 (7-1126)	80 (6-382)	84 (4-687)
Cladocera	5 (1-30)	18 (0-199)	29 (0-1236)	57 (1-2163)	0 (0-68)	22 (3-868)
Copepoda	3 (1-28)	20 (1-37)	11 (4-192)	27 (3-91)	1 (0-12)	18 (7-34)

n, number of samplings in a given year; B1, Bohuslavice 1; B2, Bohuslavice 2; B3, Bohuslavice 3.

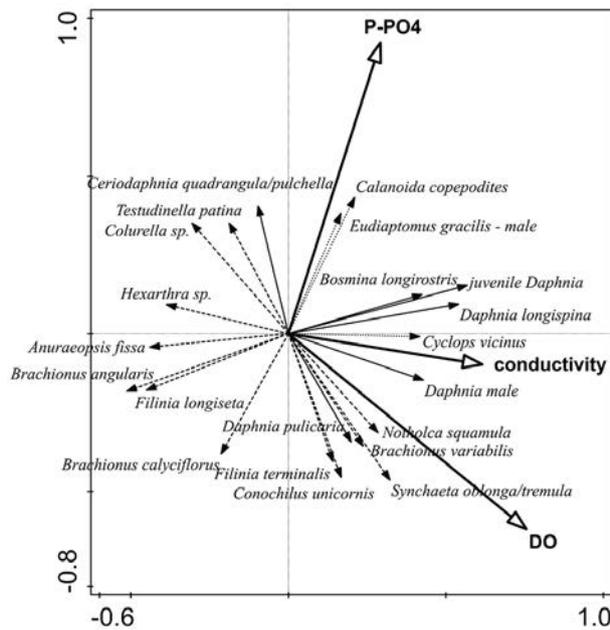


Fig. 3. Results of redundancy analysis, showing relationships between zooplankton abundance and environmental variables selected by the forward selection procedure. Dissolved oxygen concentration (DO), phosphate phosphorus (P-PO₄) and conductivity accounted for 11.6% of total variability. Dashed line, Rotifera; full line, Cladocera; dotted line, Copepoda.

Phytoplankton-zooplankton co-correspondence

CoCA revealed a significant relationship between phytoplankton and zooplankton ($p < 0.001$), with high cross-correlations between the first four axes (87%, 79%, 76% and 83%; Fig. 4, Tab. 4). In total, 40.5% of phytoplankton variation was attributable to zooplankton, with dual plots suggesting clusters of *Selenastrum bibrarianum* Reinsch 1866, *Phacus* spp., *Peridinium* sp., *Didymocystis* sp., *Euglena* sp., *Lepocinclis acus* (O.F.Müller) B. Marin & Melkonian 2003 and *Goniochloris spinosa* Pascher 1938 closely associated with summer zooplankton species (Fig. 4). Both the phytoplankton and zooplankton taxa were scattered along the second axis (from top to bottom in Fig. 4 A,B), with phytoplankton samples being arranged diagonally according to vegetation season, *i.e.* from April samples in the third quadrant (bottom left corner) to September samples near the first axis at the right site of the scatterplot (Fig. S1). In zooplankton, species scores for the rotifers *Epiphanes* sp., *Mytilina mucronata* (Müller, 1773), *Brachionus rubens* Ehrenberg, 1838 and *Lophocharis oxysternon* (Gosse, 1851) occupied the upper left corner, though these species were only found once over both years. Typical summer species, such as *A. fissa*, *P. dolichoptera* and *B. angularis* Gosse, 1851 were clustered alongside the first axis and mirrored phytoplankton taxa such as *Phacus*

Tab. 4. Summary of simple and conditional effects of plankton.

		Simple effects			Conditional effects			
		Explained variability (%)	Pseudo-F	p	Explained variability (%)	Pseudo-F	p	
Phytoplankton (canonical correspondence analysis)	DO	6.4	2.5	0.002*	DO	6.4	2.5	0.002*
	DIN	6.3	2.5	0.005*	BOD	5.4	2.2	0.002*
	BOD	5.1	2.0	0.007*	DIN	4.7	2.0	0.025*
	P-PO ₄	4.0	1.6	0.149	TP	3.3	1.4	0.242
	TP	4.0	1.6	0.105	TN	3.2	1.4	0.246
	TN	3.9	1.5	0.141	P-PO ₄	2.5	1.1	0.679
	Cond	3.8	1.4	0.325	Cond	2.2	0.9	0.843
Zooplankton (redundancy analysis)	DO	7.6	3.1	0.014*	DO	7.6	3.1	0.013*
	DIN	6.9	2.7	0.013*	P-PO ₄	6.4	2.7	0.002*
	BOD	6.3	2.5	0.005*	Cond	4.6	2.0	0.043*
	Cond	5.9	2.3	0.045*	BOD	3.2	1.4	0.113
	P-PO ₄	5.6	2.2	0.029*	TP	3.0	1.3	0.092
	TP	4.3	1.7	0.015*	DIN	3.7	1.7	0.047*
	TN	4.1	1.6	0.087	TN	1.8	0.8	0.727

