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SEASONAL CHANGES IN THE PHYTOPLANKTON TAXONOMIC STRUCTURE AND PHOTOSYNTHETIC PIGMENTS IN PELAGIAL AND LITTORAL OF TWO INTERCONNECTED LAKES IN BELARUS

Abstract. The seasonal pattern of phytoplankton taxonomical composition and phytoplankton chlorophyll *a* content from pelagial and littoral locations with and without macrophyte beds in two interconnected lakes (north-west of Belarus) under contrasting trophic conditions were studied. We estimated influence of hydrochemical parameters on phytoplankton in studied lakes. There was “a clear water phase” in pelagial and low phytoplankton abundance in littoral of mesotrophic Lake Obsterno but we revealed a brief pulse of *Chrysophyta* and “a late spring bloom” with high total phytoplankton abundance in shallow macrophyte-covered low trophic state Lake Nobisto. It was found some prominent differences in total phytoplankton abundance and taxonomic composition in littoral and pelagial locations of both lakes. We used Phyto-Pam phytoplankton analyser for analysis of algae pigments. Phyto-Pam method allowed roughly identify two types of pigments – pigments of green algae and diatoms and revealed differences in concentrations of pigments between littoral locations and pelagial in both lake types. Results indicated that total chlorophyll *a* content has a pronounced seasonal cycle with high values during the early fall and low values throughout the late spring in mesotrophic lake and have shown differences in phytoplankton pigments between lakes littoral locations.

Keywords: phytoplankton, pigments, chlorophyll *a*, seasonal changes, littoral, pelagial, macrophyte beds, mesotrophic lake, shallow lake

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СЕЗОННЫЕ ИЗМЕНЕНИЯ ТАКСОНОМИЧЕСКОЙ СТРУКТУРЫ И ФОТОСИНТЕТИЧЕСКИХ ПИГМЕНТОВ ФИТОПЛАНКТОНА В ПЕЛАГИАЛИ И ЛИТОРАЛИ ДВУХ СООБЩАЮЩИХСЯ ОЗЕР В БЕЛАРУСИ

Аннотация. Исследованы сезонные изменения таксономической структуры и фотосинтетических пигментов фитопланктона в пелагиали, а также в литорали, заросшей разными видами макрофитов, и литорали без зарослей в двух сообщающихся озерах разного трофического статуса (северо-запад Беларуси). Установлено, что для мезотрофного оз. Обстерно весной характерна «фаза чистой воды» в пелагиали и низкая численность фитопланктона в литорали. В то же время высокая численность хризофитовых водорослей (*Chrysophyta*) вызывала «осеннее цветение» фитопланктона в мелководном макрофитного типа оз. Нобисто с низкой трофностью. Выявлены существенные различия в общей численности и таксономической структуре фитопланктона между пелагиалью и литоралью в обоих озерах. С помощью специального метода с использованием прибора Phyto-Pam для определения содержания пигментов различных групп водорослей – зеленых и диатомовых и общего хлорофилла *a* установлено, что общее содержание хлорофилла *a* имело выраженный сезонный цикл с пиком ранней осенью и низким содержанием весной в мезотрофном озере, а уровни хлорофилла *a* в литорали двух исследованных озер существенно различались. Согласно полученным данным, указанный метод может использоваться только для быстрого определения или грубой оценки содержания хлорофилла *a* в водоемах.

Ключевые слова: фитопланктон, пигменты, хлорофилл *a*, сезонные изменения, литораль, пелагиаль, заросли макрофитов, мезотрофное озеро, мелководное озеро

Для цитирования: Сезонные изменения таксономической структуры и фотосинтетических пигментов фитопланктона в пелагиали и литорали двух сообщающихся озер в Беларуси / Ж. Ф. Бусева [и др.] // Вес. Нац. акад. навук Беларусі. Сер. біял. навук. – 2020. – Т. 65, № 3. – С. 310–318 (in English). https://doi.org/10.29235/1029-8940-2020-65-3-310-318

