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Periphyton Morphofunctional Indicators in the Danube's Avandelta: Long-Term Assessment of Ecological Status

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Abstract The long-term monitoring (2004-2021) at the Ukrainian part of the Danube avandelta applies measures of structural-functional organization and of morphofunctional indicators of periphyton. The latter are applied assessing the Ecological Status Class (ESC) in accordance with the requirements of the EU Water Directives. The two study areas at the Danube avandelta are the Danube-Black Sea Channel and the Ust'-Dunaisk Port. The objects of monitoring were attached living communities of algae and cyanobacteria on navigation buoys, which formed macro- and microbenthic assemblages. The aim of the study was to search for regularities of the influence of the annual volume of the Danube runoff on the floristic composition, biomass and morphofunctional indicators of periphyton and changes of the ESC categories over the 18-year study. It is shown that in years of high river flow conditions associated with an increased flowability, sensitive species of periphyton dominated, which are characterized by low functional activity and low values of specific surface (S/W) indicator ≤ 500 m^2 kg^{-1}. These species were red and green algae with lamellar thalli. In turn, under low river flow conditions, tolerant periphyton species with high functional activity and high specific surface area (surface to weight ratio, S/W ≥ 1000 m^2 kg^{-1}) seemed to take advantage, which were mainly cyanobacteria and filamentous and colonial brown algae. Further, the annual volume of the Danube runoff corresponds with values of the Surface Index (SI) of algae communities and ESC categories. SI indicated for the period from 2004 to 2021, when moving from low-water years to high-water years, concurrently decreased with the ESC from "High" to "Poor". The most pronounced deterioration of water quality described as "Bad" status by ESC categories, was observed in year 2010. In that year the river runoff indicated a strong positive anomaly of having 46% more water volume when compared with long-term averages. We conclude that studying periphyton assemblages, that are living attached on artificial substate such as navigation buoys, are well applicable as biological quality elements for monitoring the Ecological Status in accordance with the EU Water Directive.

Keywords: Danube avandelta, monitoring, algae fouling, periphyton, morphofunctional indicators, river runoff, ecological status class

1 Introduction

The Danube is the largest river of Central and South-Eastern Europe which belongs to the Black Sea basin. The catchment basin covers 19 European countries. Delta zone and adjacent maritime part of the Danube, in accordance to the Water Framework Directive (WFD, 2000/60/EC), and Marine Strategy Framework Directive (MSFD, 2008/56/EC) classification, belongs to the transition zone. The Danube Transition Zone (DTZ) is a connecting bridge between the freshwater river and the Black Sea ecosystem, which combines and strengthens biological and physico-chemical processes in the contact zone of the marine border (Zaitsev, 2015). The DTZ may be considered as an integral ecological cluster, the status of which has a direct impact on ecosystem of the adjacent northwest shelf avandelta, as well as on the entire Black Sea ecosystem.

According to EMBLAS project research, pollutants entering with river waters of Danube were discovered from northwest shelf to shores of Georgia (Slobodnik et al., 2022).

For monitoring purposes, the DTZ is an important "heartbeat place" for determining the "environmental health", which indicates the positive or negative river impact on the marine ecosystem. Considering that the Danube River and the Black Sea are EU Water Framework Directive standard zones, the main goal of which is to achieve Good Ecological Status (GES). Operational and long-term monitoring of the DTZ must be carried out in accordance with the Directive standards and requirements.

Among numerous Biological Quality Elements present in the DTZ, with the help of which the Ecological Status Class (ESC) monitoring of this area can be held, the water vegetation - Macroalgae and Angiosperm have the priority for the reason as they provide the first autotrophic link among others. Thus, the ecological transformation processes of substance and energy in aquatic ecosystems begins here. This fact is the background for monitoring the state of plant communities. In this perspective, the intensity of the primary production process can be measured, the level of eutrophication and the ESC categories that are directly related to these indicators. Phytoplankton is a sensitive autotrophic element, which quickly reacts on the changing of primarily-productional process intensity. However, in a hyperdynamic transition zone, it is a less convenient monitoring tool in comparison to the fixed forms of algae and angiosperm, which integrally reflect the ESC for a certain area during the rather long time period. The vegetation survey of the Danube delta water bodies includes mainly aquatic plants and characean algae (Dubyna et al., 2003, Dyachenko, 2011). The information about periphyton, i.e., attached living macro- and microalgae in the Danube avandelta is practically absent. One reason is that the marine zone adjacent to the delta area is almost completely devoid of solid substrate, which is a biotope for the development of macro- and microphytobenthos. In the avandelta zone multicellular algae exist in the life form of phytofouling, periphyton species, which are developed on anthropogenic origin substrates – piers, buoys and other navigation and hydra technical constructions and buildings.

By identifying a reliable monitoring tool, it is possible to develop ESC correctly. This bio-assessment method is based on selecting attached living macroalgae and, in case of the river-sea transition zone, also on dominant or key species of plant communities (often referred as indicator species) which develop biomass sensitively to species-specific environmental conditions. Phytoplankton indicators should be functional – to reflect the primarily-productional process intensity, and integral – to accumulate information about the autotrophic process intensity for the definite seasonal period for a known monitoring point. Water vegetation morphofunctional indicators have such properties of functional integrity, which allow, to judge the functional macrophyte communities' activity and the intensity of their functioning, which is directly related to the ESC categories of an aquatic ecosystem, based on the parameters of the specific surface of thallus algae (Minicheva, 1998; Minicheva, 2013).

