Seasonal Variation of Eutrophication in Some Lakes of Danube Delta Biosphere Reserve

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ABSTRACT: To understand the trophic state of lakes, this study aims to determine the dynamics of phytoplankton assemblages and the main factors that influence their seasonal variation. Sampling campaigns were carried out in three lakes from the Danube Delta Biosphere Reserve. Spectral analysis of specific phytoplankton pigments was applied as a diagnostic marker to establish the distribution and composition of phytoplankton taxonomic groups. Fluorescence spectroscopy was used to quantify changes in dissolved organic matter (DOM). The relative contribution of the main phytoplankton groups to the total phytoplankton biomass and the trend of development during succession of the seasons showed that cyanobacteria could raise potential ecological or human health problems. Moreover, fluorescence spectroscopy revealed that Cryptophyta and cyanobacteria were the main contributors to the protein-like components of DOM. It was concluded that fluorescence could be used to provide a qualitative evaluation of the eutrophication degree in Danube Delta lakes. *Water Environ. Res.*, **89,** 87 (2017).

KEYWORDS: aquatic ecosystem, algae, dissolved organic matter, fluorescence spectroscopy.

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Introduction

Phytoplankton is a main indicator of an increasing trophic state in aquatic ecosystems and the increasing biomass is the first response to nutrient pollution and the most visible effect of eutrophication. General analyses and reviews over the past two decades have identified that in the case of most shallow freshwater ecosystems, huge amount of algal biomass and frequent algal blooms have been recorded, which are an important sign for an eutrophic or hypereutrophic aquatic ecosystem (Coops et al., 2008; Huang et al., 2014; Isenstein et al., 2014; Pinto et al., 2014; Török, 2011). The development of phytoplankton in shallow lakes, as those investigated in the present study, depends on the following regulatory factors: water temperature, light availability, nutrient availability, flushing, sedimentation, resuspension, humic acids, and zooplankton grazing (Scheffer, 1998).

Seasonal variation of phytoplankton is one of the most important factors that influence the trophic state of water and contribute to the well-being and conservation of the species present in the trophic chain (Oosterberg et al., 2000). Moreover, seasonal changes in species composition due to disturbance caused by eutrophication, favors the emergence of opportunistic species such as cyanobacteria. Cyanobacteria are the Earth's oldest oxygenic photoautotrophs, being the most obvious and troublesome algal species due to their contemporary ecological "success" and increasingly frequent harmful cyanobacterial blooms, which have had major impacts on aquatic ecosystems (Paerl and Otten, 2013). Studies made over recent decades have highlighted physical and chemical seasonal drivers that provide favorable conditions for cyanobacteria development. The World Health Organization (WHO) expressed concerns about the effects of cyanobacteria and provided guidance for monitoring and management for the safety of population, while the European Commission, through its Water Framework Directive, offered an ecological approach to the water resources management in Europe. The World Health Organization established the thresholds for chlorophyll-a (chl-a) of 10 μg/L (Alert Level 1) for increased odds of irritative or allergenic effects, and 50 μg/L (Alert Level 2) for increased probability of irritative symptoms and toxic impacts, both under conditions of cyanobacterial dominance (Chorus and Bartram, 1999; Poikane et al., 2011).

Due to the large number of lakes inside the Matita-Merhei hydrological sub-unit of the Danube Delta and the isolation due to large area cover by reed, which is by far the dominant species in the Danube Delta, covering almost 160 000 ha (Oosterberg et al., 2000), a small number of lakes have been included in the monitoring program of water quality in Danube Delta Biosphere Reserve. However, gaps regarding ecological processes are considered by the researchers or authorities. To fill the gaps and to improve the knowledge on phytoplankton variation, three

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Table 1—Variation of depth and light availability during the investigation period (2014).

Lake	Shallowest depth (cm)	Deepest depth (cm)	Lowest Secchi depth (cm)	Highest Secchi depth (cm)
Dracului	100	180	80	140
Matita	100	360	40	220
Trei Iezere	90	180	50	180

uninvestigated lakes located inside the above-mentioned hydrological sub-unit have been recently included into the monitoring program and in the present study, aiming to observe the seasonal variation of phytoplankton and to determine the impact of cyanobacteria development on trophic chain. To evaluate the aquatic ecosystems characteristics and to identify the physico-chemical parameters that had the highest impact on the development of phytoplankton communities, basic limnological parameters and fluorescence indices were used.

Material and Methods

Sampling Details. Danube Delta is located in the south-eastern part of Europe, having 84% of its surface in Romania, out of the total area of 4180 Km^2. This area begins at the first bifurcation of the Danube River at Ceatal Chilia being bordered by the Black Sea to the east, Razim Lagoon to the south and Ukraine to the north. Having more than 300 lakes inside of the three main branches of the Danube River and four hydrological sub-units, it is one of the largest protected areas in Europe (Gâştescu and Ştiucă, 2008). The Danube Delta lakes are classified into three categories: type 1 lakes, which are 2 to 4 m deep and larger than 200 ha, having sand-silt substrate and an intermediate inflow of river water. These lakes are turbid with high abundance of cyanobacteria and cladocera, a low abundance of aquatic vegetation, and a predominantly eurytopic fish community; type 2 lakes, which are in the zone with a high river water input. These lakes are intermediate in size and water depth having a strong seasonal dynamics in water level; the lakes have clear water, zooplankton is scarce, but is abundant in filamentous algae, aquatic vegetation, and the fish community is predominantly eurytopic; type 3 lakes are located in the parts where reed colonization and peat accumulation are dominant. These lakes are relatively small and shallow with clear water, low abundance of zooplankton, a high abundance of aquatic vegetation, and eurytopic fish are scarce (Oosterberg et al., 2000).

Recently, Török (2011) showed that the Danube Delta lakes are not restricted to this typology and suggested a change of monitoring programs because the existing ones, which use microscopy techniques for determining algal biomass, do not allow rapid estimation of algae impact on aquatic ecosystems and preclude effective warning systems and control of eutrophication. In this respect, more lakes have been included into monitoring programs.

