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Phytoplankton Cell Size in Saline Lakes.

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ABSTRACT

We studied 62 of the most abundant species from 7 lakes and 121 phytoplankton samples collected during 2007–2013. We compared the algal cell volumes in the studied lakes with the averaged species-specific cell volume. From all studied species, we observed a downward trend in cell size in the summer, when there was an active biomass production. Salinity is a regulatory factor for the value of cell surface and, thus, for the intensity of the metabolic processes of the organisms at the first trophic level. The cell volume for most studied species in partly ephemeral lakes (Levadne, Chervone, and Ozero) was generally higher than in perennial lakes (Ripne, Veysove, Garache, and Slipne) and thus it can be assumed that the ecological succession stage of ephemeral lakes has a more significant effect on the dimensional characteristics of algae than the factors related to seasonal summer desiccation and water salinity. Previously, dimensional characteristics of algal cells in non-marine water bodies has been calculated for freshwater lakes only, therefore the data obtained for the mineralized lakes are presented for the first time and can be used for the purpose of monitoring of saline lakes ecosystem.

Keywords: Phytoplankton, limnology, physical and chemical variables, algal cell size

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INTRODUCTION

Microalgae cell size corresponds to each species' body shape and usually varied in the bright range during the vegetation season. The main cell dimensions can be different not only in different water bodies but also in time-series of samples taken from the same water bodies [1]. As a result, we can explore increases in the total cell surface with increases in cell number and decreases in cell volume when algae bloom in water body. The total cell surface is positively correlated with the intensity of metabolic processes in autotrophic organisms [2,3]. This is most clearly expressed when blue-green algae bloom. Diatoms, on the contrary, are the most conservative group of organisms in relation to its dimensions and the distribution of cell size over latitude. However, they represent basically the same tendency as other groups of organisms and show a negative correlation with latitude [4]. That is, south of the equator, the size of cells is greater than in the north where cell size decreased with increasing latitude under stressful conditions. Thus, reducing the size of microalgae cells leads not only to eutrophication, expressed in the bloom, [5], but to climatic stress also. Therefore, if we know the environmental factors that lead to a decrease in cell volume (increase in cell surface for metabolic rate), we can assume what their influence will be on the intensity of primary production processes. Previously, we have shown that algae in the studied lakes significantly affect the increase of salinity: with increasing salinity there is a decrease in species richness [6]. Thus, for algal communities of perennial lakes salinity is a factor of climatic stress. Therefore, the aim of this work was to study the effect of salinity factors on dimensional characteristics, in particular cell volume, of the dominant diatoms species of the "Slavyansky Resort" lakes.

Description of study site

Just inside the park are seven perennial lakes (Ripne, Veysove, Garache, Slipne, Levadne, Chervone, and an untitled lake – hereinafter referred to as "Ozero") and many temporary pools (Figure 1). The studied lakes are mostly of a thermokarst origin, small, and shallow. These lakes are insulated from each other and periodically dry up. They are briefly interconnected in spring. Some hydrochemical variables are given in Table 1. Sediments of the lakes are diverse, varying from sand to medical mud. Depth of the lakes is negligible (about 0.5–2.5 m) and only in the lake Ripne does it reach 8.5 m. The sediments of the lakes form a unique community of organisms, including algae, which are the basis for the formation of therapeutic mud. In the lake Ripne there is industrial fishing for mud and brine mud baths for the Slavyansky Resort – one of the oldest mud bath resorts of the Ukraine. More information about studied lakes and the area was presented earlier [6].

The water bodies are subjected to an intense recreational load, since most of the banks are designated for swimming and relaxing. Aquatic macrophyte vegetation is abundant, serving as part of the staple diet and as a habitat for protected species of birds.