The key abiotic parameter of the "river-sea" transition zone, which has a direct influence on structural-functional organization of the biological component and the ecological status of ecosystem, is the water flow intensity - the value of river runoff for a certain period. Years with different volumes of river runoff can significantly change both the portrait of biological communities and the ESC category of the ecosystem. It is related to the fact that a river runoff at one moment of time brings full nutrients in a certain concentration from a catchment basin. According to this, the volume of river runoff is varying among seasons, and drives the nutrient availability into the marine system. Dissolved and weighted compounds of nitrogen and phosphorous in river runoff, along with photosynthetic active radiation and water temperature, form the level of the primarily-productional process, which determines the ESC category.

Considering the above features of biological and abiotic components of the DTZ, the goal of this work was: to show the spatiotemporal dynamics of structural-functional organization of vegetation in different areas of the Ukrainian part of the Danube avandelta (girlo Bystroe and Ust'-Dunaisk Port) and to identify the pattern of the annual river flow volumes influence on the ESC categories in the DTZ in accordance to the EU Water Directives standards. Here we apply long-term monitoring data (2004-2021) about attached living macroalgae and microbial periphyton on artificial substrate, on the surface of navigation buoys. These life forms of algae and cyanobacteria we define in this study as morphofunctional benthic indicators for phytofouling, periphyton, different from commonly used biological quality elements of natural benthic and pelagic habitats such as phytobenthos and phytoplankton elaborating the Ecological status according to the WFD assessment of ecosystem health (Kristensen et al., 2018).

2 Material and methods

2.1 STUYDY AREA AND SAMPLING

The monitoring of structural-functional phytofouling communities, or periphyton, was held from 2004 to 2021 at two study areas of the Ukrainian part of the DTZ: Danube-Black Sea Channel (girlo Bystroe) and Ust'-Dunaisk Port. As monitoring stations, the navigation buoys in the respective sampling areas were used (Figure 1). In 2022, the monitoring of algae fouling communities was interrupted due to the Russian military aggression against Ukraine. The surface of metal buoys represented thus the man-made biotope for phytofouling communities' development (Figure 2).

Samples were taken from the side of the boat and with the help of a diver using a special periphyton frame (frame size 10 cm x10 cm) for collecting material, trimmed with sulfureous gases for keeping algal cells and thalli. In total for the period of long-term monitoring, there were 569 phytofouling samples collected and processed of on two study areas (Table 1).

Table 1: The number of samples for collecting attached living phytofouling organisms from navigation buoys at two sites from 2004-2021.

Monitoring areas	Year																	
	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021
Ust'-Dunaisk Port	9	21	-	9	36	18	27	18	9	9	18	18	18	17	18	18	9	15
Danube-Black Sea Channel	6	15	9	9	30	18	24	18	9	9	18	18	18	18	18	18	9	18
Total samples: 569	**15**	**36**	**9**	**18**	**66**	**36**	**51**	**36**	**18**	**18**	**36**	**36**	**36**	**35**	**36**	**36**	**18**	**33**

2.2 QUANTITATIVE ASSESSMENT

Besides generally accepted parameters describing water vegetation (species composition see Table 3, projective cover, biomass), for DTZ phytofouling morphofunctional assessment methods were applied according to Minicheva (1989). The value of the specific surface of species was calculated as follows: the surface square of thallus (S) is relative to its fresh weight (W), (S/W, m^2 kg^{-1}). This parameter reflects the potential ability of the species to participate in the production process and is thus a characteristic of its functional activity, which depends on the size and the morphological structure of the thallus, cell aggregates or single cells. For the Black Sea macrophytes in Minicheva et al. (2015) the coefficients of functional activity can vary within a wide range from 5 to 500 or more m^2 kg^{-1}. The combination of specific surface values with the algal community biomass allows to calculate the phytofouling surface indexes (SI, unit), which reflect the intensity of the primarily-productional process. SI characterizes the

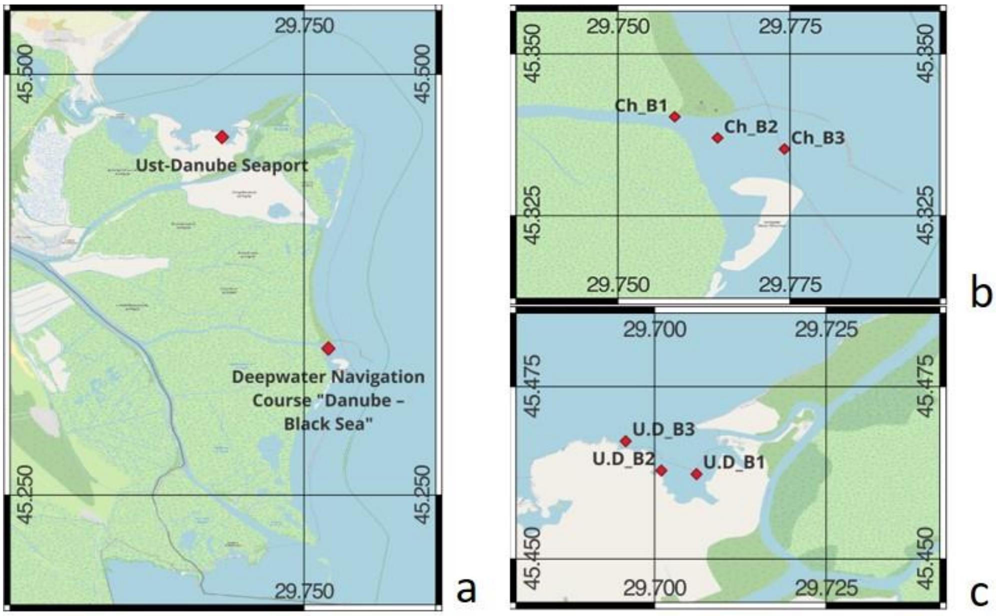

Figure 1: Map of phytofouling / periphyton sampling sites in the Danube avandelta: a – Overview about the two sampling areas at the Ukrainian part of the Danube delta, b – Danube-Black Sea Channel (girlo Bystroe), c - Ust'-Dunaisk Port. Red point in b and c mark the position of navigation buoys (three at each in b and c) from which biotic material of attached living phytofouling assemblages was sampled.

