

Phytoplankton communities in ecological assessment of the Southern Bug River upper reaches (Ukraine)

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Abstract

We conducted an environmental assessment for the first time in the upper reaches of the Southern Bug River utilizing data on phytoplankton communities and hydrochemistry. Chlorophyta (40%), Bacillariophyta (19%), and Euglenophyta (18%) were among nine identified divisions of common phytoplankters which included 281 taxa. The results of a statistical comparison of species richness showed that the investigated part of the river can be divided into two parts for follow-up monitoring. Class II-III waters reflect a trophic status equivalent to an oligo- to meso-trophic state. Using algal indicators as well as environmental variables and statistical methods we identified major factors impacting the algal communities, such as phosphate and ammonia.

Key words: Phytoplankton, species composition, indicators, abundance, biomass, statistics.

1. Introduction

Water is a prerequisite for life and a key ingredient in virtually all human economic activities. Freshwater rivers are essential sources of water and it is necessary to study, protect, and improve their ecological status (Zalewski 2000). Nowadays, the management of large rivers requires a balance between human needs and ecological integrity, although, until quite recently, ecological principles played only a minor role in river management (Edwards 1995). Now that the European Union has launched the Water Framework Directive (The Directive 2000), there is a program that requires catchment management plans to be developed for all major European rivers. The Directive also requires

that all major rivers achieve 'good ecological status' by 2015 (Sukhodolov *et al.* 2009). The Ukraine as a European country needs to monitor its rivers and take the necessary steps to minimize any harmful ecological impacts that may occur.

The most revealing components of the bioindicator-based methods used to identify the ecological state of surface waters are the species richness of algae and their diversity, abundance, and biomass (Barinova *et al.* 2006; Barinova 2011). Moreover, phytoplankton species composition is an important part of these characteristics because it plays a central role in the functioning of large rivers. Potamoplankton shows not only variations in water quality but also changes in physical variables and biotic interactions. Planktonic algae are responsive

to excessive supplies of inorganic nutrients and may pose problems in long stretches of river experiencing cultural eutrophication, although planktonic algae may also enhance water quality for humans in rivers affected by agricultural or industrial waste. The ability to apply phytoplankton-based assessments to evaluate the ecological status of large and lowland rivers lies in developing a better understanding of the ambient conditions in the river relative to undisturbed conditions in such rivers (Wehr, Descy 1998).

Europe has over 150 transboundary rivers (Whitton 1984). Eight of the 10 largest catchments in Europe are in the eastern plains of Russia and the Ukraine, and information on their present status is extremely limited. To identify the ecological state of the rivers it is necessary to know the origin of the published data to compare them with the information we have nowadays. But sometimes it isn't possible to have this historical perspective. In the case of the Southern Bug River, a number of publications presented hydrobiological data on algae in the River (Sovinsky 1876; Shyrshov 1928a, 1928b, 1928c; Pidlisnyy 1928; Radzymovskyy 1928; 1934; Roll 1937; Swirenko 1941; Pickush *et al.* 1978; Selezneva 1982; Klochenko *et al.* 1993; Klochenko, Mytkivska 1994; Davydov 1997; Zhukinskiy *et al.* 1989; Mikhailyuk *et al.* 2003; Taraschuk 2004; Gerasimuk, Kyrylenko 2006; Tkachenko 2007). However, these data are from the lower part of the river. For the middle part, the information is neg-

ligible. Phytoplankton literature data for the upper part of the Southern Bug River are known from the 1920s and 1930s (Roll 1937). Nevertheless, an ecological assessment can't be made without a comprehensive analysis of the phytoplankton.

The aim of this study was to determine the relationships between environmental factors, water quality, and the phytoplankton community's structure, species richness, abundance, and biomass in the upper reaches of the Southern Bug River in the Ukraine. The role of potamoplankton and inorganic nutrients in the self-purification process was elucidated.

2. Materials and methods

2.1. Description of study site

The Southern Bug River is one of five large fluvial systems of the Western Steppe region. Of all Ukrainian rivers, the Southern Bug is the largest river, whose basin lies wholly within Ukrainian territory (Fig. 1). Its catchment encompasses areas of the Volyn-Podolsk plateau and the Black Sea coastal plain (Sukhodolov *et al.* 2009). The catchment area of the Southern Bug River is approximately 63 700 square km, with a river length of 806 km (Vyshnevskyy 2000). This river is overregulated, with 8000 artificial reservoirs located along the river and tributaries (Vyshnevskyy, Kosovec' 2003).

