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Environmental Variables Significance for Ecological Status Assessment in the Bulgarian Eastern Continental Shallow Lakes

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ABSTRACT

Eastern Continental shallow lakes are specific with their higher nutrient concentration compared to other lentic water bodies with similar specifications. Nonetheless, their phytoplankton and macrophyte assessment systems used more often than others within state monitoring programs, are weakly influenced by nutrient concentrations, unlike all other lake types in Europe. In this study, we used all available data for Eastern Continental shallow lakes on the Black Sea coast of Bulgaria from the state monitoring for 14 years (2008–2021). The goal was to find how environmental variables influence the ecological status assessment by phytoplankton and macrophyte-related indices. Likewise, it was important to understand the studied parameter's interrelations within Dourankoulak Lake and the lake complex Shabla-Ezerets. The most important environmental parameters were determined with principal component analysis since it helps find the hidden relationships. Afterwards, those variables were used for univariate linear regression testing because of its simplicity and reliability. The aim was to find the best-suited parameters for the ecological status explanation by different biological indices, which can be used for further predictions of the ecosystem condition. In addition, nitrogen–phosphorus dependency, where possible was investigated, confirming conclusions from mesocosms studies from water bodies with similar characteristics. The algae group index or the so-called "Catalán Index", in its original form of representing results and considering its ecological quality ratio values, was influenced by conductivity concentrations the most in Ezeretsko Lake and weaker at Dourankoulak Lake. Nutrient relationship with biological quality elements was not found, confirming results on a European level. In Shablensko Lake a dose-dependent relation between nitrates and phosphates was found, which shows that augmenting the quantity of nitrogen is related to the increasing concentration of phosphates.

Keywords: eutrophic shallow lakes, environmental variables, nutrients, conductivity, Black Sea coast, biological quality elements.

INTRODUCTION

Waterbodies play a key role in the biogeochemical cycles of nutrients while maintaining biodiversity resources and ecosystem services. Unfortunately, lentic ecosystems receive nutrients in excess, and this causes eutrophication that could result in the loss of valuable aquatic biota and even specific odour [Çelekli et al., 2023]. As a result, eutrophication is still the most high-ranking pressure in lakes which is not so evident in rivers [Birk et al., 2020] where hydro-morphological alterations are of great importance [Doychev, 2023]. Nevertheless, European stagnant waters are in better condition than rivers, but still, about 40% of the natural lakes do not "achieve" good ecological status (GES) [EEA, 2018].

Shallow lakes are very distinct compared to other lake types. They have specific mechanisms of interactions with solar radiation and wind, which control thermal fluxes, creating distinct environmental conditions for aquatic biota. Apart from the physicochemical influence on the water column from heating or cooling, morphological characteristics such as the proximity of bottom sediments to the surface layer, often ensure mixing and uniformity within the pelagic habitat [Padisak and Reynolds, 2003]. In the context of climate change, those non-stratified water bodies, if eutrophic, could be synergistically affected unfavourably by biogenic elements enrichment creating higher internal loading, frequent toxic blooms, and altered zooplanktonic communities. As a result of zooplankton alteration, phytoplankton, transparency and macrophytes can be affected too [Havens and Jeppesen, 2018].

Both phytoplankton and macrophytes are amongst the most used biological quality elements (BQEs) for ecological status assessment of shallow lakes in Bulgaria [RBMP, 2016].

The Macrophyte assessment system is applying the Adapted Reference Index (RI). This metric uses indicator taxa divided into 3 groups: reference taxa, indifferent taxa and disturbance indicators. Their relative proportion provides the assessment result, considering hydrophytes, amphiphytes and selected helophytes out of Charophytes, Bryophytes, Pteridophytes and Spermatophytes [Pall et al., 2018].

For the other BQE, the Hungarian classification method for the lake phytoplankton assessment system is applied. This method includes biomass (chlorophyll-a), functional group metric, abundance of cyanobacteria and weighted average of normalized ecological quality ratio (EQR) value for the chlorophyll-a and functional group index [Borics et al., 2018].

These two assessment systems are widely used for European lakes as well. Considering the relationship between their results, expressed by EQR, with total phosphorus and nitrogen, a strong interrelation has been proven. An exception was only present in the Eastern Continental region, where these reactions were the weakest [Poikane et al., 2022].

The intercalibration group of Eastern Continental shallow lakes (L-EC1) is present in Hungary, Romania and Bulgaria [Borics et al., 2018] and is characterized by their natural eutrophic or hypertrophic regime. These regimes are specific with their naturally occurring high concentrations of several biogenic elements, which is a probable reason for primary production suppression, since the possible grazing of the phytoplankton or its self-shading. Another reason could be the high amount of nitrogen [Poikane et al., 2022] and its interaction with phosphorus compounds [Ma et al., 2023], determined as the most important, for eutrophication processes [Poikane et al., 2022; Ma et al., 2023].

According to RBMP [2016] the lakes from L-EC1, which are analysed in this article (Dourankoulak Lake and the lake complex Shabla – Ezerets) have mesotrophic to eutrophic regime, which partly coincides with the trophic assessments of Kalchev and Botev [1998]. Those assessments are made for the periods 1977–1979 for Dourankoulak Lake and 1992–1994 for the lake complex Shabla-Ezerets. Considering the nitrogen, the two water bodies are defined as mesotrophic, by phosphorus as hypereutrophic, and by chlorophyll-a concentrations as eutrophic [Kalchev and Botev, 1998].

To our knowledge, the consecutively published scientific works related to the studied here lakes, from the Black Sea coast of Bulgaria are: 1) Kalchev et al., [2002], who published an analysis related to the possibility of restoration of the lakes but again based on past periods; 2) Gecheva et al., [2011], who reported information related to the macrophyte assessment system for Dourankoulak and Shabla lakes from 2009; 3) Stoyneva et al., [2015], who compared results related to both macrophyte and phytoplankton assessment systems, based on data from 2011 and 2012; 4) Stoyneva-Gärtner et al., [2023], who based their results on a detailed study of the phytoplankton species composition from more than 40 water bodies, including the studied here, based on data from 2018, 2019 and 2021.

The mentioned articles are related either to a past period, or short-term studies or do not focus specifically on the lakes from the Eastern Continental region, which demonstrates the need for analysis in the scientific literature, related to the Bulgarian lakes, classified as L-EC1. Combining this statement with the conclusion from Poikane et al., [2022] that less well-studied water bodies must be researched in order to gain knowledge on eventual factors disturbing the relationship between nutrients and biota, was the main driving force for determining our goal in this study. We pursue evaluation and analysis of the specific ecological response, represented by phytoplankton and macrophyte-based indices, in parallel with the search for the most important environmental variables and their interrelationship on a long-term scale. All available data from the state monitoring for 2008–2021 is considered. The purpose of gaining knowledge on the main physicochemical parameters and their dependency on one another within every lake, representing L-EC1 in Bulgaria, could be beneficial for organizing more efficient monitoring programs within every water body and for the intercalibration type itself.