DO, dissolved oxygen content; DIN, dissolved inorganic nitrogen; BOD, biochemical oxygen demand; P-PO₄, phosphate; Cond, conductivity; TP, total phosphorus; TN, total nitrogen; * $p < 0.05$.

spp. and *Euglena* sp. The left part of the dual plots was occupied by *C. vicinus*, *Daphnia* gr. *longispina*, and juvenile *Daphnia*, followed by *B. longirostris* O. F. Müller, 1776 and *K. quadrata* (Fig. 4).

DISCUSSION

The fishponds in our study area can be characterised as hypertrophic ecosystems rich in nutrients and phytoplankton. Like many other fishponds in Central Europe, they suffer from high nutrient inputs from adjacent agricultural landscapes, nutrient-rich sediments and a decline in precipitation over recent years. The fishponds were generally rich in easily decomposable organic matter, and this, in combination with high nutrient inputs and reduced water input, has favoured phytoplankton growth. Chlorophyta were the most diverse group in all three fishponds; however, while these small-sized green algae were abundant in all three ponds over both years, their proportion as phytoplankton biomass was low, the highest proportions being made up of larger sized Euglenophyta and Dinophyta.

DO was a significant parameter explaining variability in both phytoplankton and zooplankton, relating to both

photosynthesis and respiration of both assemblages. DO fluctuated throughout both years, with highest values at the beginning of the season and lowest in summer, as expected in a hypertrophic environment (Jeppesen *et al.*, 1990). In both eutrophic and hypertrophic fishponds, phytoplankton blooms can lead to high diel and seasonal fluctuations in DO, and the collapse of such blooms can lead to oxygen depletion, potentially resulting in fish kills (Jewel *et al.*, 2003; Mishra *et al.*, 2022). Aquacultural waters studied by Zang *et al.* (2011) revealed DO and chlorophyll-a relationships ranging from strongly positive to no relationship at all. In such cases, other factors, such as the decomposition of organic matter in sediments and the respiration of other aquatic organisms, may also have influenced DO concentrations. As DO concentration can be influenced by processes occurring in either sediment or the water column, it can be difficult to determine which is the driving force for DO in a pond. However, Baxa *et al.* (2021), studying another pond in the Czech Republic, found that the contribution of sediment respiration to total respiration was negligible at high phytoplankton biomass.

The second parameter explaining phytoplankton variability in our ponds was BOD. Phytoplankton have