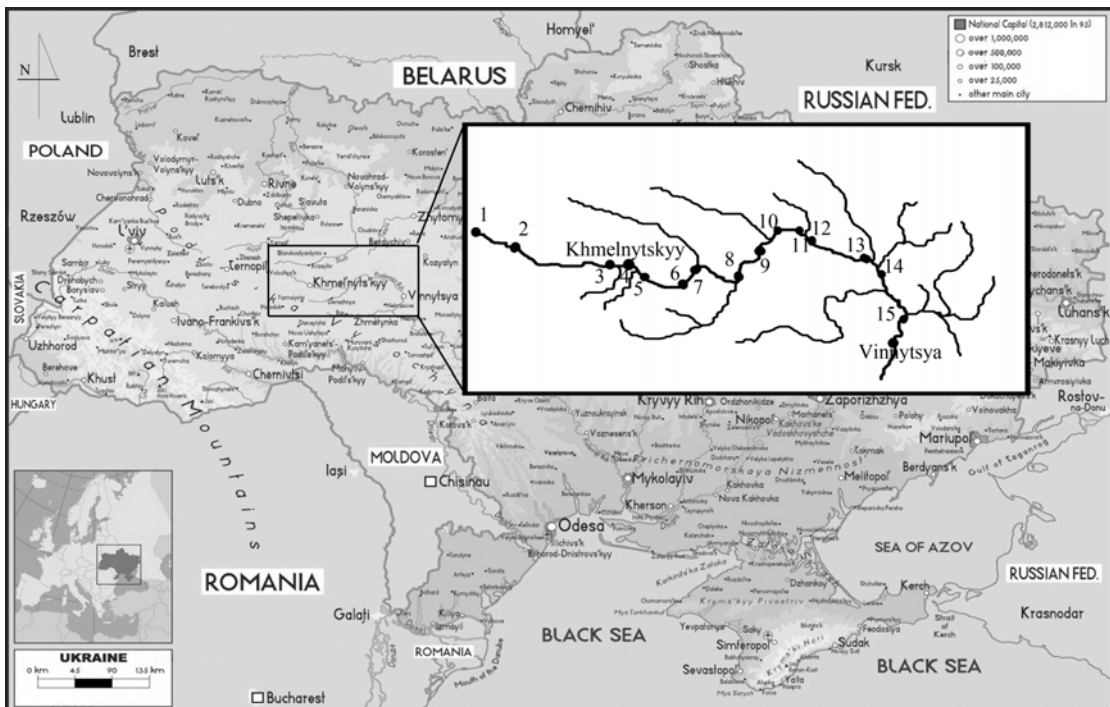


Fig. 1. Map of the upper part of the Southern Bug River.

2.2. Materials

Algological, hydrobiological, and hydrochemical investigations were conducted in the upper part (198 km) of the Southern Bug River during the summers of 2007-2011. As part of the investigation, because the river was so long, each field trip to gather samples took about two weeks during each month from the end of June until mid-August. Altogether, 118 samples of plankton were collected from 15 sampling stations. Plankton samples were collected with an Apstein plankton net (20 mesh) as well as a Ruttner sampler (Romanenko 2006). The algological samples were counted using Zeiss and PZO microscopes. Both living samples as well as those fixed with 3% formaldehyde were counted. Quantitative analyses of the plankton in the samples collected using the Ruttner sampler were carried out using a Nageotte Chamber (0.2 cm³).

For taxonomic identification the handbook series was used (Topachevskyy, Oksiyuk 1960; Kondratieva 1968; Komárek, Fott 1983; Starmach 1983; 1985; Krammer, Lange-Bertalot 1991; 1997a, b, c; Lenzenweger 1996; 1997; 1999, Komárek, Anagnostidis 1998; 2005; Krammer 2000; 2002; 2003; Lange-Bertalot 2001; Palamar'-Mordvintseva 2003; 2005; Popovský, Pfiester 2008; Kovalenko 2009; Levkov 2009). Some additional monographs and papers also were used for species identification (Asaul 1975; Tsarenko 1990; Tsarenko *et al.* 2005). Special attention was paid to the Cyanoprokaryota due to the modern conceptualization of this division (Komárek, Komárkova 2002; Wacklin *et al.* 2009; Katovsk *et al.* 2010). In our work on the taxonomy of the algae, the monographs from the "Algae of Ukraine" series were used (Tsarenko *et al.* 2006; 2009; 2011) employing the common system nomenclature derived from T. Cavalier-Smith (2004).