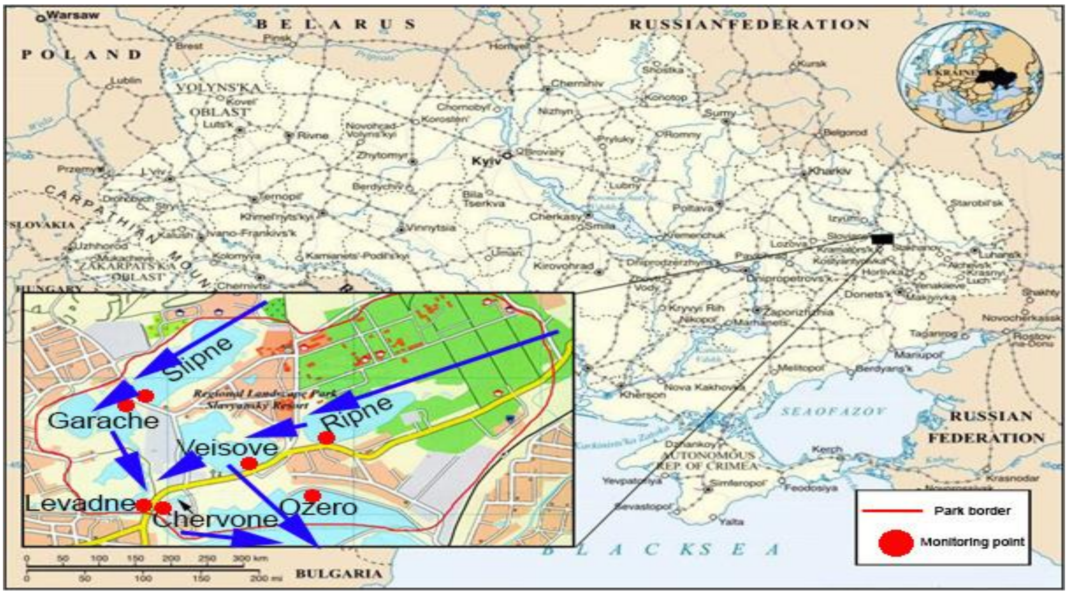

Figure 1: Study site in the Regional Landscape Park "Slavyansky Resort". Blue arrows are follow groundwater flow.

Table 1: Main hydrochemical variables in the lakes of the Regional Landscape Park "Slavyansky Resort".

Variable	Ripne	Veisove	Garache	Slipne	Levadne	Chervone	Ozero
pH	7.4±0.3	7.0±0.5	7.3±0.4	7.6±0.3	7.2±0.4	7.6±0.3	7.2±0.4
Conductivity, mSm/cm	9.5±1.6	10.8±0.2	9.3±2.1	6.6±1.1	8.0±3.1	6.7±3.6	11.0±0.2
Hardness, mgEq/L	59.6± 19.4	82.3± 13.5	51.5± 24.2	19.9± 4.7	26.1± 5.9	16.1± 5.0	102.1± 25.1
Alkalinity, mEq/L	2.1±0.5	3.1±0.6	5.5±1.1	5.1±1.0	5.4±2.8	6.3±0.7	2.8±0.6
HCO_3^-, mg/L	123.6± 26.6	181.3± 38.9	325.7± 61.9	297.9± 66.8	324.1± 165.1	369.1± 54.7	160.1± 38.4
Cl^-, mg/L	12 217± 2434	29 454± 8290	15 657± 11 506	1893± 279	8233± 1361	5550± 1212	32 583± 6857
Ca, mg/L	989.7± 327.3	1406.7± 248.6	860.2± 484.7	211.2± 50.8	365.3± 97.2	255.5± 90.4	1685.0± 434.9
K, mg/L	57.3± 82.4	99.1± 86.2	61.8± 80.0	54.4± 77.7	19.9± 9.6	50.8± 32.6	169.5± 83.4
Mg, mg/L	156.0± 56.6	162.3± 38.6	119.3± 29.0	119.4± 34.0	101.9± 26.9	64.4± 25.2	223.0± 71.2
Na, mg/L	9327± 4339	26 911± 9156	15 969± 12 026	1674± 547	6337± 4337	3715± 2525	30 450± 12 124