Figure 2: Attached living algae and cyanobacteria on the surface of navigation buoys.

square of algological surface, which develops on m^2 of substrate and may vary from a few meters in low-nutrient oligotrophic systems, to hundreds of m^2 of photosynthesizing algae surface under high-nutrient enrichment of eutrophic conditions. Indicators S/W and SI were calculated in accordance to known methodical algorithms of morphofunctional indicators for unicellular and multicellular algae (Minicheva et al., 2003). These morphofunctional indicators were used as Ecological Evaluation Indexes (EEI) to determine ESC. According to WFD and MSFD requirements, which were developed for natural habitats, here were created qualification scales for attached living algae and cyanobacteria at artificial substrate surface of buoys. ESC categories were here determined for the transition zone of Ukrainian sector of the Black Sea with salinity less than 12‰ (Table 2) (Minicheva et al., 2015). For the assessment of the interannual perennial dynamics of phytofouling or periphyton indicators and ESC categories SI was related to the DTZ water content. Using the S/W indicator, analysis was of the interannual dynamics of the species role with high (tolerant

ESC	EEI range			
	S/W, $m^2.kg^{-1}$	Ecological Quality Ratio (EQR)	SI, units	Ecological Quality Ratio (EQR)
High	S/W < 90	≥ 0,88	SI < 20	≥ 0,71
Good	90 ≤ S/W ≤ 150	0,73	20 ≤ SI ≤ 50	0,39
Moderate	150 ≤ S/W ≤ 350	0,33	50 ≤ SI ≤ 70	0,23
Poor	350 ≤ S/W ≤500	0,14	70 ≤ SI ≤ 100	0,05
Bad	S/W > 500	≥ 0	SI > 100	≥ 0

Table 2: : Scales of ecological status-classes assessment for the transition zone of Ukrainian sector of the Black Sea with salinity less than 12 ‰ for morphofunctional indicators: specific surface (S/W) and surface index (SI).

species) or low (sensitive species) S/W values in the algae communities, depending on the level of eutrophication, which is affected by the river water content.

Among various factors which have an impact on the autotrophic process in the dynamic transition zone, the annual river runoff volume (W, km^3 $year^{-1}$) was considered as the main factor as it is kinked to flowability or stagnation, and hence also controls the intensity of primary production. The impact of flowability and stagnation on the morphofunctional organization of the periphyton assemblages was analyzed using the S/W indicator.

The impact of river runoff volume on the intensity of primary production was further analyzed applying the SI indicator. In accordance with Khilchevsky et al. (2008), river runoff during 18-year study period in the DTZ (2004-2021) are divided into three stages within the boundary displayed in Table 4. For comparison of the Danube runoff volume anomaly, structures and the production process intensity provided by phytofouling, we used the indicator - Annual Anomaly (AA). The AA is the percentage of annual runoff related to annual average of the runoff. During the study period 2004-2021, the following three norms of an annual runoff were calculated: from 2000 to 2010 inclusively - norm 207.4 km^3; from 2011 to 2020 – norm 205.8 km^3 and from 2021 to 2030 – norm 203.2 km^3. In graphs monthly averages were displayed, equal to 1/12 part of the annual average value. Water content typification and annual Danube runoff anomalies calculation for the period of 2006-2022 years refer to research work by Vadym Bolshakov at the Institute of Marine Biology of the NAS of Ukraine, and is based on the Danube Hydrometeorological Observatory (Izmail) data. Statistic data processing and graphical visualization were carried out with standard Excel features.

3 Results and discussion

3.1 SPECIES COMPOSITION

During the period from 2004 to 2021 on two study areas in the DTZ 54 algae species were identified, among which - 48 species in the girlo Bystroe area, 42 at the Ust'-Dunaisk Port (Table 3). The basis of plant fouling here consist of ecologically active multicellular filamentous and thinly branched laminar forms of macro- and microalgae, for which are typical high specific surface coefficients (S/W). The thalli of multicellular macrophyte algae are the habitat physical structure for unicellular colonial diatoms (Ochrophyta) and blue-greens (Cyanobacteria), which are spread in the monitoring area due to the enhanced nutrient availability in water environment and the low level of salinity. Further, attached living microalgae on bio-surface of macrophyte algae build biofilms and can benefit from exudates produced by macrophyte thalli as discussed for biotic substrate by other studies (Han et al., 2018; Teubner et al., 2022).

Table 3: List of periphyton species (algae and cyanobacteria), their S/W values and distribution among the two study areas in the Ukrainian part of the Danube avandelta from 2004 to 2021.