In parallel with the sampling for algae, we measured hydrochemical variables including ammonia, nitrite-nitrogen, nitrate-nitrogen, and phosphate-phosphorus. Hydrochemical samples were placed in 1 liter dark glass bottles, and transported to the laboratory in an icebox. Variables were analyzed using standard methods (Semenov 1977).

2.3. Methods

Each group of species was separately assessed with respect to their significance as bioindicators. Those species that predictably responded to environmental variables could be used as bioindicators reflecting the response of the aquatic ecosystems to eutrophication, acidification (based upon pH levels), salinity, and organic pollutants (Barinova *et al.* 2006).

Saprobity indices (Sládeček 1973; 1986) were calculated on the basis of the identified species

richness and quantitative investigations of potamoplankton as follows:

$$S = \sum_{i=1}^n (s_i \cdot a_i) / \sum_{i=1}^n (a_i) \quad (1)$$

Where: S – Index of saprobity of algal community; s_i – species-specific saprobity index; a_i – species abundance.

The Shannon's diversity index (Odum 1969) was calculated as:

$$\bar{H} = - \sum_{i=1}^s \frac{n_i}{N} \log_2 \frac{n_i}{N} \quad (2)$$

Where: N = common organism abundance, s = species number; n_i = species number of every species; \bar{H} = Shannon species diversity index, bit.

Statistical methods for comparative floristic analysis were used for calculating the similarity of algal communities among the sampling stations (Novakovsky 2004).

Statistical analysis of the relationships of species diversity in algal communities and their environmental variables were calculated using Canonical Correspondence Analysis (CCA; with the CANOCO for Windows 4.5 package, Ter Braak, Šmilauer 2002). The statistical significance of each variable was assessed using the Monte Carlo unrestricted permutation test involving 999 permutations (Ter Braak 1990). The CCA biplot represents the overlap of species diversity in relation to a given combination of environmental variables. Arrows represent the environmental variables, with the maximal values for each variable located at the tip of the arrow (Ter Braak 1987).

3. Results

The chemical variables measured during the study of this 198 km portion of the river channel are representative of fresh and low alkalinity waters with high fluctuations in ammonia concentrations at stations 6 and 7 (Table I).

We identified 281 species of algae and cyanobacteria from 118 samples of potamoplankton. Chlorophyta (40%), Bacillariophyta (19%), and Euglenophyta (18%) dominated the identified divisions (Fig. 2a, Appendix I) and comprised 78.3% of all identified species in this part of the river. As can be seen in Fig. 2, bioindications on the basis of algal species presentative of the various ecological categories show that the Upper Bug River's studied communities contained mostly green algae with euglenoids and diatoms (Fig. 2a). The planktonic and plankto-benthic inhabitants of the river (Fig. 2b) were characteristic of slow flowing and moderately

Table I. Mean chemical variables measured at the sampling stations on the Southern Bug River.

Station	Name of locality	Distance between stations, km	N-NH ₄ ⁺ , mg dm ⁻³	N-NO ₂ ⁻ , mg dm ⁻³	N-NO ₃ ⁻ , mg dm ⁻³	P-PO ₄ ³⁻ , mg dm ⁻³	Coord. North, N	Coord. East, E
1	Kholodets	0	0.34	0.001	0.16	0.17	49°37'15"	26°28'05"
2	Volchya Gora	24	0.25	0.01	0.28	0.11	49°29'57"	26°45'30"
3	Oleshyn	15	0.28	0.001	0.13	0.15	49°27'32"	26°54'43"
4	Chmelnytsky	5	0.18	0.001	0.12	0.1	49°26'42"	26°56'56"
5	Kopystyn	12	1.76	0.015	0.52	0.54	49°23'31"	27°07'22"
6	Goloskov	16	4.92	0.046	0.15	1.22	49°23'13"	27°20'32"
7	Medzybodz	10	4.88	0.092	0.24	1.75	49°26'09"	27°24'51"
8	Letychev	17	0.6	0.011	0.15	1.22	49°23'08"	27°36'54"
9	Novokonstantinov	14	0.21	0.01	0.13	0.25	49°28'54"	27°43'24"
10	Berezna	16	0.03	0.01	0.28	0.14	49°33'23"	27°50'56"
11	Khmelnik	8	0.08	0.012	0.33	0.15	49°33'01"	27°57'18"
12	Shyroka Greblya	10	–	–	–	–	49°31'04"	28°02'01"
13	Uladvka	17	0.07	0.009	0.5	0.04	49°29'36"	28°13'59"
14	Guschyntsy	13	–	–	–	–	49°27'11"	28°21'26"
15	Strydzavka	21	0.28	0.009	0.13	0.2	49°17'07"	28°28'21"
Total		198						

