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Taxonomic Composition and Environmental Drivers of Phytoplankton in Earthen Fish Ponds (Bulgaria)

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Abstract. This study presents a comparative analysis of phytoplankton taxonomic structure and its relationship with environmental factors in earthen aquaculture ponds located in Plovdiv and Trivoditsi, Bulgaria, during the period May–September 2024. A total of 114 phytoplankton taxa were identified across six phyla, with Chlorophyta and Cyanoprokaryota dominating the biomass. The results showed a pronounced shift towards higher phytoplankton biomass (PhB up to 60.3 mg L^{-1}) and chlorophyll-a concentrations (up to 133 µg L^{-1}) in the Trivoditsi ponds, associated with blooms of green algae and cyanobacteria. For the first time, the invasive and potentially toxic species *Raphidiopsis raciborskii* was recorded in Bulgarian aquaculture ponds, contributing up to 15.5% of the phytoplankton biomass during bloom periods. Environmental parameters such as elevated ammonium, phosphate, and COD were positively correlated with increased PhB, chlorophyll-a, and total viable counts (TVC) of heterotrophic bacteria. Redundancy analysis (RDA) highlighted the influence of nutrient enrichment and organic load on the biological variables. The observed co-variation between PhB and TVC suggests the presence of a dynamic microbial loop, where intensive primary production supports heterotrophic bacterial growth. These findings confirm the role of eutrophic conditions and nutrient availability in shaping phytoplankton and microbial communities in earthen ponds used for aquaculture. This study provides new insights into the ecological interactions in such systems, contributing to future strategies for water quality management and sustainable aquaculture practices.

Key words: phytoplankton, cyanobacteria, *Raphidiopsis raciborskii*, aquaculture ponds, heterotrophic bacteria.

Introduction

Phytoplankton is a key part of the food chain in inland waters. It plays a big role in primary production, recycling nutrients, and building the biological community in the system (Reynolds et al., 2002; Padisák et al., 2009). The taxonomic and functional structure of phytoplankton is determined by a number of factors, including the physical and chemical conditions, the hydrological regime, the availability of nutrients, and interactions with other living things, especially heterotrophic microbes (Katsiapi et al., 2012; Laas et al., 2009). This effect is what makes up the phytoplankton's taxonomic and functional structure.

Earthen ponds are a unique subject of ecological research because they are shallow, don't let much water flow through them, and are very productive. People often use earthen ponds to farm different freshwater fish species. Seasonal blooms and the dominance of certain functional groups that are related to eutrophication conditions and human intervention are common features of the phytoplankton community in these ponds (Dochin et al., 2020a; Dochin et al., 2020b; Hadjinikolova et al., 2016). People usually think that these traits are caused by people getting involved. Studies (Stoyneva-Gartner et al., 2023; Dochin, 2022) show that the types and amounts of cyanobacteria, green algae, and diatoms in Bulgaria change a lot depending on where they are, how they are used, and what trophic levels they are at.

Padisák et al. (2009) say that phytoplankton species can be put into functional groups that show not only what kind of plant they are but also how they have adapted to live in certain environments. Also, these functional groups can be broken down into smaller groups. This makes it easier to understand the differences and similarities between the different types of water bodies and gives a more accurate ecological picture of the communities that have been seen. This is very helpful for fish farming because the conditions in earthen ponds used for fish farming are often changed by management strategies like fertilization, stocking, and water exchange (Dochin et al., 2020; Hadjinikolova et al., 2016).

A lot of people are also interested in how heterotrophic cultivable bacteria and phytoplankton interact with each other, in addition to environmental issues. These microorganisms have a big impact on breaking down organic matter, releasing nutrients, and even making phytoplankton dominant through competition or symbiosis (Kopylov et al., 2014; Laas et al., 2009). In vertically stratified eutrophic lakes, phytoplankton and cultivable bacteria have been shown to follow similar seasonal patterns. This suggests that there are complex links between their trophic and metabolic systems. Kopylov et al. (2014) showed this. Similar dependencies have also been found in ecosystems that are less deep, like those in terrestrial water bodies. However, there aren't many studies that are focused on this area.

There aren't many studies done on this topic in Bulgaria. Most of the papers focus on taxonomic analysis and checking the trophic state of water bodies (Stoyneva, 2015; Dochin, 2022; Stoyneva-Gartner et al., 2023). Dochin et al. (2020) found that phytoplankton functional groups that are resistant and grow quickly often take over fish farms. *Aulacoseira, Fragilaria, Desmodesmus*, and *Microcystis* are some of the phytoplankton groups that are part of this. These groups of phytoplankton can handle changes in light and nutrient levels very well. There are potentially toxic cyanobacteria, like *Dolichospermum, Raphidiopsis*, and *Oscillatoria*. Researchers have found that these cyanobacteria are often linked to high levels of organic load and temperature peaks (Stoyneva-Gartner et al., 2017; Pavlova et al., 2015).