MATERIAL AND METHODS

Study area

Dourankoulak Lake, Ezeretsko Lake and Shablensko Lake are the subjects of investigation for this study. The latter two form one lake system and one Water Framework Directive water body [Kalchev et al., 2002; MoEW, 2004; RBMP, 2016].

"Durankulashko Swamp", Dourankoulak Lake or Dourankulashko Lake is one of the best-preserved wetlands in Bulgaria. The coastal lake is of great international importance because it is linked to conserving over 260 plant and animal species belonging to endemic, rare and endangered taxa. It was included in the list of "Ramsar sites" in 1984 and was designated as part of the "Durankulashko Ezero" protected locality in 1980. The water body is located about 6 km from the Bulgarian-Romanian border. It is defined as a shallow closed firth occupying the low coastal parts of the deep inland areas. The open water areas are 249.8 ha and those occupied by aquatic vegetation are 193.26 ha. Its approximate volume is 2 500 000 m^3, the average depth is 1.4 m, and the maximum depth is 4 m.

The catchment area dependent on surface water runoff is 476 km^2, and the one in connection with underground aquifers is 542 km^2 [MoEW, 2002]. The strongly calcined lithological composition, the lack of slope, the small amount of precipitation and the absence of springs to form a surface runoff are the reasons for Dourankoulashko Lake not to be dependent on superficial water recharging. The underground inflow of water masses forms about 80% of the lake's supply, with the role of winter feeding being more significant. It is for this reason that the quality and quantity of groundwater in the region are key to its chemical and ecological status evaluation [MoEW, 2002].

Shablensko and Ezeretsko lakes, such as Dourankoulashko Lake, are of great importance for Bulgaria and the European Community. They are representative coastal firths, specific for the western coast of the Black Sea, in which rare and endemic plant and animal representatives are found. However, a major role for the valued nature conservation status is the protection of the ornithological fauna. The two lakes are designated as the "Shablensko Ezero" protected locality and are included in the list of sites for protection under the Ramsar Convention [MoEW, 2004].

Water Framework Directive water body (code BG2DO700L018) includes Shablensko and Ezeretsko lakes. Both are about 18 km from the Bulgarian-Romanian border [Kalchev, et al., 2002; RBMP, 2016]. The total area of the open waters in the two coastal lakes, connected by a canal, is 90.9 ha, and the area occupied by aquatic plants is about 160.8 ha. The open water area is larger in Shablensko Lake (almost 60 ha) than in Ezeretsko Lake (just over 30 ha). The catchment area of the surface runoff for the lake complex is 219.9 km^2, and that dependence on the underground aquifers reaches an area of 542 km^2. The approximate volume of Ezeretsko Lake is 2 500 000 m^3, and that of Shablensko Lake is 3 600 000 m^3. The average depth is 3.5 m at Ezeretsko Lake and 4.6 m at Shablensko Lake. The maximum depth reaches 9 m at Ezeretsko Lake, against 9.5 m at Shablensko Lake [MoEW, 2004].

According to the Bulgarian typology of surface waters and the "passport" of surface waters in the Black Sea region for basin management, Dourankoulak Lake, Ezeretsko Lake and Shabla Lake are classified as polymictic, eutrophic to hypertrophic lakes of type L7a: Black Sea freshwater lakes, characterized by salinity below 0.5‰ [RBMP, 2016]. The Geographical Intercalibration Group for the lakes is "Eastern Continental Lakes" and the intercalibration type is L-EC1. It is characterized by a low altitude (below 200 m), an average depth below 6 m, a limestone type of bedrock, etc. [European Union, 2018].

According to Lyche Solheim et al. [2019], the specified intercalibration type L-EC1 and the two water bodies considered in this study refer to aggregated lake type L-02 (lowland, limestone, very shallow, unstratified) and the broad lake type L-04 (lowland, limestone, or mixed geology, shallow/unstratified).

The monitoring was conducted at one sampling site in the Dourankoulak and Shabla lakes. In Ezeretsko Lake the sampling sites are 2 and are positioned in the east (E1) and in the west (E) part of the open water area (Fig. 1).

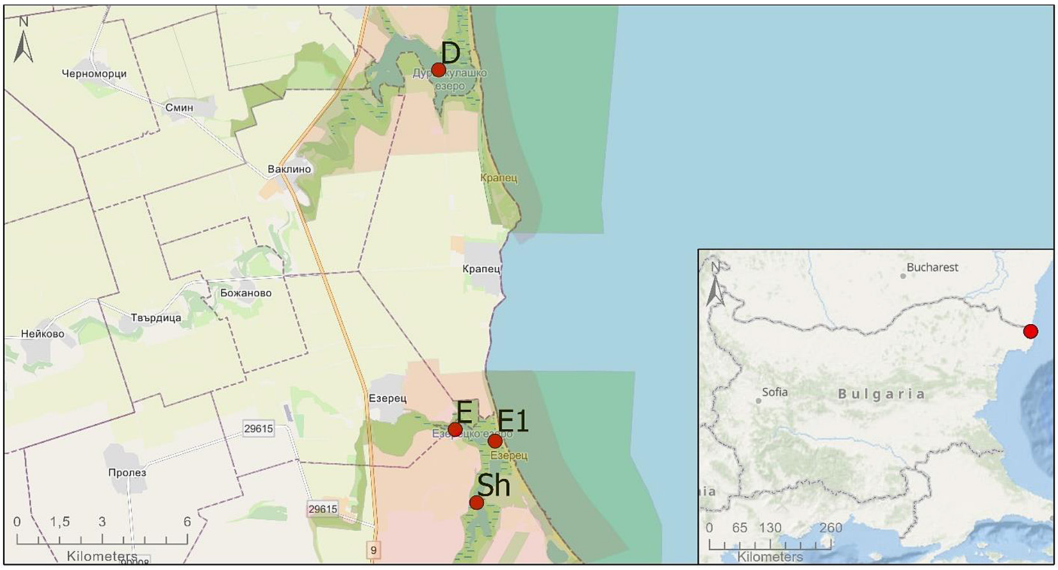

Figure 1. Map with the sampling sites. D – sampling site in Dourankoulak Lake. E – west sampling site in Ezeretsko Lake. E1 – east sampling site in Ezeretsko Lake. Sh - sampling site in Shablensko Lake. The pink area around the lakes is a protected area by the Birds Directive. The green area around the lakes is a protected area by the Habitats Directive

Physicochemical parameters

The regular state monitoring at the complex Shablensko and Ezeretsko Lakes and Dourankoulak Lake is the data source for the so-called environmental variables. Water samples were collected and analysed by an accredited regional laboratory under the control of the Executive Environmental Agency. The sampling events were conducted following the Bulgarian National Standards.

