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Impact of hydrotechnical construction on aquatic ecosystems of the Kiliia branch of the Danube Delta

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Resumption of shipping in the Bystryi branch in the Ukrainian part of the Danube Delta, one of the largest aquatic-wetland areas of Europe and the world, has made it necessary to control the anthropogenic impact on the neighboring water areas of the Danube Biosphere Reserve. The objective of the study was comparing the compositions and structure of phytoplankton, microphytobenthos, macrophytes, benthic invertebrates and ichthyofauna of the mouth area of the Bystryi branch with such communities of the mouths of the branches Vostochnyi, Tsyhanka and Starostambulskyi, which are situated in the protected zone and characterized by limited anthropogenic activity. We also determined the correspondence of the descriptors of biotic groups to the categories of the ecological status according to the Water Framework Directive of the EU. The studies were performed in the autumn and summer periods in 2020-2021. We recorded 367 species of animals and plants, the richest biodiversity was seen for the biota of the Bystryi branch - 250 species, and 180-231 species of hydrobionts were found in the undisturbed mouths. We determined 25.3% of shared species for the water areas, and therefore high values of similarity of the species compositions according to Bray-Curtis (47.5% to 81.5%). We determined no significant differences between the groups of the mouths of the examined branches according to most indicators of taxonomic and ecological structure. As the descriptors of ecological status, we chose assemblage indices of phytoplankton and microphytobenthos, which are based on ratios of biomass of functional groups of algae, and also the Macrophyte Biological Index for Rivers, saprobic index of Zelinka & Marvan and Biological Monitoring Working Party Index of Benthic Invertebrates and Representation of Species of Ichthyfauna according to vulnerability to actions of environmental factors. We determined that the range of descriptors of phytoplankton and microphytobenthos corresponded to the "high" ecological status category, such of macrophytes and benthic invertebrates to "good", and such of ichthyofauna varied "high" to "good". In general, all the mouth areas were characterized by "good" ecological status. Similarities of the species composition and the structure of biotic communities of the mouths of the studied branches of the delta indicate the absence of negative impact of the deepwater shipping on adjacent ecosystems, which may be related to the peculiarities of reactions of groups in the water areas with natural stress, as well as local impact of the hydrotechnical construction.

Keywords: transitional waters; affected area; protected area; biotic indices; ecological state.

Introduction

Deltas are unique natural ecosystems known for high biodiversity; they are rich in resources and carry out a number of ecological functions, including globally significant ones because of their high efficiency in utilizing greenhouse gases. Their position at the ends of rivers, at the borders between sharp changes in water salinity and rates of the current, creates conditions for sedimentation of suspension and localization of alluvium, purification of river water and simultaneous enrichment of adjacent water areas and land by nutrients, causing the formation of new territories and protrusion of their front edge (Syvitski et al., 2009; Bucx et al., 2014; Bănăduc et al., 2016). People have long been using the resource potential of the deltas to develop industry, transport, agricultural and fishing economies, for recreation, etc. (Yermolenko et al., 2022; Yesipova et al., 2022). Over the recent decades, special attention was paid to complex studies of delta ecosystems, and currently the focus is on the control of anthropogenic impact and quality of ecosystem services. At the same time, the consequences of human activity, the climatic changes and increase in the level of the global ocean continue to pose a threat to the sustainable existence of delta ecosystems (Bianchi & Allison 2009; Syvitski et al., 2009; Loucks, 2019).

Being located at the ends of drainage basins of rivers, the deltas are sensitive to the impact of various processes inside the basin, currently to excessive use of groundwater, cutting of forests, drying of wetlands, contamination by organic and toxic waste waters, regulation of effluent, hydroconstruction and shipping (Poff et al., 2007; Syvitski & Saito, 2007; Li et al., 2021). The needs of the latter are fulfilled and the navigable depths in water areas of the deltas are maintained by constructing navigable channels, straightening and deepening of the branches, cleaning the riffles, stream pools, shoals, and digging of pre-mouth canals. This results in redistribution of the effluent in water bodies in the delta, increase in accessibility to and therefore inflow of saline water into the deltas, increase in turbidity of water, and there is observed shoaling of water areas of concentration of stockpiles from bed-deepening works and drying of riverbank floodplain (Ohimain, 2004; Okoyen et al., 2020; Cox et al., 2021).