Therefore, Matita, Dracului, and Trei Iezere lakes were included in this study. In the case of Matita Lake, previous investigations were undertaken (Cristofor et al., 2003; Vădineanu et al., 1992); however, Dracului and Trei Iezere lakes were recently included in this program. These two lakes belong to the Matita-Merhei hydrological sub-unit of the Danube Delta, having indirect connection with the main river channel. The differences in the main morphometric features and light availability at the sampling stations are presented in Table 1, as these may be important factors that affect the phytoplankton seasonal variation.

In the Matita-Merhei complex, isolated lakes can be found, with a high residence time of 300 days depending on the distance from the Danube River (Oosterberg et al., 2000), which are independent of the water level and have a surface area of floating reed relatively constant during the seasons and a decreasing inundated reed area during the dry period. The flooding period influences the seasonal variation of water level inside the delta, being strongly correlated with the discharge of water from Danube River and having a small range in amplitude in wintertime, followed by a significant inflow of water in summertime, and having another minimum in autumn (Oosterberg et al., 2000).

The field campaigns were carried out from spring to autumn 2014. The lakes were surveyed by motorboat and the travel route included the edge of the lakes near the reed-bed shore and transects over the lake to evaluate the presence or absence of aquatic vegetation. Nine sampling points were established for physico-chemical measurements and phytoplankton biomass analyses (expressed as chl-a concentration). Chlorophyll-a measurements were undertaken by analyzing the pigments with high-performance liquid chromatography (HPLC). In situ measurements were made with the bbe Moldaenke GmbH environmental technique, which identifies the phytoplankton class based on precalibrated excitation and emission spectra, "fingerprints" programmed into the instrument (http://www.bbe-moldaenke.de/en/).

The investigated spectral groups of algae (Chlorophyta, cyanobacteria, diatoms, and Cryptophyta) are each characterized by a specific composition of photosynthetic antenna pigments and, consequently, by a five-point fluorescence excitation spectrum of a water sample. Particularly, chl-a, phycocyanobilin, phycoerythrobilin, fucoxanthin, and peridinin are relevant (Beutler et al., 2002; Catherine et al., 2012; Kring et al., 2014). The distribution of spectral algal groups was measured in the depth profile of 0.5 m from the surface of the water, due to the low depth of the lakes (Table 1). Nine measurement replications were done per site. The measurements were used to calculate the average value per each sampling site. Sampling was done at noon time, between 12 and 14 hours, once per season. Due to the impossibility of reaching sampling points in autumn (large area covered by floating and submerged vegetation), the number of sampling stations was lower (5 sample sites) compared to spring sampling (9 sample sites) The sampling sites, shown in Figure 1, are indicated by red in spring, yellow in summer, and white in autumn.

Field observation includes weather conditions: direction and intensity of wind and sky cover. Water samples were taken from

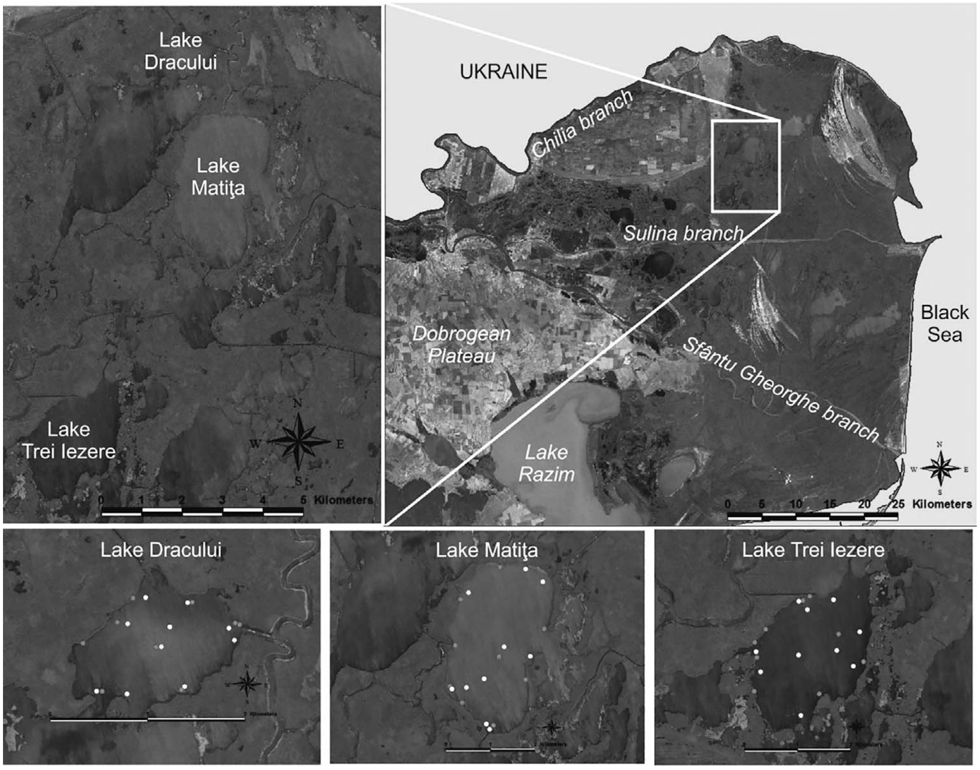

Figure 1—Sampling area in the Danube Delta Biosphere Reserve.

the surface for nutrient analysis, in which case, to stop bacterial transformations, the samples were preserved with H_2SO_4 for total nitrogen, stored in Plexiglas container and transferred to laboratory for further processing within 24 hours of arrival onshore. For fluorescence spectroscopy measurements, samples were kept in dark conditions, at 4 °C, in glass bottles.

Physico-Chemical Parameters Determination. Physico-chemical parameters were measured at each sample site, using Hanna (Woonsocket, Rhode Island) instruments, as follows: model no. HI 9146-04, serial no. B0092080 for dissolved oxygen (DO); model no. 9835, serial no. B0077175 for conductivity and temperature; pH and turbidity were measured at the same depth of water as in the case of algae biomass. Water depth and water transparency were measured with a Secchi disk.