The goal of this study is to add to what we already know by looking at the taxonomic structure of phytoplankton in earthen ponds that are used for aquaculture and looking into how this structure is related to some abiotic factors in the environment, like the total number of heterotrophic microorganisms that can be grown. In the two types of systems that were looked at, this is expected to help us understand how algal communities change over time and how they are affected by different management and ecological conditions. In addition, a comparative analysis will be done that will focus on the features and ecological importance of the ecosystems being looked at. This will be done by including published results from water bodies that are similar to those in Bulgaria and other countries.

Materials and methods

Study site

The study was conducted from May to September 2024 in six earthen aquaculture ponds located at two experimental fish farms managed by the Institute of Fisheries and Aquaculture in Plovdiv, Bulgaria. The Plovdiv (P) farm, situated within the urban area of Plovdiv (42.188333° N, 24.750278° E), is supplied with water through a channel directly connected to the Maritsa River. Two ponds (P2 and P5) were selected as representative sampling sites from this facility, which operates a typical carp polyculture system in lowland conditions. The Trivoditsi (T) farm, located approximately 26 km southwest of Plovdiv (42.133056° N, 24.459722° E), consists of a larger complex fed by spring water. Four ponds (T1, T2, T12, and T13) were selected for analysis, as the farm has a surface area approximately twice that of the Plovdiv site. The pond codes (P2, P5, T1, T2, T12, T13) reflect the official identification numbers used in the farm management records, which explains the non-sequential numbering. All ponds were chosen based on their structural similarity (size, depth, substrate type) and their function within fish polyculture systems. Each pond was sampled five times during the study period, resulting in a total of 30 samples.

Physicochemical analysis

Water temperature (TMP) and dissolved oxygen (DO) were measured in situ with an oxygen meter (WTW OXY 1970i). The depth of the euphotic layer was determined by measuring the water transparency (ZS) with a 20 cm diameter Secchi disk. Electrical conductivity (Cond) and pH were measured with a WTW Conductivity meter (Cond3310/SET) and a WTW pH-meter (315/SET), respectively. Ammonium ($N\text{-}NH_4$), nitrate ($N\text{-}NO_3$) and total nitrogen (TN), and manganese III COD (CODMn) were measured in the laboratory using standard analytical methods (ISO 8467:1993; ISO 5664:1984; ISO 7890-1:1986; ISO 6878:2004). Total phosphorus (TP) concentration was measured by the Phosphate Cell Test (114543, Merck Millipore).

Analysis of biological parameters

The phytoplankton samples (1000 mL) were collected and processed by standard methods of fixation with Lugol's solution and further sedimentation (ISO5667-1:2006/AC:2007; ISO5667-3: 2003/AC:2007). The species composition was determined on a light microscope (Carl Zeiss, Axioscope 2 plus) with magnification 400x using standard taxonomic literature with critical use of AlgaeBase (Guiry & Guiry, 2025). Counting was done on Bürker chamber with the individuals (cell, filament or colony) being the counting units, and the biomass was estimated by the method of stereometrical approximations (Rott, 1981; Deisinger, 1984). Diatoms are identified according to Cox (1996). The total biomass (mg L^{-1}) of each sample was assessed as the amount of biomass of all species, summarized by separate taxonomic groups. Chlorophyll a (Chl-a) concentration was determined by the spectrophotometric method in an ethanol extract after filtration (ISO 10260:2002). The enumeration of culturable microorganisms (total viable count) was conducted by colony count after inoculation in a nutrient agar culture medium (ISO 6222:2002).