Species	S/W (m^2 kg^{-1})	Danube-Black Sea Channel	Ust'-Dunaisk Port
Chlorophyta			
Blidingia marginata (J.Ag.) P. Dang.	110,04±1,9	+	+
Chaetomorpha capillaris (Kütz.) Börg.	110,58±2,48	+	-
Ch. gracilis Kütz.	143,47±2,77	+	+
Cladophora albida (Huds.) Kütz.	88,41 ± 4,94	+	-
C. dalmatica Kütz.	72,89±3,2	+	-
C. glomerata L. (Kütz.)	91,58±6,54	+	-
C. laetevirens (Dillw.) Kütz.	71,67±3,33	+	+
C. liniformis Kütz.	67,89±2,46	+	+
C. sericea (Huds.) Kütz.	62,98±2,72	+	+
C. sp.	56,12±2,25	+	+
C. vagabunda (L.) Van Hoek.	55,04±2,01	+	+
Rhizoclonium hieroglyphicum (Ag.) Kütz.	197,76±4,25	+	+
Rh. riparium(Roth) Harv.	241,11±6,29	+	+
Rh. tortuosum (Dillwyn) Kützing	118,63±1,86	+	-
Stigeoclonium tenue Kütz.	468,04±19,04	+	-
Ulotrix implexa (Kütz.) Kütz.	422,61±12,95	+	+
U. flacca (Dillw.) Thur.	276,66±8,43	+	+
U. sp.	425,95±21,2	-	+
U. tenerrima (Kütz.) Kütz.	560,51±30,81	+	+
U. tenuissima Kütz.	180,24±4,79	+	-
U. zonata (Web. et Morh.) Kütz.	105,46±2,25	+	-
Ulva clathrata (Roth) C. Agardh	54,03±2,56	-	+
U. intestinalis (L.) Link.	43,24±1,58	-	+
U. flexuosa Wulfen	42,78±2,01	+	+
U. linza Linnaeus	50,97±2,19	-	+
U. prolifera O. Müller	40,33±1,8	+	+
Urospora penicilliformis (Roth.) Aresch.	145,53±4,04	+	+
Rhodophyta			
Acrochaetium sp.	457,55±21,58	+	-
A. virgatulum (Harvey) Batters	395,58±15,95	+	+
Bangia atropurpurea (Mertens ex Roth) C.Agardh	114,3±4,15	+	+
Carradoriella denudata (Dillwyn) A.M.Savoie & G.W.Saunders	105,33±3,62	+	+
Ceramium diaphanum (Lightf.) Roth	39,4±1,87	-	+
Colaconema thuretii (Bornet) P.W.Gabrielson	587,34±46,49	+	-
Cyanobacteria			
Calothrix sp.	834,06±76,49	+	+
Gloeocapsa sp.	1261,2±0	+	+
Kamptonema laetevirens (H.M.Crouan & P.L.Crouan ex Gomont) Strunecký, Komárek & J.Smarda	977,16±110,2	+	+
Litonema sp.	1440,3±0	+	+
Lyngbya aestuarii (Mert.) Liebm.	528,54±20,73	+	+

L. confervoides C.Agardh ex Gomont	593,73 ± 21,97	+	-
L. lutea (Ag.) Gomont	828,09±57,1	+	+
L. semiplena J.Agardh ex Gomont	521,57±20,41	+	+
L. sp.	1070,92±34,1	+	+
Microcoleus sp.	2398,33±0	+	+
Microcystis sp.	976,66±74,85	+	+
M. splendens Hollenberg	1206,71±0	+	+
Oscillatoria sp.	654,72±15,22	+	+
Phormidium nigroviride (Thwaites ex Gomont) Anagnostidis & Komárek	581,87±23,21	+	+
Ph. sp.	1598,62±97,5	+	+
Pleurocapsa sp.	1087,4±64,23	+	+
Spirulina tenuissima Kützing	1273,31±0,00	-	+
Ochrophyta			
Berkeleya rutilans (Trentepohl ex Roth) Grunow	196,03±7,9	+	+
Ectocarpus siliculosus (Dillw.) Lyngb.	238,21±8,55	-	+
Melosira sp.	82,79±2,47	-	+
Navicula turgida Ehrenberg	142,10±5,68	+	-
N. sp.	126,8±3,75	+	-
Vaucheria dichotoma (Linnaeus) Martius	85,45±2,69	+	-
	Total:	**48**	**42**

3.2 FLORISTIC STRUCTURE OF PERIPHYTON ASSEMBLAGES

During the growing season peak from April to June, greens (Chlorophyta) and blue-greens (Cyanobacteria) dominated in the floristic structure composition of the Danube phytofouling communities (Figure 3). In comparison, genera of red algae (Rhodophyta) and diatoms (Ochrophyta) played a significantly smaller role. This floristic structure is typical for the low salinity waters of the Danube Delta. For example, in the plant communities of the Stentsovsko-Zhebriyanovsky floodplains, located near the study area of the Ust'-Dunaisk Port, the floristic structure is dominated by genera of Magnoliophyta, Charophyta, Chlorophyta and Cyanobacteria while other algae were generally absent (Minicheva, 1999). It was shown in other earlier studies at connecting channel Black Sea-Kuyalnitsky Liman (Minicheva and Kalashnik, 1999) that the contribution among the both benthic fractions, macroalgae and microalgae, can vary largely, which agrees with the occurrence of species both fractions in the recent study listed in Table 3.

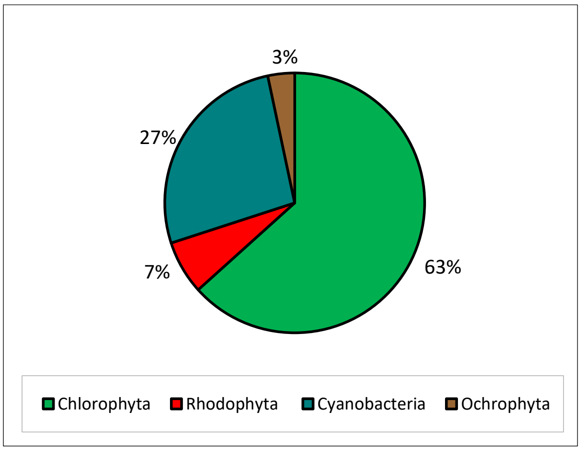

Figure 3: Phytofouling or periphyton assemblage structure of the Danube during the growing season peak (April - June) in 2004-2021.