We considered 14 of the most frequently analysed physicochemical parameters which were studied in a highly irregular manner. In some cases, the variables were measured seasonally, every month or once a year and occasionally measurements were lacking for a complete year. Because of that, we applied one of the most popular aggregating tools - annual average (AA) [Kelly et al., 2022], for every single variable measured from Dourankoulak and Shablensko lakes. For Ezeretsko Lake this was not applied due to the specifics of the conducted monitoring. Another disadvantage of the Bulgarian state monitoring is the lack of consistency in considering the "main" variables. Because of this, we established a minimum threshold of 5 non-consecutive or consecutive AA for a parameter, to be included for further consideration and data analysis.

The physicochemical quality elements (PCQEs) approved for further consideration from Dourankoulak Lake and Shablensko Lake are dissolved oxygen (DO), conductivity (CD), pH, biological oxygen demand for 5 days (BOD_5), nitrate nitrogen (NO_3-N), nitrite nitrogen (NO_2-N), ammonium nitrogen (NH_4-N), total phosphorus (TP), orthophosphates phosphorus (PO_4-P), iron (Fe) and manganese (Mn). PCQEs for further consideration from Ezeretsko Lake are 4. They are *in situ* measured and include DO, oxygen saturation (OS), CD, and pH. From the 11 variables considered from all 3 lakes, 3 (total phosphorus, oxygen regime, and pH) fall among the top 5 (total phosphorus, total nitrogen, oxygen regime, pH, and Sechi depth/transparency) identified as the most important. Those parameters refer to the most frequently used and reported ones to the European Commission, within the time frame 2009–2015 [Kelly et al., 2022].

Bulgaria has established thresholds for total nitrogen [Ordinance H-4/2012], as many other countries, that agreed on the importance of the parameter [Kelly et al., 2022]. Nevertheless, the state frequently prefers to measure other nitrogen-containing variables from the lakes. Transparency is from the parameters with more than 5 AA and is included in the analysis, but as it enters the classification of "phytoplankton ecological status/potential assessment system" in Ordinance H-4/2012 it is not included in the section "Physicochemical parameters".

Biological indices

Monitoring programs for 2008–2021 included very scarce biological status-related measurements. They were present from 2017 to 2020 and were connected to macrophyte and phytoplankton ecological status assessments.

RI is calculated for 2017, 2018 and 2020. The assessment of this criterion is based on registering type-specific species groups and their occurrence and abundance within the surface water body [Gecheva et al., 2011]. For Dourankoulak Lake the data for RI is present from 2017 and 2020, for Shabla Lake from 2018 and 2020, and for Ezeretsko Lake the information is present only from August 2020. Based on this RI was not considered for data analysis and comparison with PCQEs from Ezeretsko Lake.

In the phytoplankton assessment system in Ordinance H-4 [2012], within the monitoring programs between 2017 and 2020, were included 8 variables, classified as "main" and "additional" ones [Gecheva et al., 2011]. The Current study used all those defined as "main" and two additional variables. They are Secchi depth (SD) or the so-called transparency, total phytoplankton biovolume or total biological volume (TBV), algae group index or Catalán Index (CI), ecological quality ratio for algae group index (VGI), chlorophyll-a concentration (Chl-a) and the percentage of Cyanobacteria taxa (Cyano%).

In Ezeretsko Lake, the described phytoplankton metrics were measured and analysed in sampling events from 2016 and 2018. For "Dourankoulashko Swamp" these biological indices are calculated for 2018 and 2020 and in Shabla Lake are available solely for 2018. Based on this phytoplankton metrics were not considered for data analysis and comparison from Shabla Lake.

Ecological status assessment

The fieldwork and the laboratory work for the determination of the taxonomic composition for phytoplanktonic and macrophyte communities is done by the Executive Environmental Agency. The indices and the metrics stated in Ordinance H-4 [2012], classify surface waters in Bulgaria according to these BQEs with values in high, good, moderate, poor and bad ecological status, in compliance with the European legislation and in particular the Directive 2000/60/EC [Water Frame Directive, WFD].

In situ and laboratory-analysed environmental variables or PCQEs, are classified as accompanying information which can serve as supporting data for the ecological status assessment. Their results are separated into 3 groups – high, good and moderate environmental status [European Commission, 2005; Ordinance H-4/2012].

Data analysis

Principal component analysis (PCA) was applied to obtain the main information from environmental variables and to reduce the dimensionality of multivariate data while the results are better visualized [Pagès, 2002]. Additional motivation for implementing PCA at this stage is finding hidden relationships that are easily made visible and can help in explaining the ecological status of the freshwater ecosystem [Benkov et al., 2023].

PCA on environmental variables was performed in RStudio 2023.06.1.0 with R environment [R 4.2.3, R Core Team, 2023], using "factoextra" and "FactoMineR" packages. The physicochemical parameters which had gaps in the data for some of the years were filled automatically by RStudio with averaged results from all conducted measurements, for the given variable. Consequently, the p-value was calculated using the "dimdesc" function in "FactoMineR" and the significant (< 0.05) variables from principal component 1 (Dim 1) and principal component 2 (Dim 2) were selected for linear regression analysis.

For Dourankoulak Lake statistically significant environmental variables defined by PCA were tested for linearity with the metrics related to the "phytoplankton ecological status/potential assessment system" and RI between 2017 and 2020. This method was chosen and applied due to its simplicity and reliability [Philips et al., 2024] and was conducted in XLSTAT for Excel [Lumivero, 2023].

For Shablensko Lake statistically significant environmental variables were further analysed with RI for the period 2018–2020 and interrelationships for the period 2008–2021.

The monitoring program for Ezeretsko Lake was differently structured. Here the available data is seasonally collected in 2016 and 2018 from two sampling sites (Fig. 1). AA were not used. The measured physicochemical parameters are only 4 but are always coupled with the phytoplankton indices for the biological status assessment. Because of the scarcity of the studied environmental variables and the period matched with the analysis of the BQEs, PCA with 4 active variables and 6 supplementary ones was conducted. This methodology is chosen to investigate the correlation coefficient between the two types of variables and to define statistically significant parameters for further linear regression analysis. Consequently, every statistically significant active variable was tested for linearity with every statistically significant supplementary variable.

Only statistically significant ($P < 0.05$) linear regression models with $R^2 > 0.36$ and correlation coefficient coefficients > 0.6 were presented since these models are good enough to be used for predictions [Philips et al., 2018].

RESULTS AND DISCUSSION

Dourankoulak Lake

Eastern Continental lakes are very shallow lentic ecosystems with CD between 300 and 1000 $\mu S \cdot cm^{-2}$ [Poikane et al., 2022]. AA from Dourankoulak Lake, for the entire period, shows that CD is permanently at moderate status. The parameter registered concentrations between 930 $\mu S \cdot cm^{-2}$ in 2018 and 1264 $\mu S \cdot cm^{-2}$ in 2020. In contrast, the DO was always better than GES. BOD_5 with results fluctuating between good ecological status and moderate status, according to Ordinance H-4 [2012] (Table 1).