Since the early 21st century, the ruling document of the European Community in the sphere of water management has been the Water Framework Directive of the EU, which defines the main principles of management of aquatic resources and ways of achieving the good quality and safe condition of surface water. One of the ways of implementing the purposes of this document is assessment of ecological condition and risks that could hinder its improvement (Solimini et al., 2008; Allan, 2012). Measures of the Water Framework Directive of the EU have already been introduced for many aquatic objects of Europe: rivers (Spänhoff et al., 2012; Bilous et al., 2021; Skoulikidis et al., 2021), lakes (Søndergaard et al., 2005; Latinopoulos et al., 2021; Ntislidou et al., 2021), transitional (Pérez-Ruzafa et al., 2007; Cacciatore et al., 2019; Facca, 2020) and coastal (Giovanardi et al., 2018; Gerakaris et al., 2022) water, including river deltas (Newton et al., 2014; Stoica et al., 2014; Goulding et al., 2021). The Danube Delta is the largest delta in Europe $(4,455 \text{ km}^2)$, its Kiliia branch is at the border between Ukraine and Romania, dividing the territory into two parts, the larger Romanian – 3,370 km², and smaller Ukrainian, accounting for 1,085 km² (Baboianu, 2016). Currently, it is one of the least disturbed deltas in the world; it has retained the natural features of its ecosystemic processes. In general, its ecosystem is considered to be under low anthropogenic and climatic pressures (Bucx et al., 2014; Loucks, 2019). It is one of the least populated deltas of the world (0.22 people per 1 km²), and, at the same time, one of the largest wetlands among those left in Europe. In 1991, the Romanian part of the delta was included in the UNESCO World Heritage and the List of 200 the most valuable ecosystems of the Earth – Global 200 (Van Driel et al., 2015; Baboianu, 2016).

Wetland complex of the Danube Delta includes three large river branches, floodplain forests, limans, lakes, natural and man-made channels, sandy dunes and riparian biotopes. Wildlife in the area consists of rare fauna and flora, and no less than 30 rare types of ecosystems (Baboianu, 2016; Csagoly et al., 2016). The overall taxonomic richness of the Danube Delta is 7,402 species according to the recent estimates, including 2,383 species of plants and 4,029 species of animals (Baboianu, 2016; Csagoly et al., 2016). Since 1998, the Danube Biosphere Reserve has been in operation in the delta of the Danube. At the same time, this unique natural region, similarly to other wetlands, is subject to great risks from increasing anthropogenic activity (Ignar & Grygoruk, 2015; Van Driel et al., 2015; Sica et al., 2016). Great concerns are the consequences of contamination and hydromorphologic changes in the delta, particularly largescale irrigation works carried out over the recent decades to the intensify the land use, and introduction of shipping in its main branches (Baboianu, 2016; Shuisky et al., 2021).

In the Kiliia branch, large-scale hydrotechnical construction related to shipping began in the second half of the 20th century with the construction of navigable channel through the Prorva mouth. Supporting its functions required performance of constant dredging and significant economic investments. The economic component turned out to be decisive for the existence of the channel, for in absence of financing after the collapse of the Soviet Union, the dredging stopped and the channel has undergone rapid siltation (Boniar, 2010; Shuisky et al., 2021). Resumption of shipping in the Kiliia branch in the period of the Ukraine's independence started in 2004 with creation of the Danube-Black Sea Deepwater Shipping Canal. Implementation of the first stage of the project included the digging of a canal in the mouth of the Bystryi branch and the construction of stone-made protective levee as a continuation of the left bank into the sea for the protection of the channel from the influence of the marine water (Khomicky et al., 2020). Since that period, navigable depths are supported by the regular bed-deepening works. The construction and exploitation of deepwater shipping channels is accompanied by regular complex state ecological monitoring of the environment, and one its tasks over the recent years has been determining the ecological status of aquatic ecosystems in the influence zone, resumption of exploitation of shipping according to the requirements of the Water Framework Directive of the EU, based on the comparative principle, which is comparing the current condition with undisturbed natural locations. The main works for the support of navigable depths are being carried out in the pre-mouth area of the Bystryi branch. At the same time, three similar watercourses are located nearby (Vostochnyi, Tsyhanka and Starostambulsyi), the mouths of which - because of localization in the protected zone - are subject to lower anthropogenic impact. Comparing the compositions and the structures of biota of the mouth of the Bystryi branch and the mouth areas of other branches to assess of the impact of hydrotechnical works on freshwater ecosystems of the delta became the objective of this research.