oxygenated (Fig. 2c), slightly warm (Fig. 2d), low-alkalinity (Fig. 2e), and low-mineralized (Fig. 2f) waters. Organic pollution indicators showed two groups: species that reflected the less polluted water quality Classes I-III, and few species from Class V, indicative of polluted waters (Fig. 2g). Photosynthetic activity in the algal communities was very high, representing mostly production by autotrophic species (Fig. 2h). Indicators of ecosystem trophic state suggested eutrophication of the Southern Bug River (Fig. 2k).

Table II presents species richness, average abundance, and bio-volume of phytoplankton across the sampling stations. It can be seen that algal abundance and bio-volume increased from upstream to downstream in the river channel (Figs. 3, 4). The trend line of species richness is correlated with the trend in the Saprobity Index (S), which reflected the middle Class III of organic pollution and *vice versa* with the community complexity index of the Shannon trend line.

A statistical comparison of species richness revealed that all algal diversity can be divided into two major clusters with a similarity level of 40% (Fig. 5). The first cluster on the similarity tree shows species from stations 1-8, whereas species from stations 9-12 are in the second cluster.

A calculation of species overlap in the studied river communities showed a high level of similarity for all sampling stations, which fluctuated between 48 and 64%. The dendrite shown in Fig. 6 revealed two cores of species richness that are marked as large circles – stations 2 and 15.

We also tried to identify relationships in the community species content and chemical variables describing water quality in which the communities existed. For this, we constructed an RDA biplot using the CANOCO Program (Fig. 7) that showed two groups of variables. The first group included phosphate and ammonia and was placed in the right quadrants of biplot. The second group was placed on the left side and included nitrate and nutrient-dependent community parameters such as the number of species and Saprobity Index (S). The first group of variables was related to communities at stations 7 and 8, whereas the nitrate group corresponded to the lowermost stations 10-15.

4. Discussion

We identified a high degree of species richness within the phytoplankton communities in the upper part of the Southern Bug River, a large Ukrainian river, which had never been studied regularly until now. The green algae, euglenoids, and diatoms dominated (Appendix I, Fig. 2). At present, the middle and lower parts of the Southern Bug River can't be used for comparative analysis with the upper part. Investigations of those lower parts were not systematic, and very often we have no lists of taxa for them. But the information that we have from the upper part of the Southern Bug River can be compared with some parts of the Dnipro River, most of which lies within the Ukraine. We observed a similar pattern of rank allocation among the dominant divisions, Chlorophyta and Bacillariophyta, in its different parts

(Vladymyrova 1976; Vasenko *et al.* 2002) as in the studied part of the Southern Bug River, although the latter was different because of the inclusion of euglenoids in the dominant divisions. Moreover, this situation is common for most European rivers such as the Morava River in Czech Republic (Marvan

et al. 2004). It is important to emphasize that this ecological assessment of the Southern Bug River upper reaches was made for the first time and that the investigated conditions of the river influence the ecosystem of the whole river. The bioindicators (Fig. 2b-k) showed that the studied part of the river