Results

Environmental and Biological Conditions

Environmental parameters measured in the studied ponds showed clear spatial differences between the two aquaculture sites - Plovdiv (P) and Trivoditsi (T) - which were reflected in phytoplankton biomass and composition. Water temperature ranged from 23.5°C to 25.9°C, while dissolved oxygen varied significantly from 4.2 mg L^{-1} (pond P2, Plovdiv) to 7.48 mg L^{-1}, (T1, Trivoditsi), corresponding to oxygen saturation levels between 49.8% and 93.0% (Table 1). The pH values were consistently within the alkaline range (7.456–8.128), creating favourable conditions for cyanobacterial development. Ammonium (NH_4^+) concentrations were highest in Trivoditsi ponds (up to 0.652 mg L^{-1} in T2), while nitrate (NO_3^-) and total nitrogen (TN) concentrations were slightly elevated in Plovdiv. Chemical oxygen demand (COD) values peaked in T12 (22.89 mg L^{-1}), coinciding with high levels of Chl-a and phytoplankton biomass. Orthophosphate (PO_4^{3-}) levels varied between 0.258 and 0.4 mg L^{-1} but were not consistently limiting across the ponds. Conductivity values ranged from 333.0 to 376.0 µS cm^{-1} and were slightly higher in Plovdiv. However, the Trivoditsi ponds exhibited significantly higher Chl-a concentrations (up to 133.1 µg L^{-1} in T13) and total phytoplankton biomass (PhB), exceeding 27.6 mg L^{-1} in some ponds. These trends indicate that nutrient enrichment, elevated temperatures, and higher organic load in Trivoditsi provide optimal conditions for intensive phytoplankton growth, particularly of green algae and cyanobacteria.

Table 1. Physico-chemical and biological parameters measured in the studied aquaculture ponds.

T	O_2	O_2	pH	NH_4^+	NO_3^-	TN	NH_3	COD	PO_4^{3-}	Cond	chl. *a*	PhB	TVC	Pond	Region
°C	mg L^{-1}	%		mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	mg L^{-1}	µS cm^{-1}	µg L^{-1}	mg L^{-1}	log_{10} cfu mL^{-1}		
23.46	4.2	49.8	7.456	0.242	1.368	1.61	0.0031	8.89	0.306	361.4	19.801	6.432	6.05	P2	Plovdiv
24.58	5.88	72	7.81	0.274	1.466	1.74	0.0109	12.024	0.376	376	37.176	3.395	5.77	P5	Plovdiv
25.48	7.48	93	7.904	0.416	1.214	1.63	0.0231	20.18	0.258	333	116.646	18.496	5.92	T1	Trivoditsi
25.9	6.96	87.2	8.054	0.652	0.8	1.452	0.0352	18.866	0.4	357.6	99.808	15.464	6.1	T2	Trivoditsi
25.26	4.92	59.4	8.128	0.352	0.708	1.06	0.0253	22.892	0.318	353.4	50.541	27.598	6.55	T12	Trivoditsi
25.48	4.62	54.4	8.082	0.378	0.682	1.06	0.0215	21.816	0.294	341.8	133.077	27.126	6.49	T13	Trivoditsi

Biological parameters also showed clear differences between the sites. Chl-a concentrations, indicative of phytoplankton biomass, ranged widely across the ponds - from a minimum of 19.8 µg L^{-1} in P2 (Plovdiv) to a maximum of 133.1 µg L^{-1} in T13 (Trivoditsi). Total phytoplankton biomass (PhB) showed similar variability, ranging from 3.4 mg L^{-1} (P5) to 27.6 mg L^{-1} (T12). The total viable count (TVC) of culturable heterotrophic microorganisms, expressed in $\log_{10}$ CFU 100 mL^{-1}, ranged from 5.77 to 6.55. The highest TVC values were consistently recorded in the Trivoditsi ponds, where both Chl-a and PhB levels were also elevated. These results demonstrate that ponds with lower PhB and Chl-a levels (Plovdiv) are associated with reduced heterotrophic bacterial activity, while those with higher biomass (e.g., T12–T13, Trivoditsi) exhibit substantially higher TVC values, pointing toward a coupled increase in both autotrophic and heterotrophic microbial communities under nutrient-rich conditions.

The redundancy analysis (RDA) revealed clear gradients in the relationships between environmental factors and biological parameters across the studied ponds. Phytoplankton biomass (PhB), Chl-a concentration, and total viable count (TVC) showed strong positive associations with ammonium (NH_4^+), phosphate (PO_4^{3-}), and chemical oxygen demand (COD), suggesting that elevated nutrient concentrations and organic load play a key role in promoting both algal growth and bacterial abundance. The vectors for PhB, Chl-a, and TVC were oriented in similar directions, indicating co-variation and a shared response to eutrophic conditions. In contrast, nitrate (NO_3^-) and conductivity showed opposing trends, implying a weaker or inverse relationship with microbial productivity. These patterns highlight the importance of reduced nitrogen forms and organic enrichment as the primary drivers of phytoplankton proliferation and microbial activity in the more productive ponds.