Materials and methods

The studies were conducted in 2020 (November) and 2021 (June, September) in the mouth areas of the branches Bystryi (45°20'21.5" N, 29°45'34.4" E), Vostochnyi (45°18'12.0" N, 29°45'12.0" E), Tsyhanka (45°14'47.0" N, 29°44'28.0" E) and Starostambulskyi (45°14'47.0" N, 29°44'28.0" E). The mouth of the Bystryi branch is in the zone of anthropogenic landscapes of the Danube Biosphere Reserve and is subject to direct human impact: the mouth is the place of passage of deepwater shipping, the left bank of the branch has been extended into the sea by a man-made protective levee, and periodic bed-deepening works are carried out in the approaching marine channel, adjacent to this area, though they were not taking place during our studies. The mouths of the branches Vostochnyi, Tsyhanka and Starostambulskyi are situated south of the mouth of the Bystryi branch at the distance of 3.88, 10.47 and 13.81 km along the riverside from the place of dredging and are in the zone of regulated protection regime, where the anthropogenic activity is significantly limited.

During our research, according to the hydrochemical composition, the water was poorly alkaline in all the areas, mean salinity in the mouths of the branches Bystryi, Vostochnyi and Starostambulskyi was reduced, and the mouth of the Tsyhanka branch had low concentration of salts (Table 1). The highest values of water salinity corresponded to the olihalynna zone, recorded in the autumn seasons along the left banks of all the watercourses: at places where the branches Vostochnyi, Tsyhanka and Starostambulskyi fall into the sea, and in the shallow water in the mouth of the Bystryi branch where the natural bank turns into the levee.

Table 1

Hydrophysical and hydrochemical characteristics of the mouths of the branches subject to various levels of impact from the hydrotechnical construction ($x \pm SD$, n = 3)

Characteristics	Affected area	Protected area			
	Bystryi	Vostochnyi	Tsyhanka	Starostambulskyi	
Depth, m	1.17 ± 0.76	1.02 ± 0.64	0.69 ± 0.14	0.78 ± 0.26	
pH	7.94 ± 0.41	7.71 ± 0.45	7.52 ± 0.20	7.55 ± 0.59	
Salinity, ‰	0.47 ± 0.38	0.35 ± 0.14	1.01 ± 0.53	0.51 ± 0.24	

Note: according to results of comparing the selection within each characteristic, the Tukey test revealed no significant differences (P > 0.05).

According to their hydromorphological characteristics, the mouth of the Bystryi branch is the widest (650 m), against 80-500 m of the other mouth areas, has a much larger shallow-water area, especially along the left bank, where the mouth is protected by the levee and creates a ≈ 250 m wide zone of no more than 0.5 m depth, contains dense benthic deposits such as silted sand and grey silt and developed submerged vegetation. A similar picture was seen in the mouth of the Starostambulskyi branch, though its left-bank sandy shallow-water areas are not protected from the sea and were characterized by almost complete absence of higher aquatic plants. In the mouths of the Tsyhanka and Vostochnyi, along the left bank, at the distance of 0.5–1.0 m from the waterline, the depths begin to sharply drop to 2.0-3.0 m. Right-bank mouth areas of the Bystryi and Starostambulslkvi branches transfer into bays (Bystryi Kut and Musura respectively), which are characterized by insignificant depths (0.5–1.0 m) and loose silted benthic deposits with dense thickets of air-aquatic macrophytes. Along the right bank of the mouths of other branches, the shallow water zone reaches 5-6 m and is also being overgrown by air-aquatic and submerged vegetation.