MATERIALS AND METHODS

Material for this study cam from 121 samples of phytoplankton collected monthly during 2007-2013. In each sample, we measured dimensions more than 20 cells of each species (length, bright, and diameter). In this study we included data for measurements of 62 species only (from full species list included 238 species [6]) that were most abundant in the lake communities and had linear dimensions of which were greater than 2 microns. The cell volume was calculated with a stereo-metric method by equations corresponding to each species' body shape [7]. Average cell size was calculated from no less than 20 repetitions with standard deviation. Species-specific cell volume was calculated on the basis of each species' average dimensions [8-11] for comparison of cell size deviation in the studied lakes. The Pearson coefficients were calculated with the help of wessa.net.

RESULTS AND DISCUSSION

Volumes of algae cells were calculated for the 62 most frequently occurring algae in the studied lakes. For each species, cell volume for each of the lakes and for the lakes of the park was calculated (see Tables 2-5). Cell volumes of cells were determined in the lake Ripne for 47 species, in the lake Veysove for 55 species, in the lake Garache for 52 species, in the lake Slipne for 50 species, in the lake Levadne for 20 species, in the lake Chervone for 19 species, and in the Ozero for 17 species.

Table 2: Average cell volume of phytoplankton species (mkm^3) in the lakes of the Regional Landscape Park "Slavyansky Resort" (RLP) is lower than the species-specific average cell volume (SSACV) in communities of the lakes Ripne (R), Veysove (V), Garache (G), Slipne (S), Levadne (L), Chervone (Ch), and Ozero (O).

Species	RLP	R	V	G	S	L	Ch	O	SSACV
Acutodesmus dimorphus (Turpin) Tsarenko	1,048	-	-	1,173	923				1,454
Campylodiscus clypeus (Ehrenberg) Ehrenberg ex Kützing	84,998	89,696	84,201	103,257	62,839	-	-	-	138,474
Cocconeis placentula Ehrenberg	319	193	584	238	259	-	-	-	1,988
Cymbella laevis Nägeli	264	203	378		212	-	-	-	847
Desmodesmus communis (E.Hegewald) E.Hegewald	550	1,164	124	312	600	-	-	-	1,311
Euglena viridis (O.F.Müller) Ehrenberg	2,297	-	-	987	3,606	-	-	-	3,768
Gomphonema micropus Kützing	3,830	-	4,894	2,581	4,015	-	-	-	5,277
Lepocinclis oxyuris (Schmarda) Marin & Melkonian	83,589	126,759	-	64,640	59,368	-	-	-	145,722
Navicula salinarum Grunow	847	867	899	703	919	-	-	-	1,276
Surirella striatula Turpin	17,321	19,503	15,543	16,917	-	-	-	-	56,049

Volumes of algal cells in the lowermost levels of salinity of the studied lakes (Slipne, Chervone) are comparable to that of the same species in freshwater lakes of Israel [12], Florida [13], Brazil [14,15], Bulgaria [16], and in salt lakes of the Danube River Basin [17]. But for the more salty lakes of the park a wide range of sizes was observed: cell volume of species found in these waters can be both large and smaller than recorded in the studied lakes with lower salinity. In this regard, it has been suggested that some species may be sensitive to natural salty water in relation to its cell volume. As stated earlier [5,18-20], the factors that affect the size of algal cells in a diversity of aquatic ecosystems include:

- the rate of nitrate or ammonia input to the cell,
- the extinction coefficient of the water,
- the mixed layer depth,
- the light intensity,
- the sinking rate of phytoplankton,
- the upwelling velocity of the water,
- the compensation light intensity,
- temperature coefficients,
- the relative size selectivity of zooplankton grazing,
- the trophic level,
- the rate of reproduction,
- Sexual process for diatoms.