3.3 PERIPHYTON STRUCTURAL-FUNCTIONAL ORGANIZATION AND MORPHOFUNCTIONAL INDICATORS

The spatial differences in phytofouling indicators on the two monitoring areas, girlo Bystroe and Ust'-Dunaisk Poet, shows typically slight differences in the phytofouling or periphyton structure in the DTZ. This refers to both, the biomass indicators (B) and the phytofouling functioning intensity (SI). The average of phytofouling biomass during growing peak season 2004 – 2021 was almost on the same level in both study areas. Here, the biomass amounted on average about 0.300 kg·m^{-2} (Figure 4a). The indicator SI, which stands for the intensity of primary production of phytobenthos, also close for samples at the two sampling areas and amounted on average about 30 units (Figure 4b).

River runoff belongs to driving forces which are known to control seasonal fluctuations and interannual variability. The water volume of river runoff strongly depends on precipitation and landscape peculiarities of a catchment basin and the morphological shape of the riverbed. The river flow, alongside a seasonal regime of

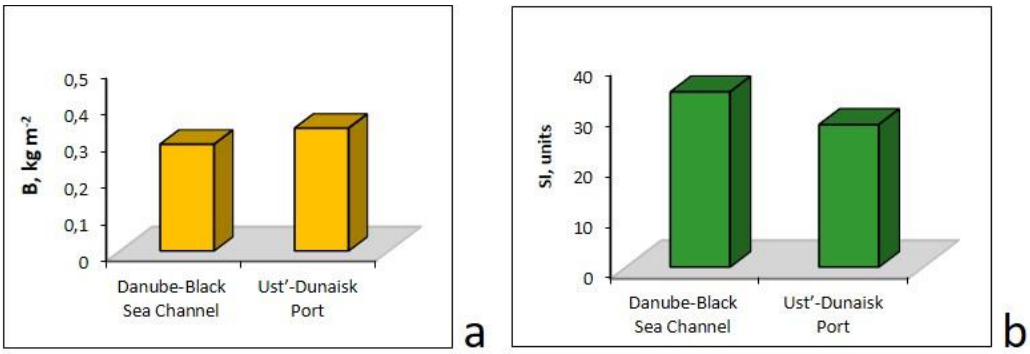

Figure 4: Structure-functional phytofouling or periphyton indicators during the growing peak season (April - June) in 2004-2001 at different monitoring points in the Danube: a – average biomass (B); b – surface index (SI).

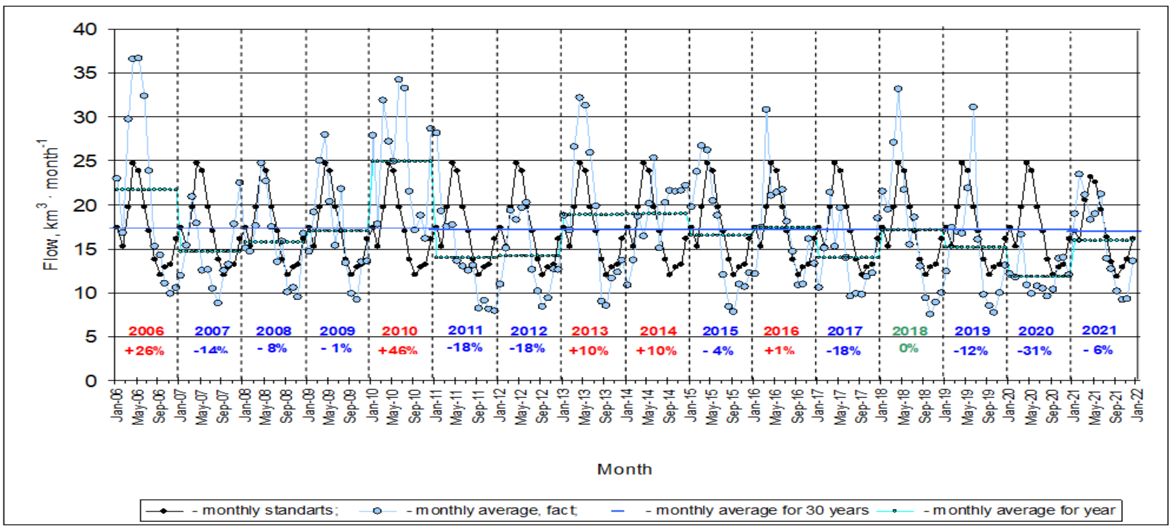

Figure 5: Danube runoff anomalies during the period of 2006-2021. The red numbers indicate the positive anomalies expressed as annual averages, and the blue numbers the negative anomalies, the green indicate indifferent years (data and graph provided by Vadym Bolshakov).

temperature and photosynthetic active radiation, are affects the succession of structural adjustment and intensity of ecological functioning by assemblages of primary producers. In this context, differences on average values on the regional level, deviations from common precipitation pattern up or down and thus fluctuating river flow regimes can cause environmental crises, and thus force anomalous deviations in biological processes. Taking into account the fact that the frequency of climatic anomalies has greatly increased over past years (Lialko et al., 2015), the probability of anomalies of Danube River runoff, also increases, which may affect the ecological status of the river itself, the transition zone, as well as the water area of the Black Sea. The most vivid example of a climate anomaly in the Danube-Black Sea area over past decades was in year 2010. The positive deviations from the long-term average of Danube flow in that year amounted 46% (Figure 5). The combination of "high water" and anomalously high temperatures in July and August 2010 led to severe environmental consequences which reduced the ESC category in the Danube avandelta to "Bad" (see further below Figure 11). In marine areas, this anomaly led to the ecological crisis related to an unprecedented phytoplankton bloom intensity in the northwestern part of the Black Sea (Alexandrov et al., 2012), as well as other negative consequences that emerged themselves in the Danube-Dnieper interfluve coastal zone (Adobovsky et al., 2012).