At each plot, phytoplankton was sampled from clean water near thickets of macrophytes, from the surface layer of water (down to 20 cm) into a capacity of 0.5 L. Microphytobenthos was sampled using microbenthometer from the commonest substrates in the area. Algological material was fixed by formaldehyde solution (40.0%). In total, we collected and analyzed 48 samples of plankton and benthic algae. Macrophytes were described in the mouths of the branches at 100 m-long areas along the both banks from the sea up and down the current. Taxonomic analysis of macrophytes was carried out within the range of taxa according to APG IV (Byng et al., 2016). Projective cover of species was indicated in percents and according to a 5 point scale for the entire area of the watercourse (where 1 point - < 0.1%; 2 - 0.1 - 1.0%; 3 - 1.0 - 10.0%; 4 - 10.0 - 50.0%; $5 \rightarrow = 50.0\%$ (Haury et al., 2006). Overall, 24 plots of thickets were described. Benthic invertebrates were sampled using the standard method for the assessment of the ecological condition (Barbour et al., 1999; Buffagni et al., 2001), which was approbated over a number of international studies of the Danube (Graf et al., 2008; Graf et al., 2015). We used Multi-Habitat-Sampling and Kick and Sweep Sampling in up to 1.0 m deep riparian areas, and collected samples using the Petersen's grab (the area of $0.10 \times 0.10 \text{ m}^2$) and dredges (1.4 m blade length) at the plots of over 1.0 m depth. In total, we collected and analyzed 84 samples of benthic invertebrates. The ichthyofauna was studied on larvae and young fish. Catches in riparian areas of up to 1.0 m depth were performed using fishnet for juveniles, and ichthyofauna from the pelagic zone was extracted using caviarharvesting net, tagboating it for 100 m. In total, we made 80 fishing net catches and 4 catches of ichtyoplankton. Along with collecting hydrobiological material, we analyzed hydrochemical and hydrophysical indicators of water using pH-meter pH-150 MI and conductometer HI 9835.

Species diversity and taxonomic structure of all biotic groups were evaluated according to their compositions during each of the seasons of the studies, as well as such for the entire period. When assessing species diversity, all taxa determined down to the lower taxonomic level were considered separate species.

The mouth areas are similar to adjacent river surface water by their hydromorphological and hydrochemical characteristics, they are inhabited live mostly by freshwater species of animals and plants (Alexandrov et al., 1998; Lyashenko & Zorina-Sakharova, 2015), they are also similar by biological quality elements – BQE, according to the characteristics of which the Water Framework Directive EU proposes assessing the ecological condition. Those elements comprise phytoplankton, microphytobenthos, macrophytes, benthic invertebrates and fishes. As descriptors of all BQEs, we chose indices that have been designed and approbated in watercourses of Europe.

As a descriptor of the ecological status according to the phytoplankton and microbenthos, we chose Q assemblage index, which is based on the dominance of representatives of functional groups of algae (Reynolds & Irish, 1997) in the overall biomass of algal groups and F coefficient (0 to 5), which was determined for each functional group of algae taking into account the impact of a broad range of ecological factors and biotopic confinement. Functional groups and F factors, necessary for the calculation of assemblage Q index were determined according to tables and formulae (Borics et al., 2007). We designated 11 groups of algae: TB (F = 5) – Bacillariophyta, confined to lotic conditions; D(F = 4) – Bacillariophyta, confined to shallow-water turbid biotopes; C(F = 4) – representatives of eutrophic lakes and rivers; TD (F = 3) – benthic and epiphytic Bacillariophyta and filamentous Chlorophyta, W2 (F = 3) – representatives of shallow-water mesotrophic limnophilous biotopes; P(F = 3) -Bacillariophyta and Chlorophyta, which develop in the conditions of eutrophic epilimnion; TC (F = 2) – epiphytous Cyanophyta in places where macrophytes emerge; J(F = 2) – Chlorophyta of shallow-water eutrophic zones; W1 (F = 1) - Euglenophyta in places of organic contamination; H1 (F = 1) – diazotrophs, tolerant to low concentration of nitrogen and carbon; W0 (F = 1) – representatives of standing water with high concentration of organic substances. Assemblage index Q was calculated according to species composition of plankton or benthic algae and biomass of functional groups of algal flora during a certain season of the studies.