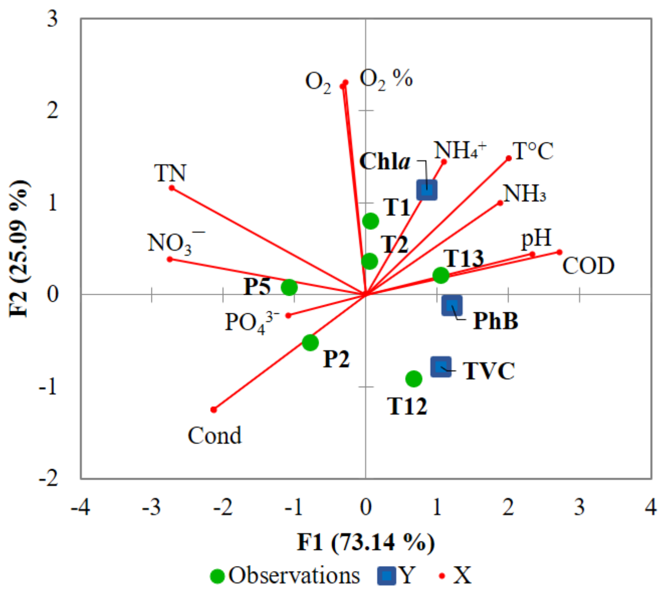

Fig. 1. RDA biplot showing the influence of key environmental variables on phytoplankton biomass (PhB), Chl-a concentration, and culturable heterotrophic bacteria (TVC) across earthen fish ponds in Plovdiv and Trivoditsi.

Taxonomic composition and biomass of phytoplankton in experimental ponds

A total of 114 plankton taxa were identified and classified into six groups: Cyanoprokaryota (35), Chlorophyta (39), Streptophyta (5), Euglenophyta (12), Pyrrhophyta (1), and Ochrophyta (22). Table 2 shows the taxonomic composition of the phytoplankton, including the most common and abundant taxa.

In May, the most abundant species in pond P2 were *Amphora* sp. (38.4%) and *Fragilaria crotonensis* Kitton (30.6%). The phytoplankton biomass was 0.829 mg L^{-1}. During the same period, *Ulnaria ulna* (Nitzsch) Compère (47.1%) and *Cyclotella* spp.

(37.1%) were the most abundant species in pond P5, with a biomass of 0.410 mg L^{-1}. *Aulacoseira granulata* (Ehrenberg) Simonsen (66.3%) was the dominant species in pond T1 at the Trivoditsi farm, with a biomass of 4.509 mg L^{-1}. *Desmodesmus communis* (E. Hegewald) E. Hegewald (23.1%) and *Nitschia* sp. (13.3%) dominated in pond T2. The value of the biomass is 4.556 mg L^{-1}. In pond T12, *Nitschia* sp. (28.5%), *Cymbella* sp. (26.5%), and *F. crotonensis* (24.6%) comprised 4.100 mgL^{-1} of biomass. *A. granulata* (37.9%) and *Mucidosphaerium pulchellum* (H.C. Wood) C.Bock, Proschold, and Krienitz (11.2%) were the two most common species in pond T13 in May. The total biomass value is 10.524 mg L^{-1}.

Table. 2. List of identified phytoplankton taxa.

Taxa	**Ponds №**					
	Plovdiv		**Trivoditsi**			
Cyanoprokaryota	**2**	**5**	**1**	**2**	**12**	**13**
Anabaena sp.					*	*
Anabaenopsis arnoldii Aptekarj				*		
Anabaenopsis circularis (G.S.West) V.V.Miller			*			
Anabaenopsis sp.	*		*	**	*	*
Anabaenopsis spp.						*
Aphanizomenon gracile Lemmermann					*	
Aphanizomenon sp.			*			
Aphanocapsa delicatissima West & G.S.West					*	
Aphanocapsa sp.		*/*	*		*	*/*/*
Aphanocapsa spp.					*	
Aphanothece sp.			*/*/*			
Aphanothece spp.						*
Chroococcus turgidus(Kützing) Nägeli						*
Cuspidothrix issatschenkoi (Usachev) P.Rajaniemi, Komárek, R.Willame, P. Hrouzek, K.Kastovská, L.Hoffmann & K.Sivonen						*
Dolichospermum flos-aquae (Bornet &Flahault) P.Wacklin, L.Hoffmann & Komárek						**
Dolichospermum planctonicum (Brunnthaler) Wacklin, L.Hoffmann & Komárek						*/**
Dolichospermum sp.						**
Merismopedia glauca (Ehrenberg) Kützing			*	*		*
Merismopedia sp.		*		*/*		*/**
Merismopedia spp.			*/*			
Merismopedia tenuissima Lemmermann			*		*/*	*
Merismopedia tranquilla (Ehrenberg) Trevisan	*				*	
Microcystis aeruginosa (Kützing) Kützing				*		
Microcystis sp.				*	*	
Microcystis wesenbergii (Komárek) Komárek ex Komárek			*		*	*
Oscillatoria sp.	*/**					*/*
Oscillatoria spp.						**