Most of the above factors are not relevant to the studied lakes, as they are shallow, warm, and transparent at the bottom. Therefore, the depth of the mixed layer has no effect on the algae of lakes, as the lakes are shallow. The sun-light intensity is irrelevant to the data, as characterized by almost the same lake transparency and sampling carried out at the same depth and at the same time. Trophic levels of the lakes is not very different, and most of the year they are eutrophic therefore, this factor also cannot have a significant impact on the difference in the amounts of algae cells. The speed of propagation of algae [21], and the diatoms sexual process have the same tendency to instances of the same species living in the studied lakes; hence, the degree of influence of these factors is the same in all studied lakes. The relative size selectivity of zooplankton grazing can have an effect only in the lake Ripne because in other lakes zooplankton is not so developed. We did not analyze the influence of other factors mentioned above.

As a result, we revealed that cells of one species of algae that live in different lakes often differ in their cell size that can be relevant to developmental factors [22]. The most stable species in this respect were frequent non-dominate species with sizes lower than the average species-specific cell volume, like *Navicula salinarum* (Table 2); the periodically dominated species with sizes lower than species-specific average cell volume, like *Entomoneis paludosa*, *Chaetoceros muelleri*, and *Monoraphidium minutum* (Table 3), and species with sizes greater than the average species-specific cell volume, like *Ankyra ocellata* and *Achnanthidium bioretii* (Table 4). Noteworthy is that cell size of species that were in the range of the average species-specific cell volume in communities of the lakes has fluctuated (Table 5).

Table 3: Average cell volume of phytoplankton species (mkm^3) in the lakes of the Regional Landscape Park "Slavyansky Resort" is lower than the species-specific average cell volume and dominated the communities. Abbreviation as in Table 2.

Species	RLP	R	V	G	S	L	Ch	O	SSACV
Achnanthes brevipes C.Agardh	6,392	4,660	6,913	4,256	-	4,293	11,113	7,119	17,486
Achnanthes brevipes var. *intermedim* (Kützing) Cleve	5,125	4,198	6,188	4,988	-	-	-	-	11,736
Achnanthes longipes C.Agardh	6,005	6,632	5,601	5,783	-	-	-	-	7,065
Amphora commutata Grunow	1,515	1,828	1,388	1,003	1,370	-	1,989	-	2,944
Ceratoneis closterium Ehrenberg	207	204	248	146	51	302	-	289	1,013
Chaetoceros muelleri Lemmermann	43	-	51	37	46	41	36	44	81
Cocconeis pediculus Ehrenberg	415	356	350	378	578	-	-	-	1,425
Craticula halophila (Grunow)	2,309	2,322	2,298	2,447	2,261	2,164	1,374	3,298	4,153

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D.G.Mann									
Cyclotella meneghiniana Kützing	1,374	1,953	1,061	1,563	1,686	1,225	754	-	4,522
Cymbella affinis Kützing	307	232	351	336	344	280	421	183	606
Diatoma anceps (Ehrenberg) Kirchner	133	185	109	69	169	-	-	-	432
Entomoneis paludosa (W.Smith) Reimer	2,390	-	2,346	-	-	-	-	2,432	5,838
Entomoneis paludosa var. *subsalina* (Cleve) Krammer	2,435	2,145	1,371	2,465	3,126	-	3,794	1,711	5,838
Gymnodinium uberrimum (G.J.Allman) Kofoid & Swezy	12,289	-	2,3013	6,625	7,229	-	-	-	65,895
Halamphora coffeaeformis (C.Agardh) Levkov	138	74	199	190	88	-	-	-	715
Monoraphidium contortum (Thuret) Komárková-Legnerová	88	90	101	84	77	-	-	-	250
Monoraphidium minutum (Nägeli) Komárková-Legnerová	27	27	20	31	27	32	-	-	206
Navicula angusta Grunow	302	119	404	382		-	-	-	873
Navicula oblonga (Kützing) Kützing	8,065	-	7,359	-	8,770	-	-	-	15,366
Nitzschia amphibia Grunow	294	374	329	326	357	168	91	414	631
Oocystis lacustris Chodat	120	132	142	-	86	-	-	-	215
Pseudoschroederia robusta (Korshikov) E.Hegewald & E.Schnepf	68	-	104	62	39	-	-	-	375
Sellaphora pupula var. *rostrata* (Hustedt) M.Aboal	1175	1,430	996	1,066	1,208	-	-	-	3,312
Surirella brightwellii var. *baltica* (Schumann) Krammer	1,264	-	-	-	1,276	1,427	1,089	-	2,564
Tabularia fasciculata (C.Agardh) D.M.Williams & Round	755	476	448	496	298	1,458	1,351	-	2,436
Tetraëdron minimum (A.Braun) Hansgirg	46	25	31	57	63	56	-	-	225