As a descriptor of the ecological status of mouth areas of the branches according to macrophytes, we chose the Macrophyte Biological Index for Rivers (IBMR), which was developed in France for assessing the ecological condition of watercourses in accordance with the Water Framework Directive of the EU; it correlates with the level of organic contamination and is broadly used for rivers in Europe (Birk et al., 2006; Haury et al., 2006; Demars et al., 2012). The index takes into account projective cover of species (according to 5-point scale) and species composition of macrophytes. For 206 species, tables were developed containing their indicatory values, expressed by both indicators: evaluation of CSi; ranging 0 (tolerant to high level of eutrophy) to 20 points (sensitive species, that tend to live in oligotrophic conditions), and coefficient of ecological amplitude, the value of which ranges from 1 (broad amplitude, grow in the conditions of three trophic classes) to 3 (narrow amplitude, occur only in one trophic class) (Haury et al., 2006a).

To describe invertebrates, using software Asterics 4.04 (AQEM, Germany, 2021), we calculated the Zelinka-Marvan water saprobity index (SZ&M) (Zelinka & Marvan, 1961), and also the index of water quality, Biological Monitoring Working Party (BMWP) (Hawkes, 1998), which were approbated in the Danube earlier (Elexová, 2003; Aristica & Constantinescu, 2006; Marković et al., 2012). The chosen descriptors of the ecological status were assessed according to species composition of invertebrates of each mouth area during a particular period of studies.

The descriptors of ichthyofauna were chosen to be the elements of the European Fish Index, EFI (Breine et al., 2005; Solana et al., 2009; Mihov, 2010). Taking into account mostly qualitative selection of samples of larvae and young fish in the riparian zone, of all metrics of this index, we used only the indicators of vulnerability of fish to the environmental factors (relative number of intolerant and tolerant species). Species were identified to particular ecological groups according to the corresponding tables (Breine et al, 2005).

The ecological state of the mouths of branches of the Delta was assessed according to average values of descriptors of each BQE by calculating the ecological quality ratio, EQR (Van de Bund & Solimini, 2007), which is the mathematical ratio of the calculated value of the descriptor to its reference value. Taking into account that the lower current of the Danube belongs to the class of very large rivers based on the river classification, value of effluent, area of drainage basin and sizes (Meybeck et al., 1996), we chose EQR gradation for the phytoplankton and microphytobenthos for this type of watercourses (Borics et al., 2007), reference value Q equaling 5 points. Reference value of IBMR index was chosen according to the literature data for a number of lowland rivers with sandy bed and equaled 10.09, and EQR gradation classes were chosen from those proposed for the methods based on macrophytes for lowland rivers (Szoszkiewicz et al., 2007). For benthic invertebrates and ichthyofauna, the reference values of the descriptors were determined using the reports for the studied region performed during the period of minimal anthropogenic impact, from which, we chose the 1940s and the 1950s (Kharchenko et al., 2001; Lyashenko & Zorina-Sakharova, 2012), when the water in the Kiliia delta of the Danube corresponded to class II (category 3) of quality (well, quite clean, meso-eutrophic, β '-mesosaprobic water). Because of lack of data regarding the qualitative parameters of benthic invertebrate species, the values of reference parameters were determined as such that correspond to those parameters of water quality class, namely the reference value of SZ&M equaling 1.80, and BMWP - 86. The reference values for ichthyofauna were determined according to the list of species of young fish in the delta for the 1940s (Liashenko, 1952), equaling 20.0% of tolerant and 10.0% of intolerant species. Gradation of EQR for benthic invertebrates was carried out according to the principle "5% - 30% - 30%- 30% - 5%" (Barbour et al., 1996; Romanenko et al., 2010; Afanasiev et al., 2020), where each range corresponds to a certain class of ecological status. Correspondence of EQR of ichthyofauna to categories of ecological status is given according to the recommendations for EFI calculation (Breine et al., 2005). Gradation of EQR within the water quality classes for all biological elements is generalized in Table 2. Correspondence of each BQE to a certain class of ecological state was determined according to mean EQR value for all descriptors, and the general condition corresponded to the worst status out of determined BQE values (Van de Bund & Solimini, 2007).