Planktolyngbya contorta (Lemmermann) Anagnostidis & Komárek				**		
Planktolyngbya limnetica (Lemmermann) Komárková-Legnerová& Cronberg			*	*	*	*
Planktolyngbya sp.	*/*		*/*/*/*	*/**/*		*/*/*
Planktothrix agardhii (Gomont) Anagnostidis & Komárek						*
Pseudanabaena sp.	*/*	**/*		*	*/*/*	*/*
Raphidiopsis raciborskii (Wołoszyńska) Aguilera & al.			*/*/**	*/*		
Romeria sp.		*	*		*	
Snowella lacustris (Chodat) Komárek &Hindák					*	
Chlorophyta						
*Actinastrum hantzschii*Lagerheim						*/*/*
Ankistrodesmus fusiformis Corda		*				*
Ankyra judayi(G.M.Smith) Fott			*/*			
Chloroidium cf. *ellipsoideum*		*				
Coelastrum astroideum De Notaris						*
Coelastrum microporum Nägeli	*	*/*/*		**	**	*/*/*
Crucigenia spp.			*			
Crucigenia tetrapedia (Kirchner) Kuntze		*/*	*		*	*
Crucigeniella pulchra (West & G.S.West) Komárek		*/*/*			*	*
Crucigeniella sp.		**				*
Desmodesmus bicaudatus (Dedusenko) P.M.Tsarenko	*	*				
Desmodesmus communis (E.Hegewald) E.Hegewald		**/**/**/*/*	*/**/*/*/*	**/**/*/*/*	*/*/**/*/*	*/**/*/*
Desmodesmus denticulatus (Lagerheim) S.S.An, T.Friedl & E.Hegewald	*			*/*		*
Desmodesmus protuberans (F.E.Fritsch&M.F.Rich) E.Hegewald			*			*/*
Desmodesmus spinosus (Chodat) E.Hegewald				*		*
Dictyosphaerium ehrenbergianum Nägeli				*		
Kirchneriella obesa (West) West & G.S.West						*
Lemmermannia triangularis (Chodat) C.Bock & Krienitz		*/*/*/*				*
Messastrum gracile (Reinsch) T.S.Garcia		*	*/*			*/*
Monoraphidium contortum (Thuret) Komárková-Legnerová					*	
Mucidosphaerium pulchellum (H.C.Wood) C.Bock, Proschold&Krienitz	*/*		*/*/*	*/*	*/*/*	**/*/*
Oocystis lacustris Chodat		**		*	*	*
Oocystis sp.		*/*				
Pediastrum duplex Meyen	*	*/**	*/*/**/**	**/*	**/**/**/**	*/**/*/*
Pediastrum simplex Meyen			*/**/**/**	*/*/**/**/*	**/**/**/**	*/*/*/*

Pseudopediastrum boryanum (Turpin) E.Hegewald				*		
Scenedesmus ecornis (Ehrenberg) Chodat	*	*				*
Scenedesmus sp.		*				
Scenedesmus spp.					*	*/*
Schroederia spiralis (Printz) Korshikov					*	
Sphaerocystis planctonica (Korshikov) Bourrelly						*/*
Sphaerocystis sp.		*				
Stauridium tetras (Ehrenberg) E.Hegewald		*	*/*	*/*		*/*
Tetradesmus lagerheimii M.J.Wynne & Guiry	*	*/*/*	*/*/* /*	**/**/*/*	*/*/*/*/*	*/**/*
Tetradesmus obliquus (Turpin) M.J.Wynne					*	*/**
Tetraëdron caudatum (Corda) Hansgirg						*
Tetraedron minimum (A.Braun) Hansgirg		**	*/*/*	*	*/*/*	*
Tetrastrum sp.		*		*/*	*/*	*/*
Tetrastrum spp.			*			
Streptophyta						
Closterium acutum Brébisson		*				
Closterium pronum Brébisson		*		*		
Cosmarium sp.	*			*	*/*	
*Elakatothrix gelatinosa*Wille		*		*		
Staurastrum sp.			*	*/*	*	*
Eulenophyta						
Euglena sp.	*			**	*	*
Euglena spp.			**	**/**	*	*/**
Euglena viridis (O.F.Müller) Ehrenberg						*
Lepocinclis acus (O.F.Müller) B.Marin & Melkonian	*		*/*/*	*		*/*
Lepocinclis oxyuris (Schmarda) B.Marin& Melkonian	*/*			*	*	
Lepocinclis sp.				*		
Phacus curvicauda Svirenko				*		
Phacus longicauda (Ehrenberg) Dujardin	*			*		*
Phacus sp.				*		*
Phacus tortus (Lemmermann) Skvortsov			*			
Trachelomonas planctonica Svirenko						*
Trachelomonas sp.				*		
Pyrrhophyta						
Peridinium sp.	*/**/*		*	*		*
Ochrophyta						
Bacillariophyceae						
Amphora sp.	**/*		*			*/*
Aulacoseira granulata (Ehrenberg) Simonsen	*/*/*	**/*/**	**/**/**/**/**	**/**/**	*/**/**/**	**/**/**/**/*
Cocconeis sp.	*	*				
Cyclotella sp.	*	**	*/*		*/*	*
Cyclotella spp.	**/*		**/*/*			