All nitrogen-containing parameters are in the spectre of GES and better than GES, excluding the nitrate nitrogen from 2008. Almost the same was observed for PO_4-P, excluding the registered "moderate" concentrations, again at the beginning of the period (2008 and 2009). The small number of TP results showed that GES was "achieved" in most cases (Table 1).

Historically, the available data reveals the following dynamics of biogenic elements for Dourankoulak Lake. The water body registered values for phosphate phosphorus of about 0.1 $mg \cdot l^{-1}$ for the period 1963–1967, about 0.2 $mg \cdot l^{-1}$ for the period 1977–1979 [Kalchev and Botev, 1998] and 0.072 $mg \cdot l^{-1}$ (Table 1) for the period 2008–2021, which shows recent reduction of phosphates.

Ammonium nitrogen registered above 0.1 $mg \cdot l^{-1}$ for 1963–1967, above 0.2 $mg \cdot l^{-1}$ for 1977–1979 [Kalchev and Botev, 1998] and 0.07 $mg \cdot l^{-1}$ (Table 1) for 2008–2021. Again, a recent reduction in the PCQE is observed. Nitrate nitrogen was above 0.5 $mg \cdot l^{-1}$ for 1963–1966, beneath 0.1 $mg \cdot l^{-1}$ for 1977–1979 [Kalchev and Botev, 1998] and 0.65 $mg \cdot l^{-1}$ for 2008–2021. This PCQE does not show a reduction in the concentrations compared to 1977–1979.

Overall, a positive tendency can be seen from this data, but considering the gaps of information in time intervals it is not possible to make firm conclusions about the levels of those nutrients.

Table 1. Annual averages (AAs) of environmental variables from Dourankoulak Lake

Parameter	DO $mg \cdot l^{-1}$	CD $\mu S \cdot cm^{-2}$	pH	BOD_5 $mg \cdot l^{-1}$	NO_3-N $mg \cdot l^{-1}$	NO_2-N $mg \cdot l^{-1}$	NH_4-N $mg \cdot l^{-1}$	TP $mg \cdot l^{-1}$	PO_4-P $mg \cdot l^{-1}$
D/08	7.55	1021	8.64	5.8	2.97	0.023	0.039		0.578
D/09	7.95	1043	8.35	4	0.38	0.033	0.067		0.166
D/10	8.44	997	8.49	5.5	0.26	0.034	0.118		0
D/11	8.57	1038	8.49	3.39	0.47	0.014	0.055		0.027
D/12	7.76	1063	8.50	3.98	0.38	0.013	0.057		0.028
D/13	7.70	1059	8.59	7.3	0.32	0.019	0.124		0.022
D/14	8.12	1039	8.52	5.05	0.36	0.02	0.088		0.019
D/15	7.18			1.73	0.8	0.02	0.06		0.03
D/16	8.9			2.8	0.38	0.02	0.06		0.02
D/17	8.62	954	8.81	4	1.03	0.057	0.07	0.035	0.022
D/18	8.46	930	8.80					0.084	0.039
D/19	7.33	997	8.44	1.66	0.13	0.012	0.103	0.056	0.024
D/20	8.98	1264	8.95	2.9	0.25	0.016	0.018	0.055	0.023
D/21	7.66	1075	8.12					0.035	0.012
Mean values	8.09	1040	8.56	4	0.65	0.02	0.07	0.053	0.072

Note: The colour code is following the ecological status classification. Blue – high ecological status. Green – GES. Yellow – moderate ecological status. D/08 – AAs from 2008. D/09 – AAs from 2009, etc.

The results from BQEs show permanent moderate status, considering RI. The phytoplankton assessment system results were worse because some of the used indices were in poor and bad status and just onetime Cyano% and TBV were in GES (Table 2).

PCA on environmental variables defined negligible differences between the contribution of Dim 1 (25.6%) and Dim 2 (23.4%) (Fig. 2). In Dim 1 two parameters are statistically significant. Nitrates with P < 7.83e-09 and phosphates with P < 1.45e-06. They are positively correlating parameters which dominate the principal component with 41 and 38% contribution. In Dim 2 the statistically significant parameters are 4: pH (P < 0.008), CD (P < 0.03), DO (P < 0.05) and NH_4-N (P < 0.0002). The greatest contribution is for NH_4-N (34.4%), followed by pH (22%), CD (17%) and DO (13%). Within Dim 2 only NH_4-N (-0.85) and pH (0.68) demonstrate well strong negative correlation (Fig. 2).

L-EC1 are normally with higher nutrient concentrations, compared to lakes from other geographical intercalibration groups with similar characteristics. For them, the positive correlation between the chlorophyll-a concentration and TP is common [Poikane et al., 2022]. Nevertheless, that relationship is poor, because the fish biomass and the macrophyte standings can have a strong impact on phytoplankton metrics [Borics et al., 2018]. Our study confirms this because the simple linear model related to the PO_4-P and Chl – a was not statistically significant, for Dourankoulak Lake, although the R^2 is 0.66.

On the European level, L-EC1 have a weak response to biogenic elements enrichment, which is visible from the R^2 < 0.36 for the linear regression models between TP and BQEs related to phytoplankton and macrophytes [Poikane et al., 2022]. On a local level, a similar trend was observed, considering Dourankoulak Lake nutrients (Table 1). They did not build significant linear regression models with the scarce BQE results for the period 2017–2020 (Table 2), even bearing in mind the criteria we set for importance [Philips et al., 2018], which confirms the reported of Poikane et al. [2022].

From our results, the condition of Dourankoulak Lake is better predicted by conductivity, one of the most preferred parameters on the European level, concerning salinity parameters [European Commission, 2024]. The linear regression models built from the 4 observations were not statistically significant and therefore are not represented as a figure. Still, AA of the CD was the only one which built linear regression models with R^2 reaching 0.781 and a p-value of the intercept < 0.01, with two indices from the phytoplankton assessment system (SD and VGI).

Although in this case, the results can only serve as a guide on which parameter to consider for more detailed future research, we can assume that particularly more extensive salinity measurements are needed even if they are only connected to conductivity. Those measurements should be synchronized with a survey of parameters related to the phytoplankton assessment system as transparency and algae group index.

The application of continuous monitoring or *in situ* measurements, solely for salinity on a monthly or seasonal basis that are only partly synchronized with the more costly phytoplankton analysis could be of great benefit to decision-makers too. It's because salinity dynamics are important for the freshwater ecosystem's biodiversity and functioning and could be altered by climate change and anthropogenic activities such as agriculture [Jeppesen et al., 2023]. In turn, climate change is altering the daily rainfall intensity and frequency, leading to their increase or decrease in different regions [Attayde et al., 2022].