Introduction. Clear differences exist in algal species composition, both between lakes and within a single lake over time, but the relative importance of nutrient competition among different taxa and purely physical processes among variable taxa in different seasons have not been clarified yet [1, 2] in the literature, a large number of methods are available to measure chlorophyll from phytoplankton [3]. Phytoplankton pigment are measured directly by means of spectrophotometric or fluorometric procedures [4, 5]. Chlorophyll *a* values are also used to calculate phytoplankton carbon biomass, assuming a fixed carbon to the chlorophyll *a* ratio. A number of older reports in the literature, mostly based on studies of the laboratory phytoplankton cultures, have demonstrated upper and lower limits on what one might expect in nutrient/chlorophyll relationships [6]. Since the early work of [7], chlorophyll fluorescence has become increasingly important for assessment of phytoplankton biomass and primary productivity [8]. Very sensitive techniques have been developed to measure chlorophyll *a* content and to analyse basic parameters of photosynthetic activity in natural surface waters down to 0.1 $\mu g \cdot Chl^{-1}$ [9, 10]. The so far available instrumentation has been limited by the fact that it cannot distinguish between different groups of phytoplankton, like green algae, diatoms and blue-green algae. In principle, such distinction is possible on the basis of the specific fluorescence excitation properties of differently pigmented phytoplankton groups [11]. We are aware that the chlorophyll *a* content generally differs between pelagic and littoral zones in the lakes and that the shallow areas are more influenced by the benthic processes than the pelagic zones [12, 13].

The goal of this study was to define seasonal patterns of phytoplankton composition of two interconnected lakes under contrasting – mesotrophic and low trophic conditions and estimated hydrochemical factors regulating phytoplankton structure during vegetation season.

Materials and research methods. Lake Obsterno (55°37′31.9″N, 27°21′55.2″E) is a mesotrophic relatively shallow lake with surface area of 9.89 km^2, max depth 12 m, mean depth of 5.3 m. The lake has a wide macrophyte beds occupying most of the shallow water area in littoral zone. Interconnected low tropic state Lake Nobisto (55°37′55.9″N, 27°24′19.9″E) with 3.75 km^2 surface area is shallow, has a max and mean depths of 2.8 and 1.4 m respectively. Lake Nobisto has a right-side shoreline with a swamped territory and the wide and dense macrophyte beds grow all around this lake, common bladderwort (*Utricularia vulgaris* L. of Lentibulariaceae family, order Lamiales) cover all the lake's bottom.

A total of 48 samples from seven habitats of two lakes in different seasons (with three replications for each habitat) were analysed for phytoplankton taxonomy. The phytoplankton samples of mesotrophic Lake Obsterno were collected in late May to September 2016 to identify the taxa in four locations – pelagial (depth of 4.5–5 m), bare littoral – an open shallow area without macrophytes, rush beds *Schoenoplectus lacustris* (L.) and yellow water-lily zone *Nuphar lutea* (L.). In Lake Nobisto, samples were collected from three habitats – one of pelagial (depth of 2.3 m) and two sites in littoral – bare littoral location and bull rush beds *Phragmites australis* (Cav.) Trinex Steud. (approximate depths of 1.2 and 0.7 m respectively) in 2017. The samples were kept in 1 liter jars and fixed with 2 % formalin solution. Each season all samples were taken during a day field trip. Data from Lake Obsterno were divided between a warm, more productive period (spring) and the cool and less productive period of the year (autumn). Species identification and counting of algal number were carried out in the Fuchs-Rosenthal chamber using a light microscope with a magnification of ×360.

Phyto-Pam phytoplankton analyser was applied to get a quick measurement of chlorophyll *a* concentration in phytoplankton samples ($\mu g \cdot l^{-1}$) without using acetone on the basis of the specific fluorescence excitation properties of differently pigmented phytoplankton groups. For this technique, due to the storage condition, we selected May and September phytoplankton samples of Lake Obsterno in 2016 and only September samples of Lake Nobisto in 2017. We performed sampling two years in different lakes but two seasons did not differ crucially in weather conditions.