Cymatopleura solea (Brébisson) W.Smith		*		*		**
Cymatopleura sp.						*
Cymbella sp.	*	*	*/*		**/*	*
Fragilaria capucina Desmazières	**					
Fragilaria crotonensis Kitton	**/**/**/*				**/*/*/*	
Fragilaria sp.	*	*	**		*	
Gomphonema acuminatum Ehrenberg	*/*/*	*/*			*/*	**
Gomphonema sp.	*		*			*/*
Navicula sp.	*/*/*	*/*	**/*/*	*/*/*	*/*/*	*
Navicula spp.	**					
Nitschia sp.	*/**/*	*	*/**/*/*	**/*/*	**/*	**/*/*/*
Nitschia spp.		*				**/*
Pleurosigma elongatum W. Smith	*	*		*		**
Pleurosigma sp.	*					
Stephanocyclus meneghinianus (Kützing) Kulikovskiy, Genkal & Kociolek					*	
Synedra sp.		*	*	*/*	*	*/*
Ulnaria ulna (Nitzsch) Compère	*/**/**	**	*			

**** dominant species; * abundant species**

In June, *Fragilaria capucina* Desmazières (44.5%) and *F. crotonensis* (21.4%) were the dominant species in pond P2 in Plovdiv, with a total biomass of 8.923 mg L^{-1}. *Oocystis lacustris* Chodat (22.4.7%) and *D. communis* (17.2%) were the most common in pond P5 during the same time. The biomass during this period was 4.426 mg L^{-1}. *A. granulata* (57.6%) was the most common species in pond T1. The PhB was 12.157 mg L^{-1}. Similarly, in T2, *Tetradesmus lagerheimii* M.J. Wynne & Guiry (24.7%) and *D. communis* (20.6%) were the most common. *Pediastrum simplex* Meyen (45.3%) and *Pediastrum duplex* Meyen (19.6%) accounted for most of the biomass in pond T12 (9.2609 mg L^{-1}). *A. granulata* (35.3%) was also one of the most common species in pond T13 during the same period. A significant bloom of Diatoms and green algae was observed, with phytoplankton biomass reaching 45.397 mg L^{-1}.

In pond P2 in July, *F. crotonensis* dominated in the community (53.8%) and had the highest biomass (7.785 mg L^{-1}). *A. granulata* dominated both in ponds P5 (43.7%), pond T1 (39.1%) and in pond T12 (40.8%). Additionally, the dominant complex in T1 and T12 included *P. simplex* with 18.5% and 17.1%, respectively. Blooms of diatoms, green and blue-green algae were observed, with a biomass of 22.268 mg L^{-1} (T1) and 44.084 mg L^{-1} (T12). During the period in T2, *P. simplex* (24.6%) and *Euglena* spp. (21.4%) dominated. Blooms of green, euglenoid, and diatom algae were observed, with a biomass of 18.593 mg L^{-1}. In T13 was detected a high percentage *A. granulata* (20.9%) and the potentially toxic *Dolichospermum planctonicum* (Brunnthaler) Wacklin, L. Hoffmann & Komárek (13.9%) and *Dolichospermum flos-aquae* (Bornet &Flahault) P. Wacklin, L. Hoffmann& Komárek (10.7%). Bloom of diatoms and blue-green algae was observed, with a total biomass of 34.137 mg L^{-1}.