For searching for the impact of Danube River runoff on both, morphofuctional phytofouling organization and ESC categories, all years of the monitoring period were divided *a priori* into three types: Low-water, Medium-water and High-water (ranges for each category see Table 4), based on The Danube water content intervals over past decades (see Material and methods).

The interannual the Danube water content dynamics during the period of 2004-2022 shows that after the annual average high-water year of 2010, there was a tendency of decreasing W of the annual Danube runoff (Figure 6). Year of 2022 was characterized by the minimum annual runoff on absolute value.

During the transition from Low-water to High-water years and an increase in the transition zone flow, almost double decrease of the average phytocoenoses functional activity (S/W), which have been developed on navigation buoys, was recorded (Figure 7). Under conditions of high flowability, a rapid metabolites outflow from the outer thallus surface occurs and favourable conditions for low functional activity algae (sensitive species) and low S/W values (mainly species of Rhodophyta and Chlorophyta) are created. And vice versa, during low-water years, the persistence of stagnation is built up, which related to an increased nutrient availability stimulating algal growth. An increase of the trophic level contributes to the favourable conditions for high ecological activity species (tolerant species) and species with high S/W values (mainly species of Cyanobacteria and Ochrophyta) development. Accordingly, in low flowability together with high trophic level, sensitive species cannot develop. And vice versa, in high flowability such indicators of high trophic waters and bad ecological conditions disappear. This process, both in the first and the second cases, reduces the total number of species in low- and high-water years. In medium-water years acceptable conditions for the full range of algal floristic composition are formed. Regarding to this, the greatest phytofouling or periphyton species diversity is observed in the medium-water period (Figure 7).

A decrease of the S/Wx indicator value during the transition from low to high water level is also followed by a water vegetation floristic structure-functional activity restructuring. For the analysis of ratio change of algae groups with different functional activities in the phytofouling floristic structure in the DTZ, all algae species were divided into 5 functional groups according to the S/W indicator value: 1- [39-100]; 2- [101-250]; 3- [251-500]; 4-[501-1000]; 5-[1000≥] ($m^2 kg^{-1}$). The first group included sensitive species with the lowest specific surface values for this area, low trophic status indicators and indicators of high ESC categories. This functional group included species of red algae (Rhodophyta), and also green lamellar algae (Chlorophyta). In turn, the last group (5th), is represented by tolerant species with maximum functional activity and the highest S/W indicator values. Species of this group 5, reach few thousands of m^2 of photosynthetic surface attributable to one kg of algae biomass only. Thus, these small species build a huge bioactive surface despite their relative low biomass. To this functional group belong blue-green algae (Cyanobacteria). Representatives of this group develop in high trophic status and low ESC categories conditions. The phytofouling floristic structure analysed over the entire monitoring period showed that in high-water years, the contribution of the1st functional group

Table 4: Typification of the Danube runoff for the period of 2004-2022.

	W, km^3.year^{-1}	Years
Low-water	[137,0 – 165,2]	2020, 2022
Medium-water	[165,3 – 236,3]	2004, 2007, 2008, 2009, 2011, 2012, 2013, 2014, 2015, 2016, 2017, 2018, 2019,2021
High-water	[236,4 - 299,6]	2005, 2006, 2010

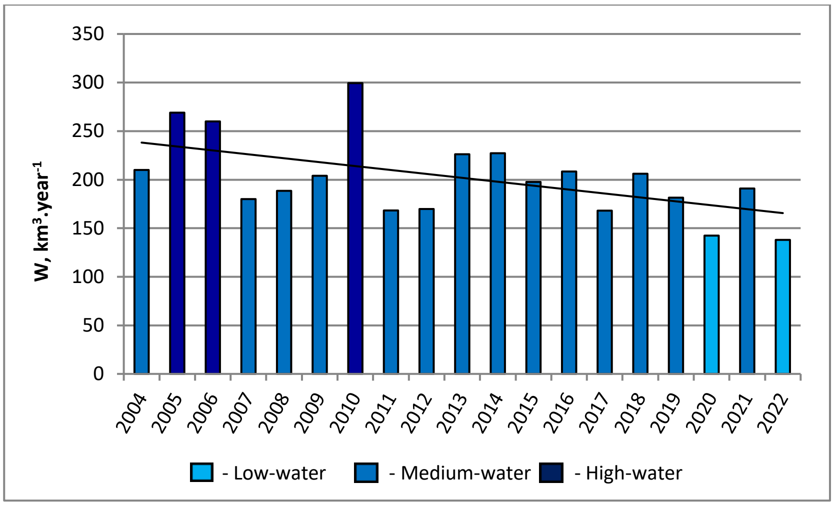

Figure 6: Typification (low-, medium- and high-water) of the long-term dynamics of the annual Danube runoff from 2004 to 2022.