The Phyto-Pam (Phyto-PAM phytoplankton Analyzer, Heinz Walz GMBH, Effeltrich, Germany) employs light-emitting-diodes (LED) to excite chlorophyll *a* fluorescence alternatingly by 10 µs light pulses at four different wave lengths (470, 535, 620 and 650 nm). The fluorescence pulses were detected by a photomultiplier and amplified under microprocessor-control, resulting in 4 separate continuous signals (4 channels).

Seston samples for carbon, nitrogen and phosphorus analysis were stored in (one liter) plastic bottles that were first washed and rinsed in distilled and deionized water. Particulate samples were collected onto precombusted (5 h in 400 °C) glass fiber filters (Microbio GF/F filters, 0.7 µm porosity, 37 mm diameter) and after filtration dried at 60 °C for 72 h. Final volume of filtering water on GF/F for seston was 0.8–1.2 l. All samples were taken once a day at around 10:00–12:00 o'clock. Flash EA 1112 NC Soil/MAS 200, Thermo Quest, Italy, CHN analyzer was used for carbon (C) and nitrogen (N) measurement. Particulate matter was analyzed for phosphorus (P) content calorimetrically after persulfate oxidation via spectrophotometer [14].

Statistical analyses. All statistical analyses were conducted using Minitab 17. To test the significant differences among habitats with phytoplankton taxonomical composition, we used one-way ANOVA with Tukey post hoc test. Eigen analysis of the correlation matrix via Principal Component Analysis (PCA) was applied to determine correlation between total abundance of phytoplankton dominant groups with elements as well as water chemistry.

Results and its discussion. During this study, water temperature varied from 21.4 °C in May with maximum of 23.6 °C in littoral location to September 14.3 °C in Lake Obsterno. Among all physico-chemical and hydrochemical parameters, TDS (115–120 ppm) and pH didn't change in a significant way but dissolved nitrates, phosphates and ammonium were not balanced in the whole season (Tab. 1). The Secchi disc transparency in Lake Obsterno differed from spring to autumn shifted from 6.5 m maximum in May to 4.2 m minimum in September.

T a b l e 1. **Hyrdochemical and physical parameters of Lake Obsterno (2016)**

Parameter	Season, location			
	Pelagial	Littoral	Pelagial	Littoral
	May		September	
T, °C	21.4	22.43 ± 0.5	14.4	14.3 ± 0.1
pH	8.4	8.5 ± 0.05	8.4	8.7 ± 0.00
Secchi depth, m	6.5		4.2	
PO_4, mg·l^{-1}	0.39	0.37 ± 0.08	3.1	1.32 ± 0.51
NO_3, mg·l^{-1}	0.3	0.6 ± 0.10	0.3	1.1 ± 0.26
NH_4, mg·l^{-1}	0.15	0.27 ± 0.01	0.03	0.21 ± 0.08

N o t e. Data for littoral is represented means from all three locations (means ± SD).

In Lake Nobisto during the autumn, temperature, pH, TDS (100–110 ppm) and nitrate (Tab. 2) did not change in a significant way among habitats while NH_4^+ and PO_4^{3-} were different in comparing with Lake Obsterno. The Secchi disc transparency in Lake Nobisto was high (2.9 m till the bottom).

T a b l e 2. **Hydrochemical parameters of Lake Nobisto (autumn 2017)**

Parameter	Location		
	Pelagial	Bare littoral	Bull rush
T, °C	9.6	9.6	9.8
pH	8.6	8.5	8.6
Secchi depth, m	2.9		
PO_4, mg·l^{-1}	2	1.84	1.04
NO_3, mg·l^{-1}	0	0	0.2
NH_4, mg·l^{-1}	0.77	0.93	1.56