In pond P2, *Peridinium* sp. (45.9%) and *U. ulna* (23.7%) dominated in August. The total biomass amounted to 10.871 mg L^{-1}. In P5, *Crucigeniella* sp. (19.3%) and *Pseudanabaena* sp. (10.6%) were the most common, with a biomass of 2.066 mg L^{-1}. In T1, the most abundant are *A. granulata* (30.47%) and *D. communis* (12.8%). Blooms of diatoms, green algae, and *Euglena* algae were observed, with biomass reaching 29.51 mg L^{-1}. In T2, *Planktolyngbya* sp. (16.9%), *Euglena* sp. (16.5%) and *Anabaenopsis* sp. (14.1%) were the most abundant. The biomass during the period was 9.914 mg L^{-1}. *A. granulata* (39.5%) and *P. duplex* (32.3%) were widespread in T12. Intense blooms of diatoms and green algae species were observed, with a total biomass of 60.328 mg L^{-1}.

In September, *Navicula* sp. (37%) and *Oscillatoria* sp. (14.3%) were the most abundant in pond P2 (3.752 mg L^{-1}). The community in P5 differed was comprised primarily of *D. communis* (19.8%) and *A. granulata* (11.5%), with a total biomass of

1.049 mg L^{-1}. *A. granulata* continued to be the dominant species in Trivoditsi experimental fish farm. In pond T1, as part of the dominant complex were detected the invasive and potentially toxic *Raphidiopsis raciborskii* (Wołoszyńska) Aguilera & al. (15.5%) and *Aphanothece* sp. (13.4%). Blooms of diatoms and cyanobacteria were observed, with a biomass of 24.038 mg L^{-1}. In T2, other common species were *Planktolyngbya contorta* (Lemmermann) Anagnostidis & Komárek (13.2%) and *P. simplex* (10.7%). Blooms of diatoms, blue-green and green algae were observed, with an estimated biomass of 31.297 mg L^{-1}. In pond 12, the dominant complex was expanded by the species *P. duplex* (28.8%) and *P. simplex* (24.7%), with a biomass reaching 20.219 mg L^{-1}. *Oscillatoria* sp. (16.35%), dominated in T13 along with *Euglena* sp. (13.5%), and *Merismopedia* sp. (11.09%). Blooms of blue-green, euglenoid, and green algae were observed, with a total biomass of 36.6 mg L^{-1}.

The maximum phytoplankton biomass during the studied period was established in June, July, and August in ponds T12 and T13 in Trivoditsi. The high levels are due to the blooms of diatoms, green and blue-green algae. The values vary widely from 0.410 mg L^{-1} in May in pond P5 in Plovdiv to 60.328 mg.L^{-1} in August in T12 in Trivoditsi. Biomass levels in the Plovdiv ponds were significantly lower than those in Trivoditsi. The maximum values recorded in Plovdiv are 2 to 6 times lower than those in Trivoditsi. In July and August, the biomass in pond T12 in Trivoditsi is significantly higher than in the other ponds due to the blooming of green and diatom species.

Discussion

Research on phytoplankton in aquaculture ponds in Bulgaria includes the study of its taxonomic composition and quantitative development. In the past, the algal flora in fish farms was investigated in the works of Vodenicharov et al. (1974), Lüdskanova & Paskaleva (1975), Paskaleva (1975), Kiryakov et al. (1982), Paskaleva & Vodenicharov (1984), among others. In more recent years, Hadjinikolova et al. (2016) presented a study on the species composition and seasonal dynamics of phytoplankton in aquaculture farms with different production systems. The publication by Dochin et al. (2020) focused on the functional classification of planktonic algae in aquaculture ponds over three years. In that study, 172 phytoplankton taxa were reported. Nevertheless, data on phytoplankton in fishponds in Bulgaria remain scarce, with only four publications addressing algal flora in this type of water body in the last decade (Hadjinikolova et al., 2016; Dochin, 2020; Dochin et al., 2020a; Dochin et al., 2020b).

According to the present study, intensive algal blooms were recorded in the Trivoditsi ponds from June to September 2024. In many cases, species from the phylum Cyanoprokaryota were among the dominant bloom-forming taxa. Some of these, such as *D. planctonicum, D. flos-aquae, P. contorta* and *Oscillatoria* spp., are well-known potential toxin producers. Previous studies have also identified potentially toxic cyanobacteria such as *Aphanizomenon flos-aquae* Ralfs ex Bornet & Flahault, *Microcystis aeruginosa* (Kützing) Kützing, *Dolichospermum spiroides, D. planctonicum, D. flos-aquae*, and *Oscillatoria limosa* C. Agardh ex Gomont as dominant species during summer months (Hadjinikolova et al., 2016; Dochin, 2020; Dochin et al., 2020a; 2020b).