Ezeretsko Lake

In situ measurements of environmental variables were conducted seasonally in 2016 and 2018, and once in 2020 (Table 3). They show CD

Table 2. Annual averages (AAs) of biological indices from Dourankoulak Lake

Parameter	SD m	TBV	CI	VGI	Chl-a mg·l^{-1}	Cyano%	RI
D/17							-4.17
D/18	0.73333	4.3025	5.48	0.9865	85.348	20.1333	
D/19							
D/20	0.6375	16.1075	14.3733	0.95725	69.857	15.4725	-15.456
Mean values	0.6854	10.205	9.9267	0.9719	77.602	17.8029	-9.813

Note: The colour code is under the ecological status classification. Green – GES. Yellow – moderate ecological status. Orange – poor ecological status. Red – bad ecological status. D/17 – AAs from 2017. D/18 – AAs from 2018, etc.

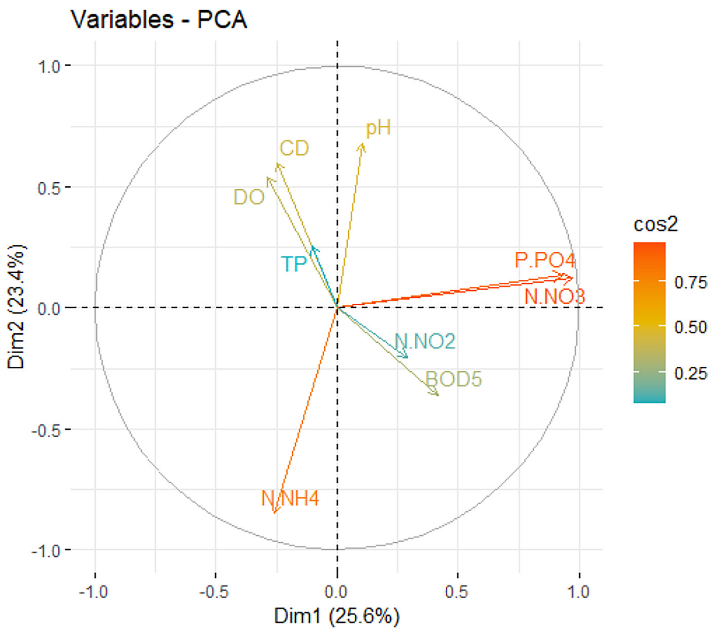

Figure 2. Dourankoulak Lake correlation circle from PCA, with vectors coloured according to cosine squared (cos^2) values

is always at "moderate" ecological status, within the range of 831 µS.cm^{-2} from September 2016 to 1131 µS.cm^{-2} from August 2020. In contrast, pH almost permanently was in the range of GES. DO occasionally registered values that were better than GES, but predominantly were in the moderate range [Ordinance H-4, 2012], due to excessively high concentrations that also corresponded with elevated oxygen saturation (Table 3). Regarding the calculated phytoplankton-based indices, it is visible

Table 3. Environmental variables and biological indices from Ezeretsko Lake

Parameter	DO mg·l^{-1}	OS%	CD µS·cm^{-2}	pH	SD m	Cyano%	CI	VGI	TBV	Chl-A mg·l^{-1}
E/5/16	6.18	78.5	882	8.49	1.95	2.28	0.71	0.998	3.298	48.07
E1/5/16	11.24	128.1	875	8.11	1.95	2.28	0.71	0.998	3.298	48.20
E/8/16	11.41	137	893	8.58	2.4	0.32	0.83	0.998	9.06	2.13
E1/8/16	12	146	887	8.6	2	0	0.78	0.998	2.81	4.26
E/9/16	9.42	104.7	881	7.49	1.95	2.28	0.71	0.998	3.298	79.90
E1/9/16	7.68	97.8	873	8.57	1.95	2.28	0.71	0.998	3.298	45.90
E/10/16	8.54	87	891	8.33	1.9	0	0.43	0.999	1.03	5.05
E1/10/16	8.3	84	891	8.32	1.5	8.8	0.81	0.998	0.29	6.1
E/4/18	11.95	123	983	8.42	2.7	0	0.84	0.998	0.1	9.48
E1/4/18	11.39	117.33	988	8.39	2.4	0	0.96	0.998	0.04	8.06
E/5/18	8.39	90.56	980	8.22	3.3	0	0.96	0.998	0.09	1.38
E1/5/18	8.58	92.066	988	8.34	3	0	1.01	0.998	0.32	3.16
E/6/18	14.07	172.66	974	8.55	2.2	0	1.69	0.996	1.84	21.92
E1/6/18	12.33	150	978	8.7	2.4	0	3.64	0.991	2.38	20.73
E/7/18	9.62	119.33	936	9.76	1	0	0.78	0.998	7.13	54.79
E1/7/18	10.89	134.33	967.8	8.59	2.5	0	1.35	0.997	1.525	15.38
E1/8/20	7.75	95.5	1131				8.88			
Mean values	9.98	115.16	941.1	8.46	2.19	1.14	1.52	0.997	2.487	23.41

Note: The colour code is under the ecological status classification. Blue – high ecological status. Green – GES. Yellow – moderate ecological status. Orange – poor ecological status. Red – bad ecological status. E/5/16 – west sampling site results from May 2016. E1/9/16 – east sampling site results from September 2016, etc.

that "high ecological status" is registered in most cases, from Cyano%, CI and VGI. Unlike them, Chl–a, was in all "colours" from the WFD evaluation system and registered bad, poor, moderate, good and high status. The lowest concentration was 1.38 $mg \cdot l^{-1}$ in May 2018 and the highest was 79.90 $mg \cdot l^{-1}$ in September 2016. TBV registered in half of the cases GES, moderate status twice and the rest were in the spectrum of better than GES (Table 3).

PCA with supplementary variables determined almost twice the percentage significance for Dim 1 compared to Dim 2 (Fig. 3). Within Dim 1, DO ($P < 4.9e\text{-}09$), OS ($P < 1.7e\text{-}11$) and VGI ($P < 2.9e\text{-}02$) are the statistically significant parameters. They have the strongest correlation coefficients and contribution for the active variables. From Figure 3 it is visible that the oxygen regime correlates negatively with VGI.

Within Dim 2, CD ($P < 0.001$), pH ($P < 0.008$) and CI ($P < 0.005$) are the statistically significant parameters. Those variables make the greatest contribution, considering active variables and with the strongest correlation coefficients, considering all. Figure 3 shows that the vector of CI is very closely located to Dim 2 and is positively correlated with the other two statistically significant variables, CD and pH.