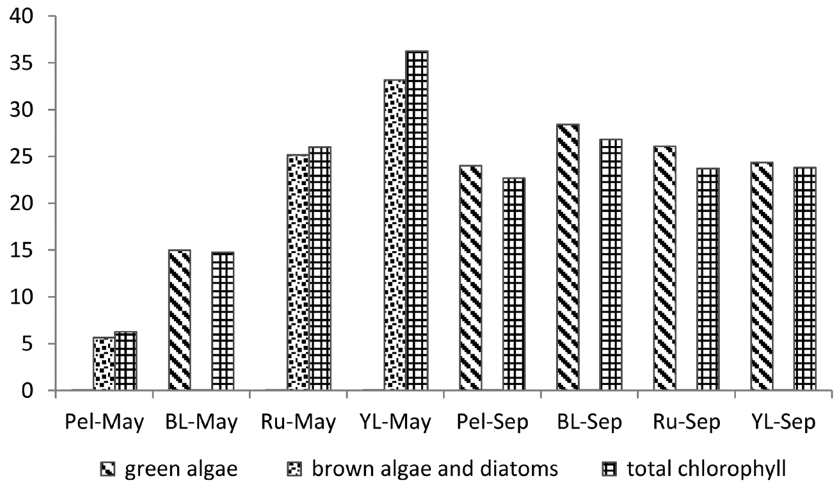

Fig. 1. Chlorophyll *a* concentration (μg·l^{-1}) of phytoplankton samples in different habitats of Lake Obsterno. Sep – September, Pel – Pelagial, BL – Bare littoral, Ru – Rush beds, YL – Yellow lily beds

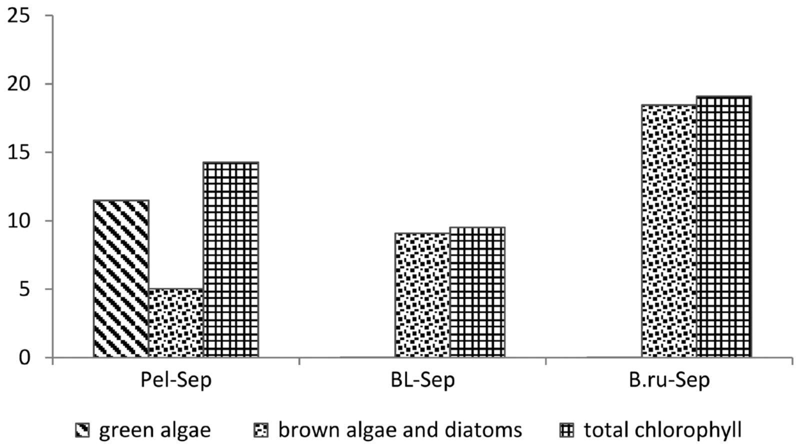

Fig. 2. Chlorophyll *a* concentration (μg·l^{-1}) of phytoplankton samples in different habitats of Lake Nobisto. Sep – September, Pel – Pelagial, B. ru – Bull rush, BL – Bare littoral

During this study, total chlorophyll *a* concentrations ranged from 6.25 to 36.24 μg·l^{-1} in Obsterno and from 9.51 to 19.10 μg·l^{-1} in Nobisto, averaging 22.84 ± 8.84 and 14.29 ± 4.79 μg·l^{-1} in Obsterno and Nobisto respectively (Fig. 1, 2). Peak concentrations occurred during late spring in yellow water-lily zone in Lake Obsterno. In general, chlorophyll *a* was lower in Nobisto than in Obsterno. The minimum chlorophyll *a* concentrations were typically observed at the pelagial zone of Lake Obsterno at the end of May where we noted "a clear water phase" as recorded in many studies [15]. Spring phytoplankton usually characterizes the "diatom bloom", so brown pigments appeared in Lake Obsterno. The shallow location without macrophyte cover (bare littoral) is more turbid lake's area where the pigments of green algae were identified properly in our phytoplankton samples of that location which is completely in agreement with Happey and Woods research in 1988 [16].