The ammonium concentration was determined at 655 nm, using the manual spectrophotometric method (SR ISO 7150-1) of a UVVIS Lambda 10 PerkinElmer (Waltham, Massachusetts) Spectrometer. The method involves the measurement of the blue compound absorption, formed by the reaction of ammonium ion with salicylate and hypochlorite ions, in the presence of sodium nitroprusside.

Nitrites were determined through formation of reddish purple azo dye, produced at pH 2.0 to 2.5 by coupling diazotized sulfanilamide with N-(1-naphthyl)-ethylendiamine dihydrochloride. The procedure was done according to method SR EN 26667/ISO 6777/2002 (Determination of nitrite. Molecular absorption spectrometric method, using the UVVIS Lambda 10 PerkinElmer Spectrometer at 540 nm).

Nitrates were determined according to method SR ISO 7890-3:2000 (spectrometric method using sulfosalicylic acid), by measuring the yellow compound absorbance, formed by reaction of sulfosalicylic acid (formed by addition of sodium salicylate in the sample and sulfuric acid) with nitrate followed by treatment with alkaline solution. The UVVIS Lambda 10 PerkinElmer Spectrometer (415 nm) was used.

For organic nitrogen, the samples were analyzed according to method SR ISO 5663-1984, using the UVVIS Lambda 10 PerkinElmer Spectrometer at 655 nm.

The ortho-phosphate (PO_4^{-3}) expressed as mg/L, was determined according to method SR EN 6878/2005 (ammonium molybdate spectrometric method), using the UVVIS Lambda 10 PerkinElmer Spectrometer at 880 nm. Filtration through a 0.45-μm-pore diameter was used to separate dissolved phosphorus from suspended forms of phosphorus. For total phosphorus (TP), the samples were treated on unfiltered water.

Phytoplankton Pigments Determination. The FluoroProbe 1.9.7 spectrofluorometer (bbe Moldaenke, GmbH, Germany) provides in situ measurements of total chl-a and further resolves this into four major phytoplankton (algae and cyanobacteria) classes using differences among fluorescence excitation spectra. The FluoroProbe uses five light-emitting diodes (LEDs, operating at 470, 525, 570, 590, and 610 nm) to excite the accessory pigments associated with photosystem II antenna system. Changes in the resulting chl-a emission allows for fluorometric estimation of algal classes based on differences in species and class-dependent peripheral antenna pigments (Beutler et al., 2002, Kring et al., 2014; Richardson et al., 2010).

For phytoplankton pigments' evaluation, the shape of the spectral fluorescence signature was used to distinguish between taxa, while the fluorescence intensity and the group-specific fluorescence/chl-a ratios were used to estimate total phytoplankton biomass (as chl-a) (Richardson et al., 2010). In this respect, phytoplankton analysis was made using the fluorescence spectrum at the following excitation wavelengths: 470 nm LED for green algae; 610 nm LED for blue green algae; 525 nm LED for diatoms; 570 nm LED for cryptophyceae.

Emission Excitation Matrix Determination. To facilitate pattern recognition and to identify the most relevant spectral properties in surface water systems, emission excitation matrices (EEMs) were recorded with the Edinburgh Instruments FLS920 spectrofluorimeter. The following parameters were used to record the EEMs: excitation wavelength range 240 to 400 nm; emission wavelength range 260 to 500 nm. In addition, fluorescence indices (humification index [HIX], biological index [BIX], and F450/F500 index) were determined to evaluate the seasonal variation of the aquatic ecosystems characteristics. HIX, BIX, and F450/500 were calculated according to Huguet et al. (2009).

Statistical Approach. Statistical data processing was performed taking into account which metrics are best to use for assessing the ecological status of European lakes, where eutrophication is the dominant pressure factor (Lyche-Solheim et al., 2013). In this respect, the most sensitive metrics for phytoplankton, established during intercalibration exercises, are chl-a, the taxonomic composition trophic index (PTI), the functional traits index (FTI), and cyanobacteria bloom intensity metric (CYANO).

$$\mathrm{PTI} = 1.247\log(\mathrm{TP}) - 1.572 \tag{1}$$

$$\mathrm{FTI} = 0.034\log(\mathrm{TP}) + 1.561 \tag{2}$$

where TP is total phosphorus and CYANO is

$$\log(\mathrm{CYANO} + 0.01) = 1.217\log(\mathrm{TP}) - 2.589 \tag{3}$$

During intercalibration exercises, chl-a data from 6532 water bodies from 22 European countries were used to develop the eutrophication-related metrics. The metrics tested for responses to eutrophication comprise five different metrics for phytoplankton. Linear regression models and *t* tests of standardized (*z*-transformed) data were used to compare regression strengths (correlation coefficient *r*) and response sensitivity of the metrics. The metrics was regressed against TP, which provided an independent variable reflecting eutrophication pressure intensity in this analysis (Lyche-Solheim et al., 2013).

In order to reveal the relationship between the phytoplankton development and physico- chemical parameters average (mean), variance (VAR), standard deviation (STDEV), coefficient of dispersion (CD), and the degree of correlation between phytoplankton biomass and abiotic parameters was calculated using Pearson's *r* correlation (Helsel and Hirsch, 2002). Data set used for the above mentioned correlation included average values of each parameter per season.

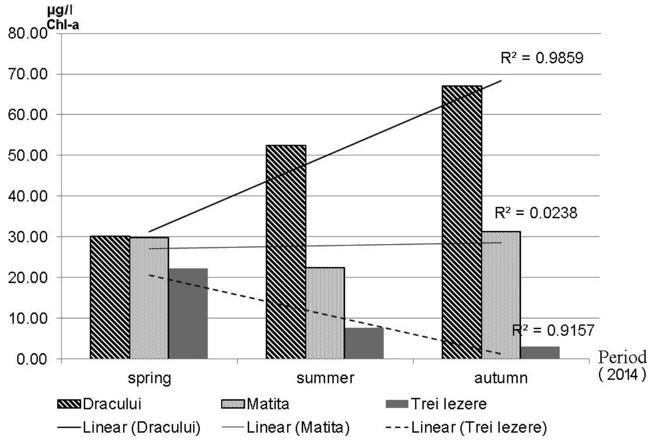

Figure 2—Seasonal variation of chlorophyll-a.