Table 4: Average cell volume of phytoplankton species (mkm^3) in the lakes of the Regional Landscape Park "Slavyansky Resort" is greater than species-specific average cell volume. Abbreviation as in Table 2.

Species	RLP	R	V	G	S	L	Ch	O	SSACV
Achnanthidium bioretii (Germain) Monnier, Lange-Bertalot & Ector	329	332	295	-	296	-	393	-	236
Adlafia bryophila (J.B.Petersen) Gerd Moser, Lange-Bertalot & D.Metzeltin	202	182	212	132	-	-	-	283	134
Ankyra ocellata (Korshikov) Fott	232	199	249	247	-	-	-	-	168
Caloneis molaris (Grunow) Krammer	3,156	-	-	-	3,156	-	-	-	1,431
Halamphora holsatica (Hustedt) Levkov	218	178	397	203	124	199	204	-	85
Hyaloraphidium contortum var. *tenuissimum* Korshikov	56	44	-	71	69	40	-	-	18
Luticola mutica (Kützing) D.G.Mann	982	-	1,267	1,163	-	-	-	515	459

Table 5: Average cell volume of phytoplankton species (mkm^3) in the lakes of the Regional Landscape Park "Slavyansky Resort" is in the range of species-specific average cell volume. Abbreviation as in Table 2.

Species	RLP	R	V	G	S	L	Ch	O	SSACV
Adlafia minuscula (Grunow) Lange-Bertalot	146	93	102	142	173	-	177	186	105
Ankyra judayi (G.M.Smith) Fott	450	-	628	272	-	-	-	-	282
Cymbella lanceolata (C.Agardh) C.Agardh	1,307	2,538	1,280	696	715	-	-	-	2,234
Diatoma tenuis C.Agardh	1,043	-	850	1,966	727	630	-	-	705
Encyonopsis microcephala (Grunow) Krammer	30	25	21	47	27	-	-	-	40
Fragilaria brevistriata Grunow	118	-	143	93	-	-	-	-	135

Kobayasiella subtilissima (Cleve) Lange-Bertalot	267	215	132	169	401	649	168	138	259
Mucidosphaerium pulchellum (H.C.Wood) C.Bock, Proschold & Krienitz	591	1,171	89	-	514	-	-	-	1,150
Navicula capitatoradiata Germain	822	727	801	800	679	-	1,105	-	1,053
Navicula gregaria Donkin	743	814	818	506	837	929	593	704	649
Navicula protracta (Grunow) Cleve	836	-	563	565	910	1,239	1,105	633	891
Navicula cryptocephala var. *veneta* (Kützing) Rabenhorst	319	233	216	258	391	417	538	184	272
Navicymbula pusilla (Grunow) K.Krammer	438	548	238	317	745	-	-	342	433
Nitzschia hantzschiana Rabenhorst	139	148	226	120	83	-	-	118	144
Nitzschia paleacea Grunow	226	105	356	397	48	-	-	-	96
Nitzschia pusilla Grunow	65	40	47	149	24	-	-	-	118
Nitzschia reversa W.Smith	447	417	243	335	-	447	795	-	455
Peridiniopsis oculata (Stein) Bourrelly	11,091	8,041	-	-	9,926	15,308	-	-	12,540
Woloszynskia pascheri (Suchl.) Stosch	8,947	2,061	5,043	-	25,478	3,205	-	-	17,544