The chlorophyll *a* content revealed differences in pigments in accordance with dominant groups such as green algae and diatoms in autumn and late May in Obsterno as well as diatoms in Nobisto during autumn. The Secchi disc transparency in Obsterno from May to September decreased greatly (from 6.5 to 4.2 m) in combination the shift in dominant groups from diatoms to green algae. Taxonomical composition of phytoplankton has shown differences in lakes during season as well as in littoral and pelagial locations. In Lake Obsterno in May *Chrysophyta* and then *Bacillariophyta* were the most abundant algae groups in pelagial, bare littoral, rush beds and yellow water-lily zone respectively. In autumn, *Bacillariophyta* was the most widespread group in all habitats (Tab. 3). According to the phytoplankton community composition of Lake Nobisto, during autumn in pelagial *Chrysophyta* but in bare littoral and bulrush, *Bacillariophyta* were the dominant groups (Tab. 4). *Cyanophyta* were absent in May in pelagial and bare littoral and had minimum values in rush beds and yellow water lily zone in Lake Obsterno. In lake Nobisto we identified minimum values of *Cyanophyta* in all habitats in autumn. Meanwhile during May and September within all habitats of both lakes, no chlorophyll content for *blue-green algae* (*Cyanophyta*) by using Phyto Pam technique was obtained. As noted by Moore in 1978 [17], numerous factors may be involved in the ecological success of blue-green algae. One proposed explanation for low dominance of blue-green algae is limited phosphorus since nitrogen-fixing species usually are those who dominate [18] which is in agreement with our data and low trophic state of both lakes.

T a b l e 3. **Taxonomical composition of phytoplankton community of Lake Obsterno: abundance (ind/l) and C:N, N:P ratios of seston within season (from May to September) 2016**

Habitat	Phytoplankton group	Mean ± SD	C:N	N:P
Pelagial	*Bacillariophyta*	162 491 ± 115 054^{AB}	5.5–7.37	107.71–35.61
	Chlorophyta	25 004 ± 27 994^{BC}		
	Chrysophyta	63 969 ± 64 405^{BC}		
	Cryptophyta	20 304 ± 15 370^{C}		
	Cyanophyta	15 612 ± 17 451^{C}		
Bare littoral	*Bacillariophyta*	98 464 ± 29 471^{B}	5.75–7.56	106.29–11.75
	Chlorophyta	17 418 ± 24 188^{CD}		
	Chrysophyta	77 290 ± 66 710^{BC}		
	Cryptophyta	35 814 ± 14 255^{BCD}		
	Cyanophyta	4 345 ± 5 397^{CD}		
Rush beds	*Bacillariophyta*	295 566 ± 195 662^{A}	5.72–8.78	101.06–6.42
	Chlorophyta	415042 ± 789 308^{A}		
	Chrysophyta	28 040 ± 17 210^{A}		
	Cryptophyta	63 796 ± 56 275^{A}		
	Cyanophyta	29 714 ± 34 133^{A}		
Yellow water-lily zone	*Bacillariophyta*	76 995 ± 54 563^{B}	6.14–7.31	82.62–1.38
	Chlorophyta	16 439 ± 15 694^{B}		
	Chrysophyta	63 057 ± 48 209^{B}		
	Cryptophyta	56 198 ± 38 639^{B}		
	Cyanophyta	5 583 ± 4 598^{B}		

N o t e. Grouping information using Tukey test for the abundance of phytoplankton community, different labels (A, B, C, D) show significant differences of main phytoplankton groups among habitats at $p < 0.05$.

In Obsterno during May, we achieved a range of 6.47, 6.73, 6.68, 7.16 for C:N and 243.3, 239, 224 and 193.61 for N:P ratio respectively in pelagial, bare littoral, rush beds and yellow water lily zone. In September, C:N ratio of seston was greater than in May for pelagial, bare littoral, rush beds and yellow water lily zone followed by 9.39, 9.36, 8.65 and 8.47. In September N:P ratios were measured by an exceed range of above 1000 (8227, 2348, 2945 and 6392) respectively for pelagial, bare littoral, rush beds and yellow water lily zone which could be a reason of great phosphorus depletion and/or high nitrogen concentration. In May, phosphorus content of seston varied from 6.69 μg/l in littoral and 6.59 μg/l in pelagial but in September, it was recorded as 4.20 μg/l in littoral and 0.80 μg/l in pelagial locations.