The linear correlation coefficient (r) was tested at $P = 0.05$ and $P = 0.01$ in the table for the Product Moment Correlation Coefficient for a certain df $= (n - 2)$

According to value (r) the relationship between phytoplankton biomass and abiotic parameters was classified as follows: very weak correlation 0.00 to 0.19, weak correlation 0.20 to 0.39, modest correlation 0.40 to 0.69, strong correlation 0.70 to 0.89, and very strong correlation 0.90 to 1.00.

Results and Discussion

Dynamics and Seasonality of the Phytoplankton Based on Environmental Conditions. Using chl-a as a proxy for phytoplankton biomass, previous evaluations in the Danube Delta, showed that the extraction and fluorimetric methods, performed with in situ fluorescence and HPLC instruments, presented a highly significant correlation ($r^2 = 0.97$) (Nemeth et al., 2002; Török, 2014).

In the present study, decreasing or increasing trends of phytoplankton growth, over the seasonal cycle, emerge through comparison of chl-a concentrations reported in the three lakes on the same aquatic complex of the Danube Delta (Figure 2). The results are in agreement with the findings of Xu and Xu (2015), who showed that, generally, chl-a presents a seasonal variation, with higher values in autumn compared to summer and spring.

The evidence of opposite trend of phytoplankton development is noteworthy when we compare the seasonal variations of chl-a concentration between Dracului and Trei Iezere lakes. The main proxy indicator for phytoplankton biomass recorded during the investigation period has shown that the average concentration per lake/season had huge variation in the case of Dracului Lake, which was the most eutrophic lake. The level of eutrophication in Dracului Lake had an increasing trend during seasonal cycle. According to Romanian water quality classes (Official Monitor of Romania, Part I, no. 511 [13 June 2006]), Dracului Lake is a hypereutrophic lake having its mean values of the chl-a concentration around 30.06 μg/L (variation between 18.73 μg/L to 48.64 μg/L) in spring, 52.40 μg/L (variation between 40.92 μg/L to 59.01 μg/L) in summer, and 67.08 μg/L

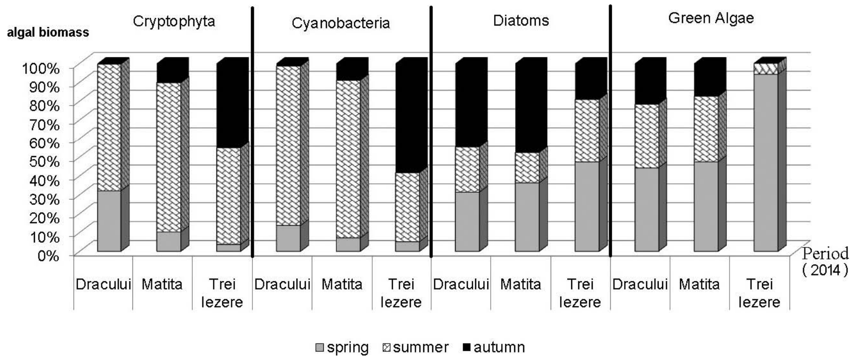

Figure 3—Seasonal variation of algae taxonomic groups.

(variation between 34.37 μg/L to 85.21 μg/L) in autumn. Submerged plants covered the bottom of the lake during the entire investigation period. All three lakes were almost completely filled with vegetation (typically *P. trichoides*) due to the indirect connection with the Danube River and to no or low turbidity for the entire investigated period. The distribution of submerged vegetation in the water column showed the dominance of species with upright growth associated with non-limiting light conditions (Cristofor et al., 2003). The presence of aquatic vegetation inhibits the development of phytoplankton due to competition for the same nutrient resources. Consequently, it might be assumed that the cause for the seasonal increase of chl-a could be the presence of the mixed populations (phytoplankton and periphyton stemmed or scraped from the vegetation) into the water column, which contribute to the increasing concentration of chlorophyll.

The mean trophic state of the Matita Lake has been recorded at 29.76 μg/L in spring (13.54 μg/L to 47.8 μg/L), 22.37 μg/L in summer (4.28 μg/L to 54.99 μg/L), and 31.22 μg/L in autumn (15.07 μg/L to 47.47 μg/L). The seasonal chl-a variation for Trei Iezere Lake showed a decreasing trend with a mean concentration of 22.34 μg/L (15.47 μg/L to 41.44 μg/L) in spring, 7.60 μg/L (3.57 μg/L to 12.44 μg/L) in summer, and 3.02 μg/L (2.54 μg/L to 4.56 μg/L) in autumn. The likely cause for the decreasing trend of the phytoplankton biomass in Trei Iezere Lake, in comparison with Dracului Lake, could be the surrounding reed bed and the influence of the humic substances' release and circulation below the floating reed bed. Other potential contributors to this decreasing trend could be the flushing of the phytoplankton due to water circulation through the reed bed and the inadequate light exposure due to the presence of the aquatic vegetation (species as: *Trapa natans*, *Stratiotes aloides, Potamogeton crispus,* and *P. trichoides*), which covers almost 80% of the water surface.

In the case of selected lakes, the relative contribution of the different taxonomic groups of the phytoplankton community to total chl-a has shown that cyanobacteria (or blue-green algae) varies between 17.97% in Dracului Lake to 38.12% in Trei Iezere Lake reaching the maximum concentration during summer in Dracului Lake (about 9.42 μg/L cyanobacteria) (Figure 3). The recorded value was below the threshold limit of phytoplankton biomass established by WHO (Chorus and Bartram, 1999; Poikane et al., 2011).

Taking into account all of the above-mentioned aspects, the trend of seasonal succession in the Danube Delta suggests that the development of cyanobacteria may give rise to potential ecological or human health problems. The threshold for Alert Level 2 (Chorus and Bartram, 1999; Poikane et al., 2011), where limits for the presence of toxins were confirmed by chemical or bioassay techniques, was recorded in the case of Dracului Lake, both in summer and in autumn.