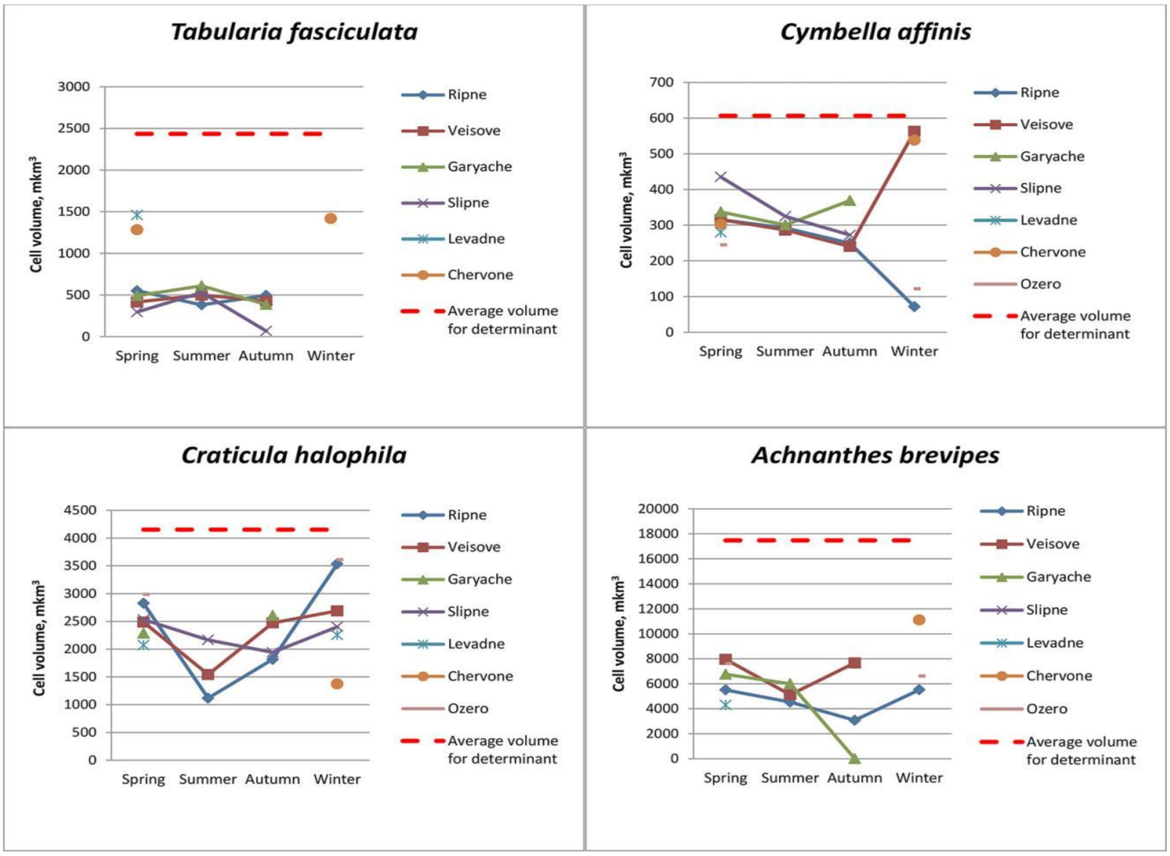

Figure 2: Seasonal dynamics of cell volumes in the studied lakes for *Cymbella affinis, Craticula halophila*, *Tabularia fasciculata, Achnanthes brevipes* in comparison with their species-specific average volumes.