A major and novel finding of this study is the first recorded invasion of the toxin-producing Cyanoprokaryota species *Raphidiopsis raciborskii* in Bulgarian aquaculture ponds. This species was detected in pond 1 at the Trivoditsi base in September 2024 and was present in three out of four sampling events during July, August, and September. During intense bloom conditions, its share of total phytoplankton biomass ranged from 5% to 15.5%. In recent years, *R. raciborskii* has been increasingly reported in various Bulgarian waterbodies. It is considered a typical invasive species that continues to expand its geographic range and stands out as alien to the native algal flora of Bulgaria (Stoyneva, 2014; 2015; Stoyneva-Gärtner et al., 2017). In Bulgaria, it was first recorded in Lake Srebarna (Draganov & Stoyneva, 1992; Stoyneva, 1995; 1998a; 1998b; 2003), and its presence has since been confirmed in many lakes and reservoirs (Stoyneva, 2003; Dimitrova et al., 2014; Pavlova et al., 2015; Stoyneva-Gärtner et al., 2017; Stoyanov et al., 2012; 2013; Stefanova et al., 2020; Dochin, 2021; 2022; 2023; Stoyneva-Gärtner et al., 2023a, b, c; Belkinova et al., 2024; Dochin, In Press).

The results of the present study reveal a clearly expressed seasonal dynamic and taxonomic differentiation of phytoplankton in the studied earthen ponds. The dominance of genera such as *Desmodesmus, Pediastrum, Coelastrum* and *Scenedesmus* in spring and summer samples, as well as the presence of nitrogen-fixing cyanobacteria (*Dolichospermum, Raphidiopsis* and *Oscillatoria*), emphasize the high trophic status of the waterbodies and the critical role of temperature and light availability as main drivers. Similar results have been reported by other authors studying small and shallow artificial ponds used for aquaculture (Dochin et al., 2020; Hadjinikolova et al., 2016).

The observed strong co-variation between phytoplankton biomass (PhB, up to 27.6 mg.L^{-1}) and Chl-a concentrations alongside elevated total viable counts (TVC up to 6.55 CFU.100 ml^{-1}) suggests a dynamic and interactive microbial environment in the Trivoditsi ponds. This pattern is characteristic of eutrophic systems, where increased primary productivity provides ample organic substrates for heterotrophic bacteria to thrive. Such relationships have been widely reported: Kopylov et al. (2014) found positive links between culturable heterotroph counts and phytoplankton abundance in stratified eutrophic lakes. Similarly, in shallow freshwater ecosystems, green algae blooms were accompanied by significant increases in *Bacillus* and *Exiguobacterium* populations (Messyasz et al., 2015). These findings underscore a probable mechanistic link in our study, where nutrient-enriched, warm, and high-light conditions triggered phytoplankton blooms that, in turn, fueled heterotrophic bacterial growth. The elevated TVC values further support the presence of an active microbial loop, facilitating the rapid recycling of nutrients released from algal biomass degradation - a process documentted in numerous aquatic microbial ecology studies (Laas et al., 2009; Katsiapi et al., 2012).

Classical models of phytoplankton functional classification (Reynolds et al., 2002; Padisák et al., 2009) provide a robust framework for interpreting the observed groups, which in this case correspond to groups P, S1, and LM – typical for eutrophic, well-lit, and weakly stratified waterbodies. This confirms the importance of abiotic structure and morphometric characteristics of the ponds in determining the algal community structure.

Of particular interest is the positive correlation found between the number of culturable heterotrophic microorganisms and Chl-a concentration, which aligns with the findings of Messyasz et al. (2015), who demonstrated that bacterial species such as *Bacillus megaterium* and *Exiguobacterium* co-occur with increased phytoplankton biomass. Although no direct taxonomic link was observed between specific taxa, metabolic interdependencies are present, especially during periods of high primary production.

An additional perspective is provided by the study of Meseck et al. (2007), which showed that under limited nitrogen availability and altered pH, phytoplankton and bacterioplankton may compete for ammonium. These results are relevant to the context of earthen ponds, where organic loading and fertilization may trigger similar resource competition.

Climatic factors also play a significant role. Elliott (2009) found that rising temperatures increase the likelihood of toxic cyanobacterial dominance, especially when phosphorus and ammonium concentrations are high. In our study, in the samples from the summer period, the genus *Microcystis* stands out as part of the dominant complex, represented by several taxa, which aligns with this model and highlights the need for controlling trophic levels in aquaculture-oriented ponds.

Conclusions

In conclusion, the analysed data confirm the importance of a multifactorial approach in assessing the status of phytoplankton and its interaction with the microbial environment. The use of functional groups, combined with data on other biological parameters as TVC and Chl-a, enables a deeper understanding of ecological mechanisms operating in artificial aquatic ecosystems.

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