The physico-chemical parameters measured during the investigated period, are different between the three lakes. To identify how much oxygen is produced during photosynthesis of algae (Table 2) the DO and water temperature calculated to percent DO saturation shows that in Dracului Lake, percent DO saturation was high in all three seasons (86.84, 86.19, and 87.86%); in Matita Lake, the saturated DO increased from spring to autumn (57.48, 70.01, 77.61%); meanwhile in Trei Iezere Lake, the saturated DO showed a decreasing trend (percent saturated DO in spring, summer, and autumn were 86.04, 33.16, and 74.41, respectively). These percentages of DO saturation corresponded to the chl-a results. The spring concentration of orthophosphate, PO_4^{-3}, is quite low (0.006 to 0.013 mg/L), probably due to its immediate utilization by phytoplankton, being 3 to 5 times higher in summer.

Within lakes, the temporal (seasonal) natural variation for phytoplankton was low for the metrics of PTI ($R^2 = 0.097$ in Matita Lake, $R^2 = 0.11$ in Trei Iezere Lake, and $R^2 = 0.097$ in Dracului Lake). There was no variation recorded for FTI metrics, a very low variation for chlorophyll-a in Matita lake ($R^2 = 0.0642$), and high variation in Trei Iezere Lake ($R^2 = 0.7928$) and Dracului Lake ($R^2 = 0.995$).

Between lakes, the temporal natural variation for phytoplankton was high in spring ($R^2 = 0.7895$) and very low in summer ($R^2 = 0.0151$) and autumn ($R^2 = 0.0439$) for the metrics of PTI. In the case of FTI metrics, no variation was recorded. In the case of chl-a, very high variation was observed, as follows: $R^2 = 0.953$ in spring, $R^2 = 0.8429$ in summer, and $R^2 = 0.9198$ in autumn. Cyano bloom intensity was not considered an important variance factor due to the presence of aquatic vegetation.

Table 2—Average value/seasons 2014.

Lakes	Season	Depth (cm)	Secchi depth (cm)	Turbidity (NTU)	DO (%)	EC (μS/cm)	t °C	TN (mg/L)	PO_4^{-3} (mg/L)	TP (mg/L)
Dracului	Spring	180	120	0.00	10.62	443	6.7	0.866	0.008	0.022
	Summer	140	120	0.00	8.49	355	16.1	0.593	0.031	0.071
	Autumn	140	100	0.00	10.28	393	8.5	0.284	0.015	0.039
Matita	Spring	360	140	0.00	11.27	432	6.3	1.246	0.006	0.023
	Summer	220	220	0.00	6.84	394	16.5	0.367	0.030	0.053
	Autumn	220	200	0.00	9.15	388	8.2	0.467	0.011	0.030
Trei Iezere	Spring	190	100	0.00	10.42	444	7.1	1.397	0.013	0.051
	Summer	170	170	0.00	3.22	376	16.8	0.559	0.049	0.075
	Autumn	160	160	0.92	8.58	412	9.1	0.658	0.027	0.042

In the Danube Delta lakes, seasonal variation of nutrients, especially for phosphorus is more pronounced than in the Danube River, which is influenced by the river flood pulse and the connectivity with the main channels. Historical data showed that the average of TP concentration over the changing season (April–October) varied between 0.08 to 0.15 mg TP/L and total nitrogen varied between 1.4 to 2.4 mg N/L. Differences between lakes in phosphorus are low. There is a seasonal dynamics (with low—possibly limiting—concentrations of phosphorus in spring, and summer concentrations significantly higher), which influence the development of phytoplankton. Along with this seasonal pattern, a phosphorus concentration of accumulation and release appears to be present in the Danube Delta lakes. The extensive inundated reed beds, around many of the lakes, are efficient removers of nitrogen in spring and summer, but may release significant amounts of phosphorus in summer (Oosterberg et al., 2000).

Differences between lakes in nitrogen are significant. The lakes located closer to the river have higher quantities of nitrogen, while lakes situated farther from the river present low nitrogen. Spring nitrogen values are high and summer values are 1.5 to 3.5 times lower.

The oxygen state of the lakes has a strong diurnal variation, being very low in the morning, especially at Dracului and Trei Iezere lakes due to the biological processes and the consumption of the oxygen by the submerged vegetation. Typically low values are recorded at sunrise and high values are recorded in the afternoon. The analysis of Pearson correlation highlighted the response of the phytoplankton biomass expressed as chl-a and explanatory variables (Table 3). For all the lakes, very strong and strong correlation between chl-a depth, transparency, and total nitrogen has been established.

Dracului and Trei Iezere lakes do not present important processes as flushing of phytoplankton and nutrient input during the flood pulse. Also, nutrient-induced algal blooms after the flood pulse, caused by the significant distance from the Danube River and by the strong hydrological relationship with the River, have not been observed. The low influence of the flood pulse is essential for mass development of aquatic vegetation, which contributes to decreasing the nutrient concentration and light availability, releasing allelopathic substances, which in turn contribute to the inhibition of cyanobacteria development.

Dissolved Organic Matter Fluorescence. Two main fluorescence peaks in the EEM were assessed: peak T ($\lambda_{excitation}/\lambda_{emission} = 280/330$ nm) representing the protein-like fraction of DOM and peak C ($\lambda_{excitation}/\lambda_{emission} = 310/420$ nm) associated with the humic-like component. Generally, peak T indicates the microbial activity in the water system, while peak C relates to microbially reprocessed DOM (Hudson et al., 2007). Protein-like and humic-like components were reported in previous studies on algal and phytoplankton cultures (Ferrari and Mingazzini, 1995; Fukuzaki et al., 2014; Korak et al., 2015;

Table 3—Correlations between phytoplankton biomass and physico-chemical parameters (STDEV = standard deviation, CD = coefficient of dispersion, r = correlation coefficient).