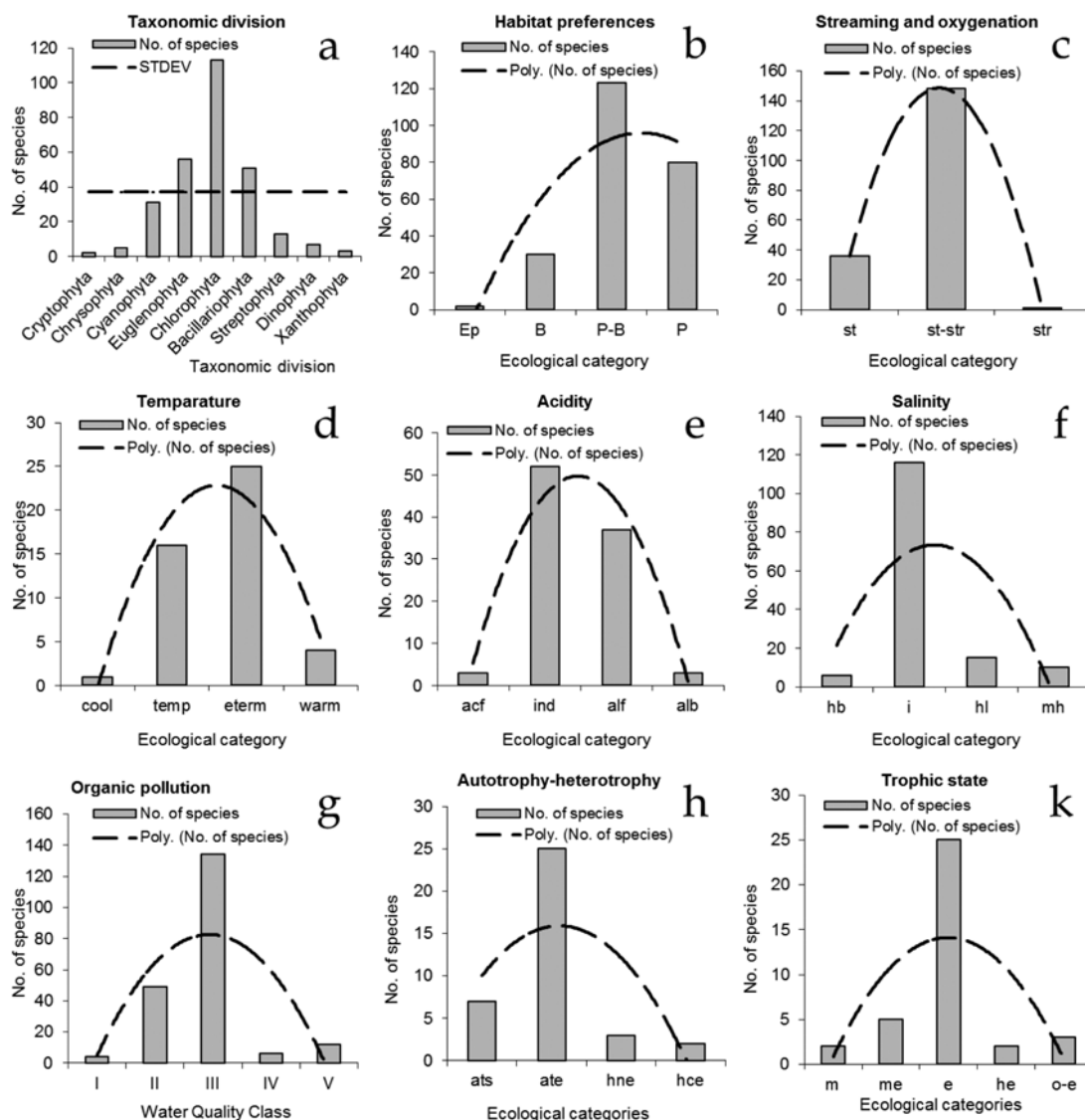


Fig. 2. Bioindicator plots for the ecological analysis of the Southern Bug River phytoplankton communities (on the basis of data presented in Appendix I and Barinova *et al.* 2006). (b) Ecological types: Ep, epiphytes; B, benthic; P-B, planktonic-benthos; P, plankton. (c) Stream flow and oxygenation: st, standing water with low oxygenation inhabitants; st-str, low-flow moderately oxygenated water inhabitants; str, flowing water enriched by oxygen-loving inhabitants. (d) Temperature: cool, cool-water; temp, temperate; warm, warm-water; eterm, eurythermic. (e) Acidity (Hustedt 1957): acf, acidophile; ind, indifferent; alf, alkaliphile; alb, alkalibiont. (f) Salinity: hb, halophobes; i, indifferents; hl, halophiles; mh, mesohalobes. (g) Organic pollution water quality class (Sládeček 1986): I, clean water – V, very polluted water. (h) Nitrogen uptake metabolism (Van Dam *et al.* 1994): ats, nitrogen-autotrophic taxa, tolerating very small concentrations of organically bound nitrogen; ate, nitrogen-autotrophic taxa, tolerating elevated concentrations of organically bound nitrogen; hne, facultatively nitrogen-heterotrophic taxa, needing periodically elevated concentrations of organically bound nitrogen. (k) Trophic state (Van Dam *et al.* 1994): m, mesotraphentic; me, meso-eutrathentic; e, eutrathentic; he, hypereutrathentic; o-e, oligo- to eutrathentic (hypereutrathentic).

was low in alkalinity and low in dissolved solids, slow flowing and moderately oxygenated, falling within water quality Class I-III like most regional rivers (Vasenko *et al.* 2002; Marvan *et al.* 2004). Our analysis reflected the eutrophic state of the river ecosystem in which photosynthetic activity is high, mostly due to the presence of green algae and diatoms, and that trophic condition increased from the source of the river to downstream stations.