Mismatch volumes of algal cells of one species in different lakes may be due to the various reasons listed above. We hypothesized that, at least for certain species, resizing could be due to differences in the degree of salinity between the lakes, which may also be responsible for differences in the total mass of phytoplankton. To do this, we calculated Pearson correlation coefficients. The analysis has revealed a negative correlation of the electrical conductivity of water with the cell volume of five algae species: *Cocconeis pediculus* (-0.95*), *Navicymbula pusilla* (-0.88*), *Entomoneis paludosa* var. *subsalina* (-0.95**), *Navicula cryptocephala* var. *veneta* (-0.91**), and *Nitzschia reversa* (-0.93*). Analysis of the correlation between chloride concentrations in the water and the amount of cells was statistically significant for: *Halamphora holsatica* (0.92**), *Entomoneis paludosa* var. *subsalina* (-0.87*), and *Navicula cryptocephala* var. *veneta* (-

0.79*). Thus, we assume that cell size of *Entomoneis paludosa* var. *subsalina* and *Navicula cryptocephala* var. *veneta* are impacted mainly by chlorides, which greatly enrich the water of the studied lakes. *Halamphora holsatica* cell volumes were positively correlated with the chloride content, regardless of the total dissolved solids. The amount of cells in *Cocconeis pediculus*, *Navicymbula pusilla*, and *Nitzschia reversa* are likely to be reduced, depending on the total salt content or by the influence of certain cations or anions, except chlorides. Previously continental aquatic objects for such aspects were investigated only in freshwater lakes; so the data obtained for the mineralized lakes are represented for the first time.

We have selected eight species of diatoms, which are found in almost all lakes in different seasons of the year, in order to study the seasonal dynamics of their sizes (Figures 2, 3). Also, we compared the average volume of algal species cells in the studied lakes with average species-specific cell volume of relevant species calculated on the basis of species diagnosis [8-11].