	chl-a (μg/L)	Depth (cm)	Secchi depth (cm)	Turbidity (NTU)	O_2 (%)	EC (μS/cm)	t °C	TN (mg/L)	PO_4^{-3} (mg/L)	TP (mg/L)
Dracului										
STDEV	28.45	23.09	11.55	0.00	1.14	44.14	4.99	0.29	0.01	0.03
CD	14.62	3.48	1.18	0.00	0.13	4.91	2.39	0.15	0.01	0.01
r		–0.90	-0.83	0.00	–0.22	-0.62	0.25	–0.99	0.36	0.40
Matita										
STDEV	7.99	80.83	41.63	0.00	2.22	23.86	5.42	0.48	0.01	0.02
CD	3.23	24.50	9.29	0.00	0.54	1.41	2.85	0.33	0.01	0.01
r		0.70	-0.85	0.00	0.97	0.61	–1.00	0.77	–1.00	–1.00
Trei Iezere										
STDEV	13.74	15.28	37.86	0.65	3.74	34.02	5.12	0.46	0.02	0.02
CD	16.72	1.35	10.00	0.92	1.89	2.82	2.38	0.24	0.01	0.01
r		0.96	–0.98	–0.54	0.66	0.82	–0.62	0.98	–0.80	0.70

Table 4—Fluorescence intensity for peaks T and C, and fluorescence indices.

Samples	Peak T fluorescence intensity (a.u.)	Peak C fluorescence intensity (a.u.)	T/C	HIX	BIX	F450/F500
Dracului Lake						
Summer	45 183	9000	5.02	2.49	0.55	1.27
Autumn	13 370	22 890	0.58	3.39	0.76	1.19
Spring	8441	14 480	0.58	4.67	0.8	1.29
Matita Lake						
Summer	42 690	15 008	2.84	4.49	0.44	1.05
Autumn	17 540	26 850	0.65	4.51	0.77	1.22
Spring	9017	13 920	0.65	4.31	0.82	1.27
Trei iezere Lake						
Summer	7108	12 914	0.55	4.76	0.77	1.31
Autumn	11 960	22 170	0.54	4.92	0.77	1.24
Spring	8907	14 390	0.62	4.57	0.81	1.25

Liberzon et al., 2016; Nguyen et al., 2005; Rochelle-Newall and Fisher, 2002; Zhou et al., 2015). In particular, high protein-like fluorescence is generated by cyanobacteria (Korak et al., 2015), Cryptophyta, and diatom cultures (Fukuzaki et al., 2014). Also, Liberzon et al. (2016) found that protein-like fluorescence correlated strongly with phytoplankton biomass and suggested that this fraction could serve as an indicator of aquatic production.

Both peak T and peak C showed seasonal variation at the three lakes (Table 4). Peak T fluorescence intensity was inconsistent between lakes, displaying high values in summer at Dracului and Matita lakes, but for Trei Iezere the highest values were recorded in autumn. Peak T presented high values at the samples with high quantities of Cryptophyta, showing a strong correlation with this parameter ($r = 0.89$) (Table 5). These results suggest that Cryptophyta could be the main contributor to the protein-like component, among the studied phytoplankton species. These findings are confirmed by the study of Fukuzaki et al. (2014) where Cryptophyta species showed significantly higher protein-like fluorescence compared to the diatom species. Peak T also correlates with cyanobacteria ($r = 0.77$), which is in accordance with the findings of Korak et al. (2015). No other significant correlation has been obtained for peak T with other phytoplankton species.

To gain better understanding of the algal-derived DOM, the ratio between peak T and peak C was calculated (Table 5). This parameter indicates the predominance of DOM components. A close relationship was found between T/C ratio and the quantity of Cryptophyta ($r = 0.98$) and of cyanobacteria ($r = 0.92$). No clear relation of phytoplankton with other parameters was observed. These findings confirm that, among the analyzed phytoplankton species, Cryptophyta and cyanobacteria are the main contributors to the protein-like fluorescence. Moreover, T/C ratio could be used to indicate the presence of Cryptophyta or cyanobacteria in particular water systems.

Table 5—Correlation coefficients between fluorescence and phytoplankton parameters.

	T	C	T/C	HIX	BIX	F450/F500
Green algae	0.24	–0.33	0.42	–0.84	–0.06	0.17
Cyanobacteria	0.77	–0.55	0.92	–0.67	–0.65	0.05
Diatoms	0.00	0.26	0.02	–0.65	0.10	–0.07
Cryptophyta	0.89	–0.54	0.98	–0.64	–0.79	–0.15
chl-a	0.19	0.04	0.27	–0.83	–0.05	–0.02

Peak C showed a consistent seasonal variation, with higher values in autumn compared to spring and summer, at all three lakes. No significant positive correlation was observed between peak C and phytoplankton. These results could be explained by the study of Rochelle-Newall and Fisher (2002), who assessed the fluorescence of algal-derived DOM at wavelengths close to the position of peak C. They found that, although the algal cultures showed high DOM fluorescence, this signal was not originating from DOM directly produced by phytoplankton. Thus, in the current study, the humic-like component could partly be the result of a subsequent process in algal activity and degradation, making it difficult to differentiate the algal derived humics from the background humic substances originating from other sources.

The fluorescence indices, HIX, BIX, and F450/F500 were calculated to obtain better understanding of DOM characteristics from the three lake samples (see Tables 4 and 5). Based on the classification provided by Huguet et al. (2009), with regard to HIX, most samples, from the current study, present a weak humic character and an important recent autochthonous component. The summer and autumn samples from Dracului Lake contain DOM of biological or aquatic bacterial origin. These results confirm that part of DOM is derived from algal activity, as shown by peak T fluorescence intensity and T/C ratio. On the contrary, the highest HIX value, indicator of a more humic and allochthonous character compared to the other samples, was observed at the autumn samples from Trei Iezere Lake, which also has the highest preponderance of humic substances. Humification index values also showed a strong inverse correlation with green algae and chl-a. This could indicate that high quantities of phytoplankton could potentially reduce the humification process. However, more studies are needed to validate this assumption.