Comparing abundance and bio-volume over the stations, we identified a tendency for growth downstream (Table II). Species numbers increased downstream. Saprobity indices, which reflect organic pollution that stimulate photosynthetic activity on the one hand, and species richness on the other (Barinova 2011), reflected high self-purification capacities in the ecosystem within the studied part of the river (Fig. 3). However, the structure of

the phytoplankton communities visibly degraded, which is indicated by lower Shannon's diversity index values (Fig. 4) and moderate cell volumes (Table II). Therefore, it is possible to conclude that the self-purification ecosystem ability of the river is high, in spite of its anthropogenic pressures, which increase downstream. Correlation analysis characterized the river ecosystems in the southern regions as Eastern Mediterranean (Barinova 2011), which is characterized by high year-round insolation as well as high summer water temperatures.

In a comparison of species composition in the communities, using statistical methods to evaluate all of the stations, we can divide the river into two sections – upper and lower – with a break point between stations 8 (Letychev) and 9 (Novokonstantinov). These sections differ not only in their hydrology (Vyshnevskyy 2000; Denysyk, Gusak 2002) but

Table II. Mean biological variables over sampling stations of the Southern Bug River.

Station	No. of species	Abundance (cell dm ⁻³)	Bio-volume (mg dm ⁻³)	Cell volume (mg)	Saprobity Index	Shannon Index
1	95	7875	10.57	0.0013	2.01	3.54
2	73	1720	0.69	0.0004	1.94	2.69
3	60	2575	3.15	0.0012	1.56	2.45
4	87	2240	1.18	0.0005	1.72	2.59
5	66	4176	3.14	0.0008	1.91	2.99
6	97	4760	4.78	0.001	2.05	3.34
7	73	3496	2.57	0.0007	1.96	3.08
8	65	10360	3.36	0.0003	2.03	1.63
9	116	46925	8.55	0.0002	1.93	2.46
10	93	3233	1.54	0.0005	2.08	2.11
11	81	3900	1.52	0.0004	2.09	2.13
12	97	3693	6.1	0.0017	2.2	2.42
13	90	32444	11.65	0.0004	2.06	3.08
14	95	132977	30.53	0.0002	1.94	1.47
15	134	5076	5.1	0.001	2.11	2.53

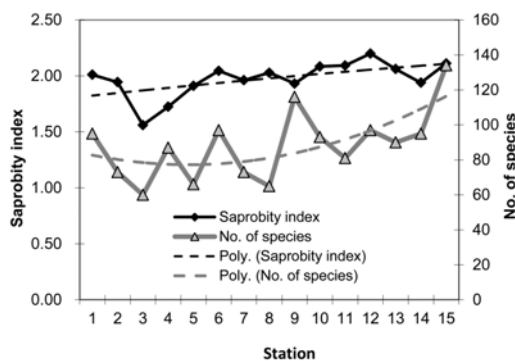


Fig. 3. Mean species richness of the plankton communities and the Saprobity Index (S) of the sampling stations in the Southern Bug River. Polys are functions that describe general trends.