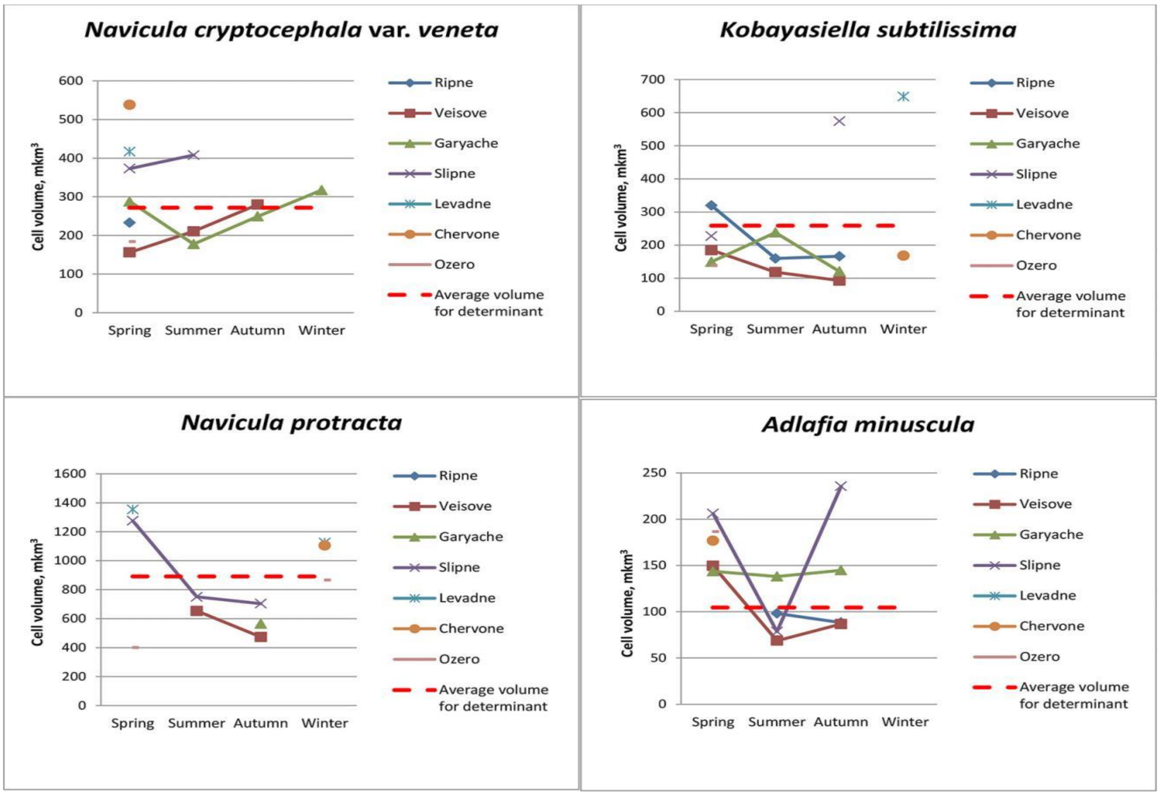

Figure 3: Seasonal dynamics of cell volumes in the studied lakes for *Navicula cryptocephala* var. *veneta*, *Kobayasiella subtilissima*, *Adlafia minuscula* and *Navicula protracta* in comparison with their species-specific average volumes.

As can be seen in Figures 2 and 3, averaged cell volumes for *Cymbella affinis* Kützing, *Craticula halophila* (Grunow) D.G.Mann, *Tabularia fasciculata*, and *Achnanthes brevipes* C.Agardh were lower than species-specific average volume (Table 3), but average cell size of *Navicula cryptocephala* var. *veneta*, *Kobayasiella subtilissima*, *Adlafia minuscula* (Grunow) Lange-Bertalot, and *Navicula protracta* (Grunow) Cleve fluctuated around its species-specific average volume in different lakes and in different seasons (Table 5).

For almost all species in all lakes a downward trend was observed in the cells in the summer, when there has a larger capacity of the biomass to bloom, which usually happened with phytoplankton in boreal water bodies [23-25], and in Ukraine especially [26]. Only *Tabularia fasciculata* and *Kobayasiella subtilissima* have an upward trend, and its cell volumes are higher in summer. This tendency can be seen for *Tabularia fasciculata* in the lakes Veisove, Garache, and Slipne, and for *Kobayasiella subtilissima* in the lake Garache only. There is only one species, *Cymbella affinis*, which as a gradual decrease in cell volume at the end of the year in lakes Ripne and Slipne.

CONCLUSION

In conclusion, it should be noted that the majority of species, for which we calculated the dimensional characteristics that are predominantly benthic or plankton-benthic inhabitants [6]. That is, the conclusions drawn on the basis of samples of phytoplankton can be attributed to all identified diversity of algae in the lakes, regardless of the substrate. That factor represents the salinity values for regulating cell surface and, thus, for the intensity of the metabolic processes of organisms in the first trophic level. We conclude that water salinity inhibit phytoplankton metabolic rate because the total cell surface is positively correlated with its intensity [2,3], but in the most saline of the studied lakes the cell size is larger. This finding is new, because previous [1] patterns of the relationship between primary production processes and the dimensions of the cells by other methods were not revealed. The calculated cell volume for most of the studied species was usually higher in the intermittently drying lakes (Levadne, Chervone, and Ozero) than in the perennial lakes (Ripne, Veysove, Garache, and Slipne). Thus, we can conclude that other factors, such as the ecological succession stage, affect the dimensional characteristics of phytoplankton cells in ephemeral lakes, and therefore override the drainage factors and related dynamics of water salinity. In the ephemeral winter lakes Levadne, Chervone, and Ozero, the recovering process of algal community repeats at least two times per season, apparently reducing the salinity stress. In affect the cell size increases in comparison with the perennial lakes. Populations of ephemeral lakes develop discontinuously, therefore never reaching the diminution stage at the end of continuous reproduction process characteristic of perennial populations. The data obtained for the mineralized lakes are presented for the first time and can be used for the purpose of monitoring lake ecosystems.

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