Biological index values show that most samples present intermediate (0.7 to 0.8) or strong (0.8 to 1.0) autochthonous components. However, the summer samples from Dracului and Matita lakes, which have the highest peak T, present the lowest BIX values. The results could indicate low DOM production and the presence of "old" organic matter (Huguet et al., 2009). The low BIX samples could also indicate other sources of DOM, non-algal derived. Considering that BIX and HIX use different regions within the EEM, the two parameters investigate different fractions of DOM, which could explain the different results. In addition, Zhou et al. (2015) found that the protein-like fraction could have a secondary source of algal scums. These finding are also supported by the work of Rochelle-Newall and Fisher (2002) regarding DOM as not being directly derived from phytoplankton. F450/F500 has further shown that all samples contain a major source of allochthonous DOM and, according to Nguyen et al. (2005), only values above 1.6 would indicate algal-dominated waters. Hence, phytoplankton generates a large quantity of protein-like and humic-like fluorescence, but it contributes, together with other major autochthonous and allochthonous sources, to the complex pool of DOM.

Conclusions

Techniques applied in this study significantly improve the eutrophication assessment and control system by extension of the areas (lakes as Dracului, Trei Iezere, and Matita, which have not been frequently monitored previously) that can be monitored in real-time, by providing relevant information on the state of water quality in the Danube Delta Biosphere Reserve. This is the first study that assesses the relationship between the seasonal variation of phytoplankton and the fluorescence of DOM components for samples collected from Delta lakes. In addition, basic limnological parameters such as water depth, transparency, temperature, turbidity, oxygen concentration, and nutrient concentration highlighted the highest impact on the development of phytoplankton communities. The present analysis revealed that total phosphorus concentration might not be the primary driver of phytoplankton biomass abundance. The analysis showed that chl-a increased during the seasons even if, in the summer–autumn period, the bottom of the lake was covered almost entirely by aquatic vegetation. The growth of aquatic vegetation should have an inhibitory effect to the development of algal biomass due to the competition for the same nutrient sources.

The results of this study also showed that the surrounding reed bed might generate a decrease in the phytoplankton biomass. Reed bed presence is responsible for the changes in humic substances' release and circulation, phytoplankton flushing, or inadequate light exposure.

Fluorescence spectroscopy showed that, among the phytoplankton species, Cryptophyta and cyanobacteria were the main contributors to the protein-like fluorescence. Humic substances were produced indirectly by phytoplankton and high quantities of algae could potentially reduce the humification process. Also, it proved that algal-derived organic matter contributed along with other autochthonous and allochthonous sources to the production of DOM. Therefore, fluorescence could be used to provide a qualitative evaluation of the eutrophication degree in Danube Delta Lakes.

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References

Beutler, M.; Wiltshire, K. H.; Meyer, B.; Moldaenke, C.; Lüring, C.; Meyerhöfer, M.; Hansen, U.-P.; Dau, H. (2002) A Fluorometric Method for the Differentiation of Algal Populations *in vivo* and *in situ*. *Photosynth. Res.*, **72,** 39-53.

Catherine, A.; Escoffier, N.; Belhocinea, A.; Nasri, A. B.; Hamlaoui, S.; Yéprémian, C.; Bernard, C.; Troussellier, M. (2012) On the Use of the FluoroProbe, a Phytoplankton Quantification Method Based on Fluorescence Excitation Spectra for Large-Scale Surveys of Lakes and Reservoirs. *Water Res.*, **46,** 1771-1784.

Chorus, I.; Bartram, J. (1999) *Toxic Cyanobacteria in Water: A Guide to Their Public Health Consequences, Monitoring and Management*; St Edmundsbury Press: Bury St Edmunds, Suffolk, U.K.

Coops, H.; Buijse, L. B.; Buijse, A. D. T.; Constantinescu, A.; Covaliov, S.; Hanganu, J.; Ibelings, B. W.; Menting, G.; Navodaru, I.; Oosterberg, W.; Staraş, M.; Török, L. (2008) Trophic Gradients in a Large-River Delta: Ecological Structure Determined by Connectivity Gradients in the Danube Delta (Romania). *River Res. Appl.*, **24,** 698-709.

Cristofor, S.; Vadineanu, A.; Sarbu, A.; Postolache, C.; Dobre, R.; Adamescu, M. (2003) Long-Term Changes of Submerged Macrophytes in the Lower Danube Wetland System. *Hydrobiologia*, **506,** 625-634.

Ferrari, G. M.; Mingazzini, M. (1995) Synchronous Fluorescence Spectra of Dissolved Organic Matter (DOM) of Algal Origin in Marine Coastal Waters. *Mar. Ecol.: Prog. Ser.*, **125,** 305-315.

Fukuzaki, K.; Imai, I.; Fukushima, K.; Ishii, K. I.; Sawayama, S.; Yoshioka, T. (2014) Fluorescent Characteristics of Dissolved Organic Matter Produced by Bloom-Forming Coastal Phytoplankton. *J. Plankton Res.*, **36,** 685-694.

Gâştescu, P.; Ştiucă, R. (2008) *Danube Delta—Biosphere Reservation*; CD PRESS: Bucharest, Romania [in Romanian].

Helsel, D. R.; Hirsch, R. M. (2002) *Statistical Methods in Water Resources Techniques of Water Resources Investigations*; Book 4, chapter A3; U.S. Geological Survey: Reston, Virginia.

Huang, D. S.; Pan, S. L.; Zang, H. C.; Wu, C. J.; Lin, M.; Guo, C.; Gao, Y.; Scholz, F. (2014) Monitoring Algal Bloom Through Relationships Between Chlorophyll α and Phytoplankton. *Environ. Eng. Manage. J.*, **13,** 805-815.

Hudson, N.; Baker, A.; Reynolds, D. (2007) Fluorescence Analysis of Dissolved Organic Matter in Natural, Waste and Polluted Waters—A Review. *River Res. Appl.*, **23,** 631-649.

Huguet, A.; Vacher, L.; Relexans, S.; Saubusse, S.; Froidefond, J. M.; Parlanti, E. (2009) Properties of Fluorescent Dissolved Organic Matter in the Gironde Estuary. *Org. Geochem.*, **40,** 706-719.

Isenstein, E. M.; Trescott, A.; Park, M.-H. (2014) Multispectral Remote Sensing of Harmful Algal Blooms in Lake Champlain, USA. *Water Environ. Res.*, **86,** 2271-2278.