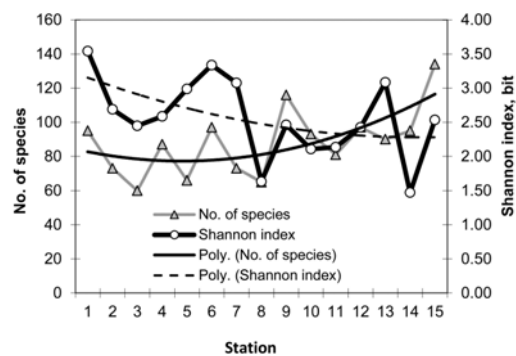


Fig. 4. Mean species richness in the plankton communities and the Shannon Diversity Index across the sampling stations in the Southern Bug River. Polys are functions that describe general trends.

also in their anthropogenic pressures, which are reflected in the plankton community's structure.

The programs used for our analysis, such as CANOCO and GRAPHS, represent the first use of these programs for identifying the ecological status of the rivers in Ukraine. These methods yielded significant results and, thus, could be used in future investigations. With the help of comparative floristic methods, we hoped to expose not only identical communities, but we also tried to divide the river system into similar parts, to reduce the number of stations in subsequent monitoring surveys. This approach was productively used earlier for the rivers of the Mediterranean region (Barinova 2011).

Using comparative floristic methods, we did an analysis of the plankton communities (Novakovsky

2004), which indicated a high degree of overlap in species composition among the stations (Fig. 6) that reflected the potamoplanktonic community continuum. Nevertheless, our analysis showed that communities in the upper reaches of the Southern Bug River could be divided into two different parts in which station 2 and station 12 were enriched by species from the other stations and which belonged to both the upper and lower sections. These stations could be used as reference stations for monitoring ecosystems based on phytoplankton assemblages.

The CANOCO statistical program helped us to identify the major factors that impacted the studied communities on the basis of the species-environmental variables relationships. It was found that two groups of variables were important for separating

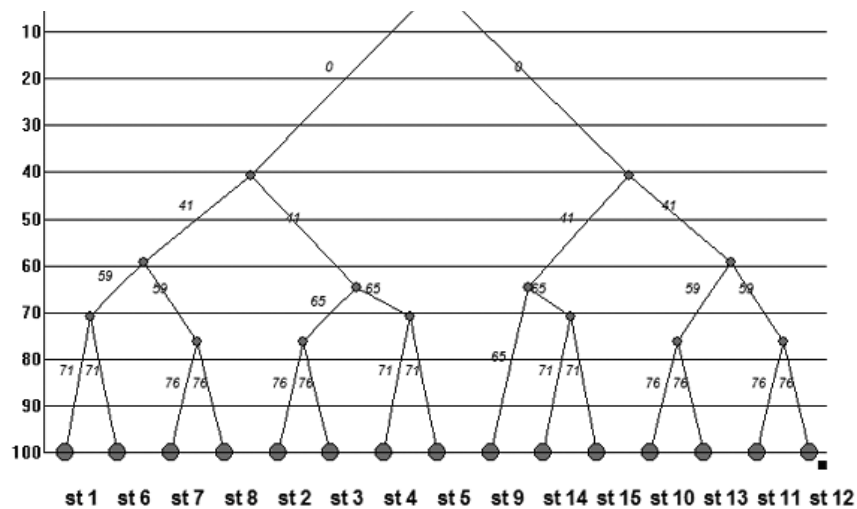


Fig. 5. Tree of similarity of the phytoplankton communities in the sampling stations of the Southern Bug River calculated on the basis of Sørensen-Czekanowski indices. At the similarity level of 40% two clusters are cut off.