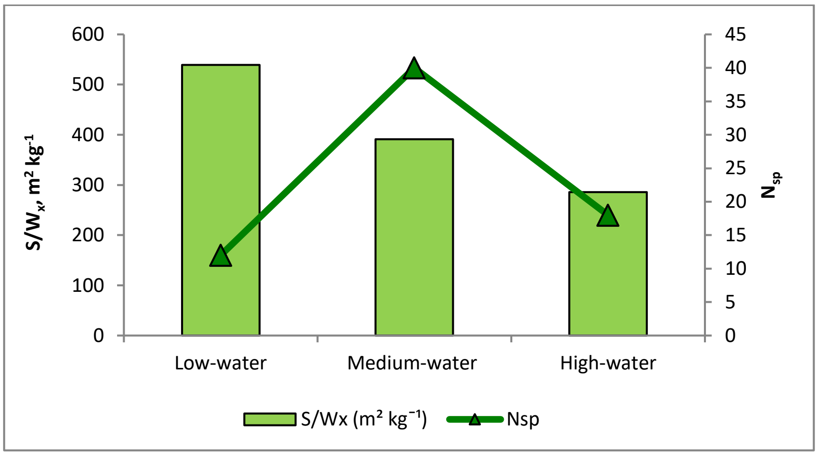

Figure 7: Species diversity change (N_{sp}) and average phytofouling floristic composition functional activity decrease (S/Wx) during the transition from Low-water to High-water conditions in the Danube avandelta.

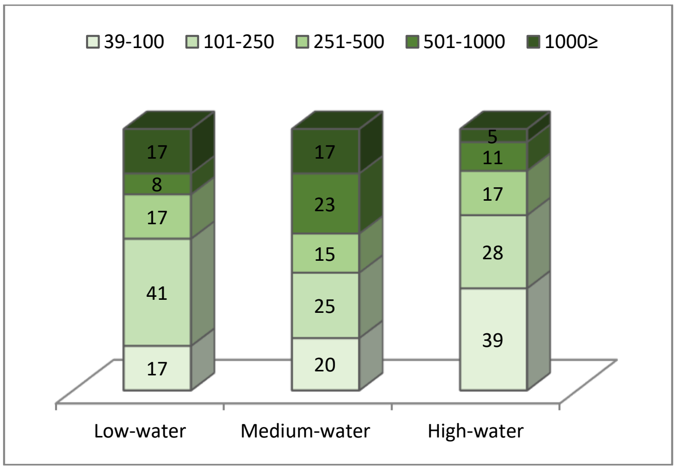

Figure 8: Percentage ratio of 5 functional algal groups with different surface index value (S/W, $m^2\ kg^{-1}$) in the phytofouling floristic structure in the Danube avandelta during the period of 2004-2021 in dependency of water content of the year.

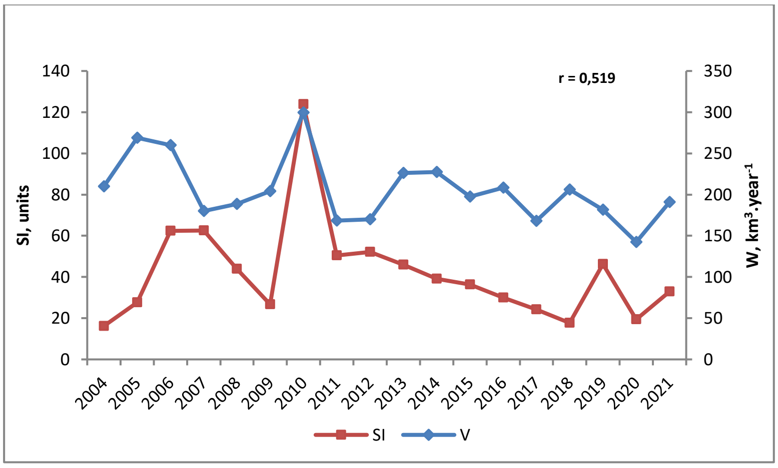

Figure 9: Synchronization of the Danube annual runoff volume (W) and phytofouling surface index (SI) in the DTZ during the period of 2004-2021.

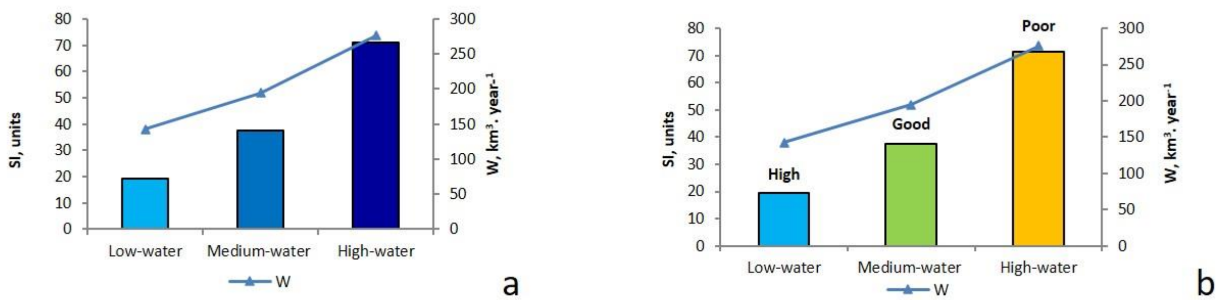

Figure 10: Dependency of phytofouling autotrophic process (SI) (a) and ESC categories (b) on the Danube water content during the period of 2004-2021.

floristic structure increases and decreases in case of the 5th functional group (Figure 8). The highest levelness in terms of percentage ratio of 5 functional algae groups is observed in Medium-water years (see Figure 8).

The SI indicator includes the value of specific surface of species as well as the biomass indicator, which reflects how the species realizes its potential opportunities in particular ecosystem conditions.