Kring, S. A.; Figary, S. E.; Boyer, G. L.; Watson, S. B.; Twiss, M. R. (2014) Rapid in situ Measures of Phytoplankton Communities Using the bbe FluoroProbe: Evaluation of Spectral Calibration, Instrument Intercompatibility, and Performance Range. *Can. J. Fish. Aquat. Sci.*, **71,** 1087-1095.

Korak, J. A.; Wert, E. C.; Rosario-Ortiz, F. L. (2015) Evaluating Fluorescence Spectroscopy as a Tool to Characterize Cyanobacteria Intracellular

Organic Matter Upon Simulated Release and Oxidation in Natural Water. *Water Res.*, **68,** 432-443.

Kring, S. A.; Figary, S. E.; Boyer, G. L.; Watson, S. B.; Twiss, M. R. (2014) Rapid in situ Measures of Phytoplankton Communities Using the Bbe Fluoroprobe: Evaluation of Spectral Calibration, Instrument Intercompatibility, and Performance Range. *Can. J. Fish. Aquat. Sci.*, **71,** 1087-1095.

Liberzon, J.; Carmel, Y.; Angel, D. L. (2016) The Elusive Nature of Fluorescent Organic Matter in Lake Kinneret, Israel. *Hydrobiologia*, **763,** 53-68.

Lyche-Solheim, A.; Feld, C. K.; Birk, S.; Phillips, G.; Carvalho, L.; Morabito, G.; Mischke, U.; Willby, N.; Sndergaard, M.; Hellsten, S.; Kolada, A.; Mjelde, M.; Böhmer, J.; Miler, O.; Pusch, M. T.; Argillier, C.; Jeppesen, E.; Lauridsen, T. L.; Poikane, S. (2013) Ecological Status Assessment of European Lakes: a Comparison of Metrics for Phytoplankton, Macrophytes, Benthic Invertebrates and Fish. *Hydrobiologia*, **704,** 57-74.

Ministry of Environment and Water Management no. 161 of 16.02.2006, approving the Norms concerning the classification of surface water to determine the ecological status of water bodies. In: Official Monitor of Romania, Part I, no. 511 (13 June 2006). Bucureşti, România [in Romanian].

Nemeth, J.; Boyanovski, B.; Traykov, I. (2002) *Phytoplankton.* Joint Danube Survey. Technical Report of the International Commission for the Protection of the Danube River; pp 102-122.

Nguyen, M. L.; Westerhoff, P. E.; Baker, L.; Hu, Q.; Esparza-Soto, M.; Sommerfeld, M. (2005) Characteristics and Reactivity of Algae-Produced Dissolved Organic Carbon. *J. Environ. Eng.—ASCE*, **131,** 1574-1582.

Oosterberg, W.; Staraş, M.; Bogdan, L.; Buijse, A. D.; Constantinescu, A.; Coops, H.; Hanganu, J.; Ibelings, B. W.; Menting, G. A. M.; Năvodaru, I.; Török, L. (2000) *Ecological Gradients in the Danube Delta Lakes—Present State and Man-Induced*; Evers Litho & Druk: Lelystad, Netherlands.

Paerl, H. W.; Otten, T. G. (2013) Harmful Cyanobacterial Blooms: Causes, Consequences and Controls. *Environ. Microbiol.*, **65,** 995-1010.

Pinto, U.; Maheshwari, B. L.; Morris, E. C. (2014) Understanding the Relationships Among Phytoplankton, Benthic Macroinvertebrates, and Water Quality Variables in Peri-Urban River System. *Water Environ. Res.*, **86,** 2279-2293.

Poikane, S.; van den Berg, M.; Hellsten, S.; de Hoyos, C.; Ortiz-Casas, J.; Pall, K.; Portielje, R.; Phillips, G.; Lyche Solheim, A.; Tierney, D.; Wolfram, G.; van de Bund, W. (2011) Lake Ecological Assessment Systems and Intercalibration for the European Water Framework Directive: Aims, Achievements and Further Challenges. *Proc. Environ. Sci.,* **9,** 153-168.

Richardson, T. L.; Lawrenz, E.; Pinckney, J. L.; Guajardo, R. C.; Walker, E. A.; Paerl, H. W.; MacIntyre, H. L. (2010) Spectral Fluorometric Characterization of Phytoplankton Community Composition Using the Algae Online Analyser. *Water Res.*, **44,** 2461-2472.

Rochelle-Newall, E. J.; Fisher, T. R. (2002) Production of Chromophoric Dissolved Organic Matter Fluorescence in Marine and Estuarine Environments: an Investigation into the Role of Phytoplankton. *Mar. Chem.*, **77,** 7-21.

Scheffer, M. (1998) *Ecology of Shallow Lakes*; Springer: New York.

Török, L. (2011) The Trend of Phytoplankton Development in Danube Delta's Lakes. *Scientific Annals of the Danube Delta Institute*, **17,** 89-98.

Török, L. (2014) Assessment of Phytoplankton Variation by Spectral Fluorescence in the Danube Delta Biosphere Reserve. *J. Environ. Prot. Ecol.*, **15,** 517-525.

Vădineanu, A.; Cristofor, S.; Ignat, G. (1992) Phytoplankton and Submerged Macrophytes in the Aquatic Ecosystems of the Danube Delta During the Last Decade. *Hydrobiologia*, **243/244,** 141-146.

Zhou, Y.; Jeppesen, E.; Zhang, Y.; Niu, C.; Shi, K.; Liu, X.; Zhu, G.; Qin, B. (2015) Chromophoric Dissolved Organic Matter of Black Waters in a Highly Eutrophic Chinese Lake: Freshly Produced from Algal Scums? *J. Hazard. Mater.*, **299,** 222-230.

Xu, Z.; Xu, Y. J. (2015) Rapid Field Estimation of Biochemical Oxygen Demand in a Subtropical Eutrophic Urban Lake with Chlorophyll a Fluorescence. *Environ. Monit. Assess.*, **187,** 4171.