The two significant supplementary variables from the conducted PCA were qualitative enough for linear regression modelling with all 4 PCQEs, measured in Ezeretsko Lake from 17 sampling events (n = 17) (Table 3). As a result, one model was good enough to be used for predictions [Philips et al., 2018], and therefore to be presented. Figure 4 shows that the increase in CD is related to higher results of CI in 63% of the cases. The key coefficients have the following values: $R^2 = 0.633$; Adjusted $R^2 = 0.608$; $P < 0.001$; $F = 25.819$; $t(CD) = 5.081$. From this result, it is demonstrated that an increase in the conductivity may be related to the relevant degradation of the ecological status, evaluated by the algae group index – CI.

In Ezeretsko Lake, as in Dourankoulak, the salinity parameter seems most important. We lack data about nutrient content within the lake waters, but the results are quite logical since the main pressure in Ezeretsko Lake is the same as in Dourankoulak Lake. Again, the excessive groundwater abstraction from the lake's catchment creates favourable conditions for intrusion [MoEW, 2004].

That means that for the quality of the surface waters in Ezeretsko Lake, the aquifer is vitally important. The influence of the Black Sea is very prominent in this water body since salinization

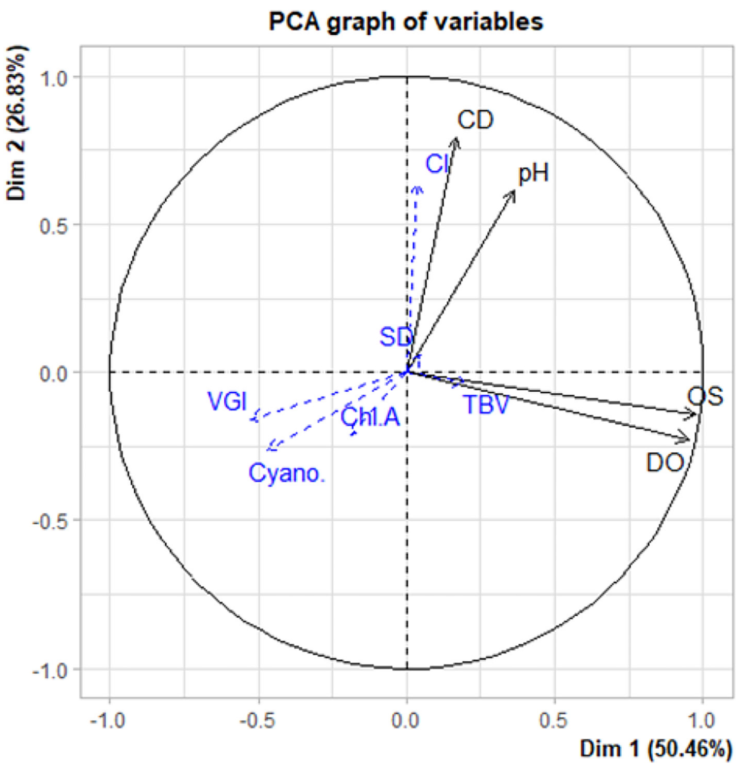

Figure 3. Ezeretsko Lake correlation circle from PCA with supplementary variables. Black vectors – active variables. Blue vectors – supplementary variables

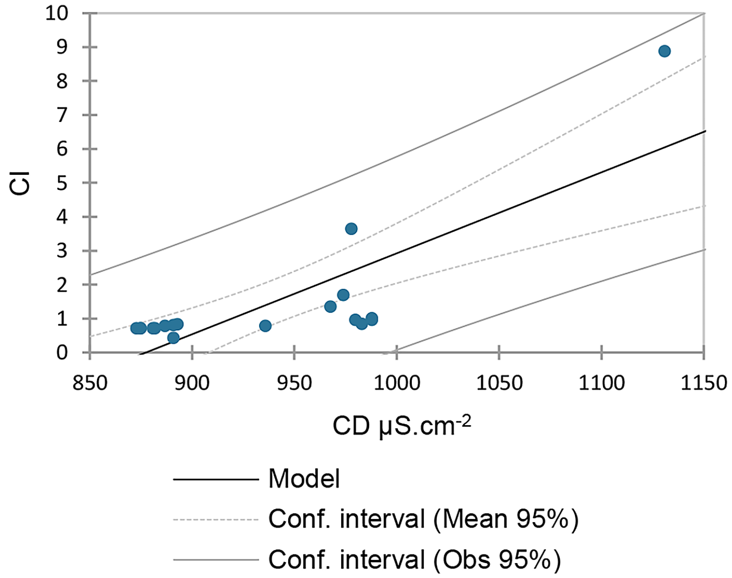

Figure 4. Linear regression model for Ezeretsko Lake

can occur on a sporadic or seasonal basis as well, by sea "splashes" and "transfer" in the presence of high waves, or indirectly by the intrusion. In addition, the intensive extraction of groundwater throughout the lake catchment intensifies the intrusion significantly. During those processes in the inland water body, chlorine and sodium ions increase their concentrations and the overall salinity of the shallow lake [MoEW, 2004].

The salinity is the total concentration of all ions. Since it is not very sustainable from a financial point of view for member states to measure all major ions, they prefer conductivity or chlorine ions, which are interchangeable if local data about the relationship between both parameters is clarified [European Commission, 2024]. For the current study and the time frame 2008–2021, that kind of information is not available. Nevertheless, CD is a salinity parameter that in shallow lakes such as Ezeretsko Lake could serve as evidence for a regime transition of a water body from "high alkalinity" to "brackish" if the rainfall is reduced or intrusion occurs [European Commission, 2024]. The latter is not an unusual phenomenon for the lake [MoEW, 2004], nor for brackish water bodies in the Antalya River basin, where the reasons for CD augmentation could be nutrient enrichment or increased evaporation [Çelekli et al., 2023]. Another example of the role of nutrient enrichment on salinization was found in Hubei province where a study on 29 shallow lakes found a significant positive correlation between nutrients in the form of total nitrogen and total phosphorus with electrical conductivity [Peng et al., 2021].

In addition, a strong negative correlation in the PCA between oxygen concentrations and VGI (Fig. 3) could be commented on as well, because increased abstraction could lead to a lowering of water levels, which in turn can make the water more turbid and more susceptible to hypoxia. It should be stated that climate change as well can also reduce the water levels because of heat waves leading to increased evaporation and altered rainfall regimes, or both impacts can act simultaneously and therefore the reduced oxygen content be the result of a multi-stressor effect [European Commission, 2024].

The above-mentioned interactions between parameters, the time gaps in data about variables of all kinds and the total lack of information about nutrient levels point out that the conduction of synchronized monitoring on several salinity and nutrient parameters (continuous, *in situ* or by sampling) could be important to shallow lake management. The derivation of locally specific dependencies from at least seasonally gathered data could ensure appropriate decisions, in the probable absence of data in the future. This would allow the regional basin directorate to consider some restrictive or restorative measures if needed.