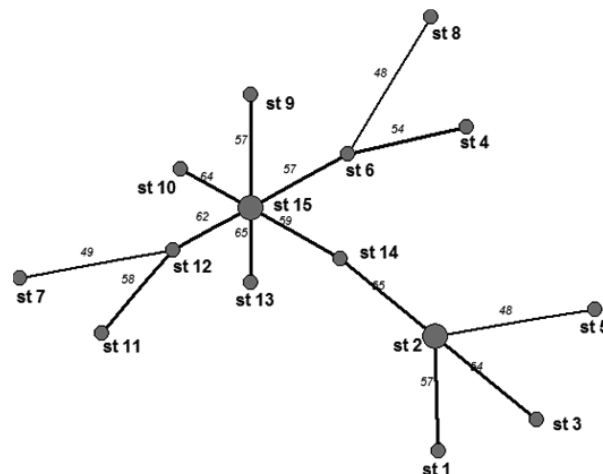


Fig. 6. Dendrite of phytoplankton species richness overlapping in the sampling stations of the Southern Bug River calculated on the basis of Sørensen-Czekanowski indices. The bold lines represent the most similar communities. Communities that included most of the species from the others are represented in large circles.

communities – the first group (phosphate and ammonia) for the upper section, and the second group (nitrate and nutrient dependent community parameters) for lower section of the studied parts of the river (Fig. 7). Despite ammonium concentrations which were relatively low, communities not only reacted to ammonium as such, but also to the sum of river pollutants. The sources of the pollutants, perhaps, are the livestock farms located along the river and at the mouths of tributaries. Furthermore, the most likely, anthropogenic pollution impacts were determined to already have begun in the upper section of the river; however, the ability of the Southern Bug River ecosystem to moderate the negative impacts of phosphate and ammonia in its lower section is quite high.

Thus, based on phytoplankton communities and hydrological similarities, the studied part of the river can be characterized as an upper section – from the river source to the station near Novokonstantinov with a monitoring point at station 2 (Volchya Gora) – and a lower section – with a monitoring point at station 12 (Shyroka Greblya).

Therefore, according to the analysis of the phytoplankton communities and hydrological similarity, we divided the river into two different sections with two monitoring points, which reflect the changes in community structure and water quality in the studied parts of the river's upper reaches.

Conclusion

For the first time, the upper reaches of the Southern Bug River's potamoplankton and chemi-

cal conditions were studied. The river reaches has a high species richness, which consisted of 281 taxa from 9 identified divisions with Chlorophyta (40%), Bacillariophyta (19%), and Euglenophyta (18%) being dominant.

It is very important to study the upstream reaches of large rivers; it is exactly these parts that determine water quality in the lower parts of rivers. The studied part of the Southern Bug River is different from other Ukrainian large rivers because of the inclusion of euglenoids. In this case, we can conclude that euglenoid species are included as a major part of algal diversity in Class II-III of quality which reflects their oligo- to meso-trophic state. At the same time algal diversity in the studied part of the river was similar to that in other large European rivers, such as the Morava River in Czech Republic. The data obtained from the Southern Bug River's upper part can be used as a model for ecological and floristic analyses of the large Ukrainian rivers. The revealed information highlights the importance of river monitoring, comparative analysis of the middle and upper parts of the river, and comparison of these data with other Ukrainian and regional rivers. Therefore, our ecological assessment is relevant not only for our studied river but it can also be used as an example for large European rivers. Bio-indicator methods that were implemented in our analysis were enriched by calculation of the statistical relationships between phytoplankton diversity and environmental variables. As a result, we can conclude that ammonia, as a result of anthropogenic pollution from the river tributaries and from factories, had the most critical impact.

Acknowledgements

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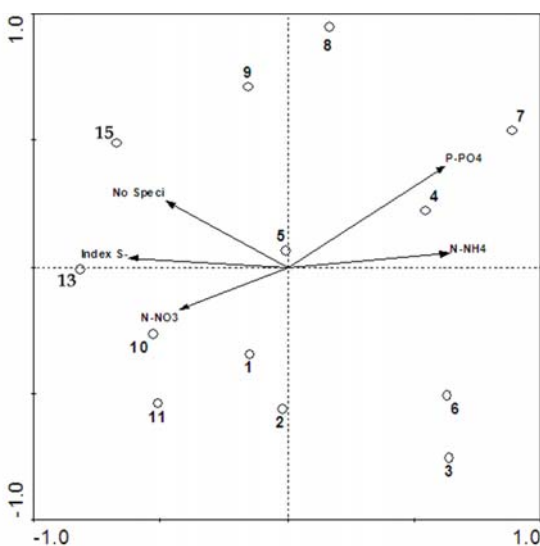


Fig. 7. Redundancy analysis biplot (RDA) for phytoplankton communities and environmental variables at the sampling stations on the Southern Bug River.

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