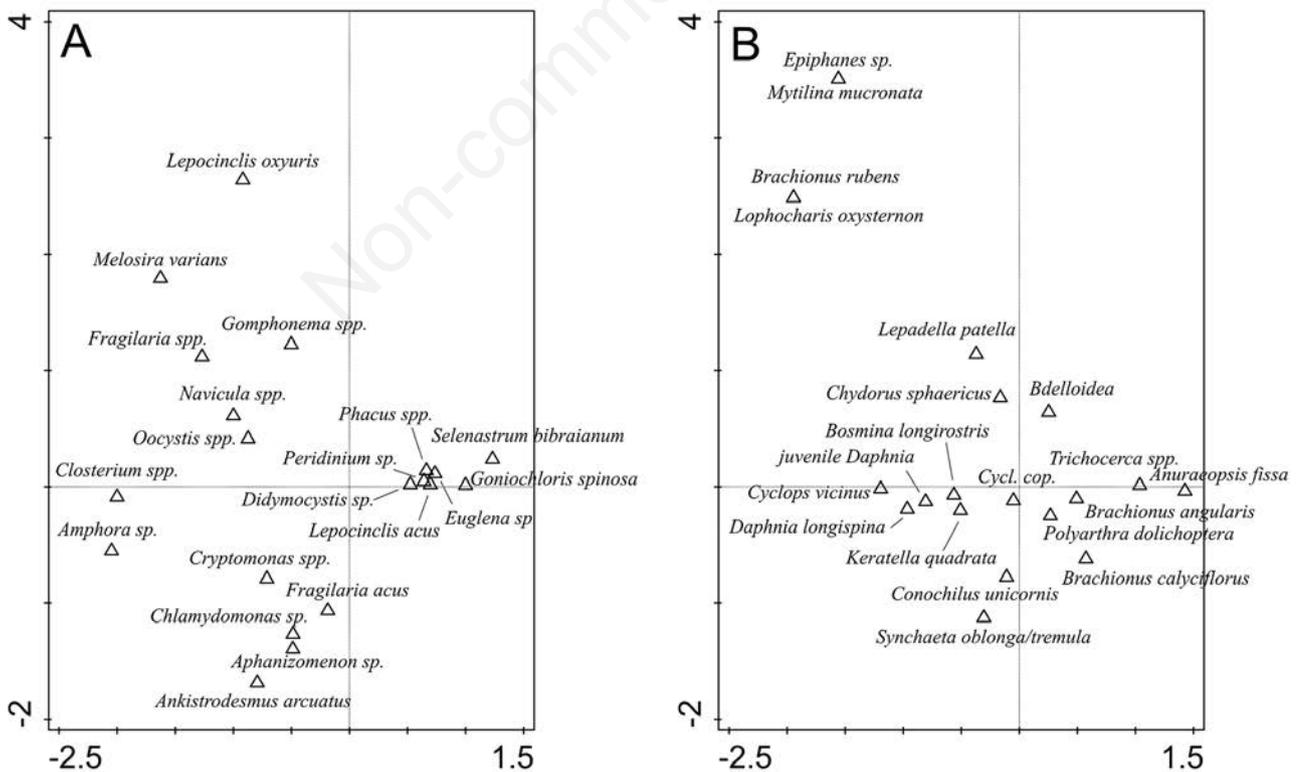


Fig. 4. Dual plot of co-correspondence analysis, showing weighted average scores for phytoplankton biomass (A) and zooplankton abundance (B). The twenty best-fitting phytoplankton and zooplankton taxa are plotted.

been shown to contribute to variability in BOD in a range of water ecosystems (Mallin *et al.*, 2006). However, BOD is usually strongly correlated with phytoplankton biomass under eutrophic conditions (Wang *et al.*, 2007; Li *et al.*, 2018), with *Euglena* and representatives of Chrysophyceae, for example, often associated with small organic ponds as they can tolerate high BOD levels (Reynolds *et al.*, 2002). Indeed, in our own study, these same taxa were positively correlated with BOD.

Zooplankton also display complex interactions with DO, with respiration in particular influencing the oxygen regime. Karpowicz *et al.* (2020) showed that freshwater zooplankton are relatively tolerant to anoxic conditions and that an anoxic zone may even serve as a refuge for zooplankton; however, while zooplankton may be tolerant to anoxic environments, DO has been shown to limit zooplankton occurrence to the surface layer in shallow ponds where DO concentrations are usually not limiting (Vad *et al.*, 2013).

Nutrient availability has been shown to play an important role in phytoplankton and zooplankton development in all aquatic ecosystems (Carpenter *et al.*, 2001). In our study, DIN, a primary nutrient component, was an important factor explaining the development of phytoplankton in the fishponds. Despite previous studies showing that phytoplankton-zooplankton relationships are often weaker in eutrophic and hypertrophic ecosystems (*e.g.*, Elser *et al.*, 1990; Hessen *et al.*, 2006), our results showed a strong association between these two plankton assemblages (Fig. 4). Here, both the development and relationships of phytoplankton and zooplankton were likely to have been influenced by the presence of macrophytes and fish biomass, which were not included in our analyses due to a lack of relevant data. However, macrophytes and phytoplankton generally show a negative relationship, primarily driven by resource competition for light and nutrients (Van Donk and Van de Bund, 2002; Barrow *et al.*, 2019). The effect of macrophytes on zooplankton, on the other hand, can be complex. On the one hand, macrophytes provide microhabitats where zooplankton can hide from predatory fish (Jeppesen *et al.*, 1998), while on the other, young fish inhabit macrophyte beds as they serve as shelter against larger piscivores. In this case, zooplankton would suffer from fish predation within macrophyte beds. Furthermore, food quality for zooplankton filtrators can differ between the macrophyte-rich littoral zone and open water zones where phytoplankton is predominant. In the study of Muylaert *et al.* (2010), for example, zooplankton grazing pressure was shown to be significantly positively correlated with presence of submerged macrophytes. Though fish data were unavailable for our sites, zooplankton and phytoplankton coupling is known to be influenced by fish presence (Williams and Moss, 2003). In our own case, we can assume that predation pressure on

zooplankton was relatively high as the fishponds were highly stocked with carp for production purposes. Further, as the main fish stocked were yearling carp, the potential impact on zooplankton is likely to be high as carp of this age preferentially select zooplankton as food (Rahman *et al.*, 2009).

CONCLUSIONS

Dissolved oxygen, biochemical oxygen demand, dissolved inorganic nitrogen, phosphate and conductivity were shown to be the most significant environmental variables influencing phytoplankton and zooplankton in a cascade of hypertrophic fishponds. Though the literature reports a weak relationship between phytoplankton and zooplankton in such hypertrophic lakes, our results suggest a strong trophic relationship between them, with a potentially strong bottom-up effect on the fishpond food web.

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Online supplementary material:

Tab. S1. Spearman rank order correlations table.

Fig. S1. Co-correspondence analysis: position of fishponds (samples) based on phytoplankton taxa.