Shablensko Lake

AA from Shablensko Lake, for the entire period (2008–2021) show that CD is permanently at moderate status, with the lowest registered value of 764 $\mu S.cm^{-2}$, from 2010 and the highest value of 2003 $\mu S.cm^{-2}$, from 2020. DO concentrations had results in the moderate status from 2013, two times in the GES range and the rest of the years were at high ecological status. The pH was always at GES. BOD_5 was predominantly better than GES but in 2008 at the worst possible status for the PCQE (Table 4). NO_3-N registered results between 1.11 mg·l^{-1} in 2010 and 4.97 mg·l^{-1} in 2008. GES was "achieved" in 6 of the years. NO_2-N and NH_4-N registered results in the range of GES and better than GES. PO_4-P registered "moderate" concentrations twice, again at the beginning of the period (2008 and 2009) and in the rest of the cases was in GES and better than GES with concentrations between 0.01 and 0.032 mg·l^{-1} (Table 4). Only in Shablensko Lake, enough measurements were conducted for Fe and Mn to consider their AAs for data analysis. (Table 4).

Historically phosphate phosphorus registered low concentrations of about 0.01 mg·l^{-1} for the period 1965–1966, about 0.4 mg·l^{-1} for the period 1992–1994 [Kalchev and Botev, 1998] and 0.054 mg·l^{-1} (Table 4) for the period 2008–2021, which shows clear reduction compared to 1992–1994. Ammonium nitrogen registered about 0.2 mg·l^{-1} for 1965–1966, about 0.3 mg·l^{-1} for 1992–1994 [Kalchev and Botev, 1998] and 0.08 mg·l^{-1} (Table 4) for 2008–2021. Again, a recent reduction in the nitrogen-containing parameter is observed. Nitrate nitrogen was almost 0.9 mg. l^{-1} for 1965–1966, beneath 0.3 mg·l^{-1} for 1992–1994 [Kalchev and Botev, 1998] and 2.12 mg·l^{-1} (Table 4) for 2008–2021. This PCQE shows augmentation of the concentrations compared to both previous periods.

PCA on PCQEs determines 35.8% significance for Dim 1 and 20.3% for Dim 2 (Fig. 5). In Dim 1, PO_4-P ($P < 7.4e-6$), NO_3-N ($P < 2.2e-5$), BOD_5 ($P < 6.2e-4$) and Mn ($P < 1.8e-3$) are the statistically significant parameters. They reached about 75% contribution to Dim 1 and obtained the strongest correlation coefficients. In Dim 2, pH ($P < 0.001$), DO ($P < 0.014$), CD ($P < 0.031$) and Fe ($P < 0.007$) are the statistically significant variables. They reached about 86% contribution to Dim 2 and had the strongest correlation coefficients.

The statistically significant parameters from Dim 1 are strongly and very strongly positively correlated in between (Fig. 5). As well, phosphate phosphorus and nitrate nitrogen are negatively correlated with ammonium nitrogen (Fig. 5).

Linear regression testing between RI, calculated in 2017 and 2020, and the PCQEs do not assure valuable results. Phytoplankton-related metrics were not considered because they were measured for several months only in 2018. Therefore, valuable comparisons between PCQEs and BQEs for Shablensko Lake are impossible.

All negatively or positively correlating PCQEs from the PCA (Fig. 5) were tested for linearity. Figure 6 shows the only statistically significant regression model based on nitrates and phosphates interaction. Figure 6 as well demonstrates, that in almost 73% of the cases, nitrate augmentation is related to phosphate saturation in the water column of Shablensko Lake. The coefficients from the linear regression analysis have

Table 4. Annual averages (AAs) of environmental variables from Shablensko Lake

Parameter	DO mg·l^{-1}	CD µS·cm^{-2}	pH	BOD_5 mg·l^{-1}	NO_3-N mg·l^{-1}	NO_2-N mg·l^{-1}	NH_4-N mg·l^{-1}	PO_4-P mg·l^{-1}	Fe mg·l^{-1}	Mn mg·l^{-1}
Sh/08	8.76	769	8.48	6.17	4.97	0.04	0.05	0.29	0.05	0.01
Sh/09	7.4	837	8.22	2.25	2.07	0.03	0.07	0.12	0.32	
Sh/10	6.93	764	8.3	3.25	1.11	0.03	0.11		0	0
Sh/11	7.84	797	8.34	3.33	1.38	0.03	0.08	0.03	0.03	0
Sh/12	6.42	996	8.37	3.55	2.19	0.03	0.08	0.03	0.021	0
Sh/13	5.83	963	8.31	2.54	1.95	0.02	0.06	0.02	0.04	0.006
Sh/14	7.56			1.18	1.9	0.05	0.04	0.01		
Sh/15	8.3			0.9	2.3	0.02	0.18	0.02		
Sh/17	8.77	913	8.47	3.2	2.07	0.025	0.028	0.025	0.002	0.001
Sh/18	8.56	905	8.62		1.66	0.037		0.027		
Sh/19	7.87	1887	8.34	1.47	2.05	0.027	0.145	0.032	0.001	0.004
Sh/20	8.49	2003	8.5	3.3	1.68	0.041		0.023	0.001	0.003
Sh/21	7.23	1431	8.35		2.24	0.02		0.019		
Mean values	7.69	1115	8.39	2.83	2.12	0.03	0.08	0.054	0.052	0.003

Note: The colour code is in accordance with the ecological status classification. Blue – high ecological status. Green – GES. Yellow – moderate ecological status. Sh/08 – AAs from 2008. Sh/09 – AAs from 2009, etc.

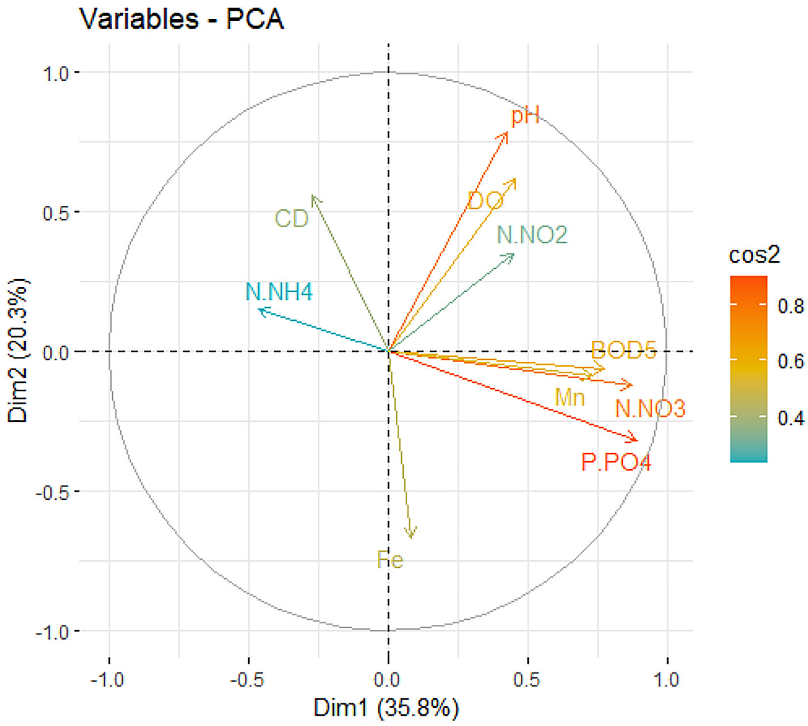

Figure 5. Shablensko Lake correlation circle from PCA, with vectors coloured according to cosine squared (cos^2) values

the following values: $R^2 = 0.727$; Adjusted $R^2 = 0.704$; $P < 0.001$; $F = 31.941$; t (PO_4-P) = 5.652.