Principal Component Analysis of C:N and N:P ratios with phytoplankton abundance expressed some positive but weak correlation in May and September which is followed as: C:N with *Dinophyta* (PC = 0.394), *Chrysophyta* (PC = 0.362), *Bacillariophyta* (PC = 0.305), *Chrysophyta* (PC = 0.362) and *Chlorophyta* (PC = 0.261); N:P with *Cyanophyta* (PC = 0.031), *Dinophyta* (PC = 0.281), *Cryptophyta* (PC = 0.598), *Chrysophyta* (PC = 0.015) and *Bacillariophyta* (PC = 0.409). Principal Component Analysis of phosphate and *Cryptophyta* abundance (PC = 0.640) showed a strong correlation but *Chrysophyta* (PC = 0.024), *Cyanophyta* (PC = 0.007) and *Dinophyta* (PC = 0.174) revealed a poor correlation in Lake Obsterno.

Analysis of variance with post hoc and Tukey test showed the significant differences among phytoplankton groups seasonally (F = 6.09, $p = 0.000$). Grouping information using Tukey method in Lake

T a b l e 4. **Taxonomical composition of phytoplankton community of Lake Nobisto: abundance (ind/l) and C:N, N:P ratios of seston in September 2017**

Habitat	Phytoplankton group	Mean ± SD	C:N	N:P
Pelagial	*Bacillariophyta*	128 151 ± 64 114^{AB}	10.74	31.09
	Chlorophyta	35 182 ± 25 732^{AB}		
	Chrysophyta	349 740 ± 382 704^{AB}		
	Cryptophyta	24 583 ± 22 678^{AB}		
	Cyanophyta	12 396 ± 11 408^{B}		
	Euglenophyta	53 516 ± 71 750^{AB}		
Bare littoral	*Bacillariophyta*	106 270 ± 42 018^{B}	12.13	73.73
	Chlorophyta	31 329 ± 6 917^{B}		
	Chrysophyta	129 959 ± 110 072^{B}		
	Cryptophyta	24 392 ± 20 845^{B}		
	Cyanophyta	8 655 ± 8 352^{B}		
	Euglenophyta	20 122 ± 19 254^{B}		
Bull rush	*Bacillariophyta*	104 453 ± 80 017^{B}	11.61	15.28
	Chlorophyta	28 802 ± 18 720^{B}		
	Chrysophyta	95 117 ± 48 946^{B}		
	Cryptophyta	30 234 ± 28 200^{B}		
	Cyanophyta	11 185 ± 7 984^{B}		
	Euglenophyta	35 729 ± 30 108^{B}		

N o t e. Grouping information using Tukey test for the abundance of phytoplankton community, different labels (A, B) show significant differences of main phytoplankton groups among habitats at $p < 0.05$.

Obsterno showed the abundance of *Bacillariophyta* is significantly different from the other groups in pelagial and bare littoral. As showed in the Tab. 3, mean abundance of *Bacillariophyta* is higher than others. Beside it, *Bacillariophyta* had a significant difference with rest of phytoplankton groups during seasons (Tab. 3).