Regarding to this, unlike the S/W indicator, fixed evolutionarily in the morphological species status, SI - reflects a realized water autotrophic intensity dependent from abiotic conditions. During periods of increased volume of river runoff, the total volume of dissolved and suspended compounds of nitrogen and phosphorus increases, which stimulates the primary production. The adsotrophic type of nutrition - the absorption of nutrients through the external contour of the thalli (Khailov et al., 1992) contributes to a total increase in the algal surface that develops on the surface of the substrate, respectively, the values of the SI indicator increase. The 18-year period data analysis showed that there is a synchronization between annual runoff W and the SI indicator value. This regularity is most strongly expressed in anomalous periods, as it was in 2010 (Figure 9).

Based on the connection between water content throughout a year and the intensity of potential primary production, the direct dependency of SI indicator on the Danube annual runoff W is confirmed. The long-term time series showed that the transition from low-water to high-water periods is followed by a double increase of SI values (Figure 10 a). Since for the morphofunctional SI indicator a classification scale for the Black Sea transition zone was developed (details see Material and methods), it is possible to define ESC categories for assessing specifically the ecological status for the DTZ. The ESC categories defined for different water content periods shows that the Danube annual runoff W increases contemporary with increasing nutrient availability, stimulating the growth and production of algal primary producers and effects algal turbidity and therefore reduces the ecological status in the transition zone. During the research period for the Ukrainian part of the Danube avandelta, ESC categories decreased from «High» to «Poor» in the transition from Low-water to High-water period (Figure 10b).

Using the SI indicator for ESC categories, long-term time series data for the ecological status for DTZ is displayed in Figure 11. Based on the EU Water Directives, which is here applied for a benthic algal community on artificial substrate, the ecological status obviously varies within years. Assessing off-seasonal and interannual changes of ESC categories from 2004 to 2021, an integral picture of fluctuating natural-climatic and anthropogenic conditions is depicted, i.e., the direct influence of the Danube River on the Black Sea ecosystem becomes evident (Figure 11).

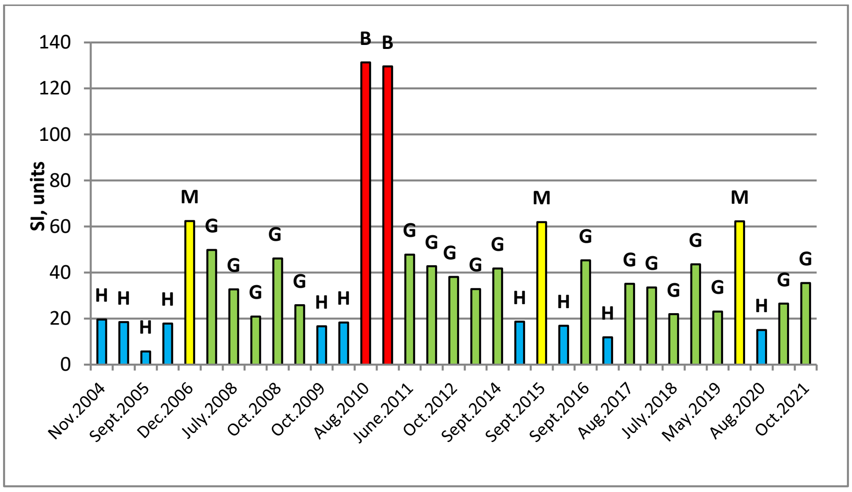

Figure 11: Long-term seasonal dynamics for values of ESC categories (H- High, G- Good, M- Moderate, P- Poor, B- Bad), based on phytofouling SI values of the Ukrainian part of the Danube avandelta from 2004 to 2021.

4 Conclusions

Morphofunctional periphyton indicators (macroalgae and microalgae) were derived from navigation buoy samples, which thus refer to algae and cyanobacteria living attached on artificial substrate in the Ukrainian part of the Danube avandelta. These monitoring data from 2004 to 2021 were assessed in accordance with the EU Water Directives standards. This long-term assessment by ESC allowed to obtain following results:

- It is shown that in high-dynamic conditions of the Danube avandelta with the absence of solid natural substrate, all anthropogenic constructions, which are used by plant fouling as a habitat, can be used as monitoring stations for ESC categories assessment based on morphofunctional phytofouling indicators.
- It is shown that in the both study areas, Danube-Black Sea Channel (girlo Bystroe) and Ust'-Dunaisk Port, the long-term averages of floristic diversity, biomasses and algae fouling surface indexes, were not significantly different throughout the 18- year study period.
- It is shown that the annual Danube runoff, which mainly depends on precipitation, has a significant impact on the structural-function of the benthic assemblage of primary producers in the transition zone. During high flow conditions associated with an increased flowability, sensitive species with low functional activity and low specific surface values (S/W) - red and green algae with lamellar thalli have advantage of dominance and development. In contrast, during the lowered flowability conditions, tolerant species with high functional activity and low specific surface values (S/W) - cyanobacteria, filamentous and colonial brown algae seem to benefit by growth.
- Monitoring data of morphofunctional indicators of algae fouling on navigation showed that, during the transition from Low-water to High-water years, the ESC categories decreased from “High” to “Poor”.
- Based on the long-term seasonal dynamics of the ESC categories for the Ukrainian part of the Danube avandelta using the morphofunctional indicator of fouling algae - SI, it was confirmed that the most significant decrease in the ESC category to “Bad” is observed under the influence of abnormal climatic conditions associated with an increase in river runoff, as observed in 2010 with a positive anomaly of 46% compared to the regional average level over the last 30 years.
- Studying periphyton assemblages, that are living attached on artificial substate such as navigation buoys and thus are fixed in their habitat position at the delta or channel stretches, are suitable as biological quality elements for monitoring the Ecological Status in accordance with the EU Water Directive.

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