The addition of nitrates to the water masses of shallow lakes can lead to phosphorus augmentation within the water column or its reduction. If phosphorus is reduced this can be a consequence of oxidation of the sediment and if phosphorus elevates its quantity the reason could be the response of the phytoplanktonic community and their augmenting abundance, which causes dissolved inorganic phosphorus reduction and an enhancement of the diffusion of phosphorus from the sediment towards the water column. When concentrations of nitrates are above 5–7 $mg \cdot l^{-1}$ positive correlation with phosphates is expected [Ma et al., 2021]. In the case of Shablensko Lake, the average concentration of NO_3-N is 2.12 $mg \cdot l^{-1}$ (Table 4), which is equivalent to 9.385 $mg \cdot l^{-1}$ of nitrates molecule, when converted. Therefore, the regression model (Fig. 6) demonstrates that in 73% of sampling events nitrates augmentation was related to phosphate enrichment within the water column of Shablensko Lake. This confirms the conclusion of Ma et al., [2021], related to the internal loading of phosphorus from the sediment in a dose-dependent activity of phosphates, because of the nitrate's quantity.

Other factors such as phytoplankton biomass, fish biomass, organic matter in the sediment etc. could influence internal loadings as well, nevertheless, one of them is the total nitrogen concentration [Ma et al., 2023]. Considering that most European lakes are highly responsive to nutrient enrichment, with their phytoplankton and macrophyte assessment systems, especially from phosphorus [Poikane et al., 2022] the need for nitrate reduction in Shablensko Lake is justified.

The most effective practices to reduce internal loadings could include excavation of sediments, aeration, oxygenation, hypolimnetic withdrawal or adding substances [Poikane et al., 2024], but even giving more space for grasslands and forests in the areas bordering agricultural surroundings could be beneficial. They are currently very scarce (Fig. 7), but their augmentation could contribute to nitrogen reduction, if we refer to the negative correlation between the areas occupied by natural vegetation and the nitrogen quantity in shallow lake waters [Peng et al., 2021].

CONCLUSIONS

Lakes provide a great spectrum of essential services for the surroundings [Poikane et al., 2024]. Unfortunately, anthropogenic pressure as excessive groundwater abstraction, agricultural activities, fish stocking and others could worsen their quality. This

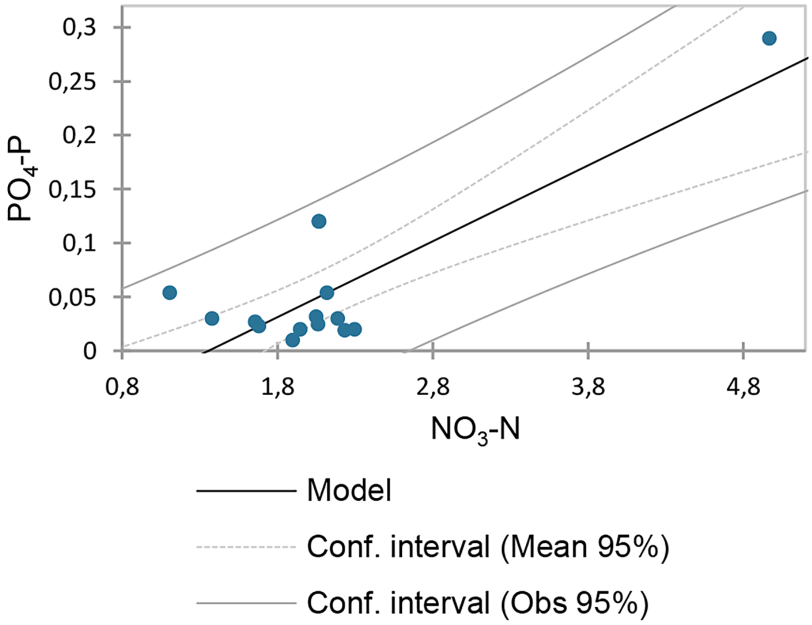

Figure 6. Linear regression model for Shablensko Lake

Figure 7. Drone photography at Shablensko Lake from the beginning of 2024 in a northwest direction

is why in the drainless area of northeast Bulgaria where rivers are not present even in the smallest scales, Dourankoulak Lake, Shablensko Lake and Ezeretsko Lake must be maintained at the present status or even restored, to ensure sustainable services and ecosystem health.

All data that we used for analysis in the current study allowed us to find that in Ezeretsko Lake the increase in the conductivity acts as the main pressure on the phytoplanktonic community, which is reflected by the algae group index or the Catalán Index – CI (Fig. 4). For Dourankoulak Lake results were similar, because conductivity influenced the algae group index expressed by its EQR value – VGI. Unlike Ezeretsko Lake, in Dourankoulak Lake this was less pronounced due to the lack of statistically significant linear regression models.

Those results suggest that for L-EC1 in Bulgaria, further groundwater abstraction from their water bodies should be restricted or better managed, to ensure adequate groundwater recharging by the aquifer, which could result in a decrease in the overall salinity within the inland water bodies.

Shablensko Lake demonstrated that nitrate augmentation in comparison to a long past period, is connected to phosphate enrichment in the water column. This could be a consequence of increased evaporation and altered rainfall regime due to climate change, nutrient resuspension from available fish stocks, internal loading processes, diffusive pollution from cultivated lands and reduced recharging from the aquifers, due to an increased abstraction.

We cannot neglect the fact that recreational fishing could be the main reason for the enrichment with phosphorus, since Poikane et al., [2022] pointed to a main pressure and main lake use of this type of activity within the intercalibration group L-EC1. As an example can be given that the feeding behaviour of common carp could lead to nutrient enrichment by resuspension from the bottom layer [Qui et al., 2019]. Unfortunately, fish monitoring was not included in the state monitoring for the period, nor data about the stocking activity was available. Therefore future projects or monitoring programs should be more complex and to consider the results described here if better understanding is the main goal.

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