In Lake Nobisto lake during September, C:N ratios were recorded in 10.47, 12.13 and 11.61 respectively for pelagial, bare littoral and bull rush and were higher than its values in Lake Obsterno. N:P showed a range of 31.09, 73.73 and 15.28 for mentioned habitats and were different from those in Lake Obsterno. PCA analysis didn’t express an average or strong correlation between phytoplankton groups and CNP as well as water chemistry. *Chrysophyta* (349–740 ind/l) in May and *Bacillariophyta* (104–453 ind/l) in September were identified as the most abundant groups from spring to autumn. Grouping information using Tukey method in Lake Nobisto showed that abundance of *Bacillariophyta*, *Chlorophyta*, *Chrysophyta*, *Cryptophyta* and *Euglenophyta* in pelagial are significantly different from the abundance of other groups in bare littoral and bull rush during autumn (Tab. 4). Occurrence of *U. vulgaris* may indicate the lack of nitrate nitrogen in Lake Nobisto in autumn as well as low phosphates in comparison with pelagial of Lake Obsterno. Phosphorus content of seston in Lake Nobisto was measured as 7.43 µg/l in pelagial, 3.43 µg/l in bare littoral and 9.51 µg/l in bull rush. In temperate lakes, in mesotrophic systems, most algal taxonomic groups are represented over the growing season, especially *Bacillariophyta*, *Chlorophyta* (green algae), *Cryptophyta* and *Dinophyta*, as well as *Cyanophyta* and *Chrysophyta* [19]. *Cryptophyta*, *Chrysophyta*, *Chlorophyta* and *Bacillariophyta* increase with phosphorus concentration. Some taxonomic groups increase significantly over a wide phosphorus range (*Cyanophyta*, *Bacillariophyta*), others exhibit decelerating rates of increase at low (*Chrysophyta*) or moderate P levels (*Cryptophyta*, *Dinophyta*) [20]. As it is shown in our survey, phosphate and *Cryptophyta* showed a strong correlation but a poor correlation with the rest of phytoplankton groups in Lake Obsterno. This result in marked shifts in average phytoplankton taxonomic composition across this intermediate phosphorus range. McQueen and Lean [21] claimed that *Chrysophyta* and *Dinophyta* show a decelerating rate in their growth when P is low or moderate, as they decreased in May and in early summer but then decelerated toward autumn in Lake Obsterno. High light, N:P and intermediate pH and temperatures may favour *Chlorophyta* [22]. *Chlorophyta* in Lake

Obsterno appeared more abundant in late summer and early autumn where temperature and light were higher than spring [16]. Both *Cryptophyta* and *Chrysophyta* exploit nutrient and light gradients [23] and conditions associated with enrichment (increased turbidity and organic materials) favour their growth which is in agreement with our obtained data with highest abundance of *Cryptophyta* and *Chrysophyta* in May for both lakes. On the other hand, most cryptophytes and many chrysophytes are small monads, and grazing regulation should significantly modify their response to nutrient enrichment [24]. *Chrysophyta* have been shown to be frequently phosphorus limited, but they respond unpredictably to enrichment [23]. The comparatively strong relationship between phosphate and *Cryptophyta* abundance (PC = 0.640) in our data suggests that this group is more influenced by phosphorus. On the other side a poor correlation between phosphate with *Chrysophyta*, *Cyanophyta* and *Dinophyta* suggest that they could be affected by pH and alkalinity [23] which is common among many of these taxa than phosphorus [25, 26]. According to the PEG-model of plankton seasonal succession toward the spring, nutrient availability and increased light permit unlimited growth of phytoplankton, especially in *Cryptophyta* and small diatoms. In the middle of warm season, by more soluble phosphorus, *Cryptophyta* become predominant as its clear in studied lakes. By seasonal changes and light limitation from spring to autumn, macrophytes and vegetation become important particularly in shallow lakes as it happened in both our studied lakes [27]. Macrophytes are known to affect nutrient cycling in lakes causing changes in phytoplankton biomass, growth and leading to competition among different taxa [28], but there is no effect on the presence or absence of macrophytes on the total biomass of diatoms [29] as its shown in our survey suggesting not only hydrochemical but other factors affect on phytoplankton dynamics.

Conclusion. A synthesis of patterns in average spring and autumnal abundance of major phytoplankton taxonomic groups in mesotrophic Lake Obsterno show that all groups increase in abundance with soluble phosphorus at different degrees, but only *Cryptophyta* showed a strong correlation over the season. However, neither abundance of dominant phytoplankton groups, nor chlorophyll *a* content didn't show any correlations of taxonomic composition with the range of nutrient levels observed. Contrary, in shallow low trophic Lake Nobisto mainly nutrient deficiency and competition with macrophytes leads to development and changing defined phytoplankton groups especially in pelagial. We also approve that Phyto-Pam has been limited by the fact that it cannot distinguish between different phytoplankton groups, like green algae, diatoms and *blue-green algae* and it's just a quick method for analysis of the main algal pigments.

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