Table 2

Gradation of the indicators of ecological quality for the descriptors of biological elements of quality in classes of ecological status

Biological	Borders of EQR in classes of ecological state					
quality elements	high	good	moderate	poor	bad	
Phytoplankton, microphytobenthos	1.00-0.70	0.69-0.60	0.59-0.50	0.49-0.40	< 0.40	
Macrophytes	1.00-0.90	0.89-0.65	0.64-0.40	0.39-0.15	< 0.15	
Benthic invertebrates	1.00-0.95	0.94-0.65	0.64-0.35	0.34-0.05	< 0.05	
Ichthyofauna	1.00-0.67	0.66-0.45	0.44-0.28	0.27-0.18	< 0.18	

Note: EQR-ecological quality ratio.

All statistical calculations were carried out using Past 4.11 software 11 (Hammer et al., 2001; University of Oslo, Norway). Similarity of species compositions was evaluated using the results of cluster analysis (Bray-Curtis distance equation, Simple Average cluster method, without standardization, presence/absence transformation) in BioDiversity Pro 2.0 pack for analysis of biological data (Scottish Association for Marine Science and the Natural History Museum London, Great Britain, 1997). The data were statistically analyzed using ANOVA and the Tukey test, criteria of significant difference between mean values. The results are expressed as mean values and standard deviations ($x \pm SD$). The results

are expressed as mean values and standard deviations ($x \pm SD$). The differences between the data were considered significant at P < 0.05. **Results**

Species diversity. In the composition of phytoplankton, we recorded 122 species of algae, including 72 in the mouth of the Bystryi branch, 57 in the Vostochnyi branch, 56 in the Tsyhanka branch and 65 species in the Starostambulskyi. Bacillariophyta was in general represented by 84 species, including 57 found in the mouth of the Bystryi branch, 46 in the Vostochnyi branch, 44 in the Tsyhanka branch and 48 in the Starostambulskyi branch. As with Chlorophyta, we recorded 13 species, including 8 in the mouth of the Starostambulskyi distributory and 4 in phytoplankton in each of the other mouths. In the composition of Euglenophyta, 11 species were found: 7 were recorded in the mouth of the Bystryi branch, 2 in each water areas in the protected territory. We found 10 species of Cyanophyta, including 6 species in the mouth of the Starostambulskyi branch, 3 each of the mouths of the Bystryi and the Vostochnyi

branches, and 2 were seen in the mouth of the Tsyhanka branch. Other divisions were represented by 1-2 taxa, including Cryptophyta (Cryptomonas sp.) occurring in all the examined water areas, whereas others were seen only in the protected water areas: Chrysophyta (Pseudokephyrion ovum (Pasch. et Ruttn.) Schmid) - in the mouths of the Vostochnyi and Tsyhanka branches, and Dinophyta (Gonyaulax spinifera (Clap. et Lanchm.) Dies and Peridinium sp.) - in the mouth of the Tsyhanka branch. We determined no statistical difference for mean values of species richness of the phytoplankton divisions in various areas (P was within 0.08-0.43, Table 3). Also, such difference was absent for the values of the overall species richness (P = 0.49, Fig. 1). Shared phytoplankton species for all the examined plots were 21 species of algae (17.2%), and except the indicated species of Cryptophyta, all of them belonged to the Bacillariophyta order. Three species of phytoplankton, Synedra acus Kütz., Navicula placentula f. rostrata A. Mayer and Nitzschia vermicularis (Kuetz.) Grun., were present in the protected zone and absent in the mouth of the Bystryi branch.

Table 3

Impact of hydrotechnic construction on taxonomic structure (% of the overall number of species) of phytoplankton, microphytobenthos, macrophytes, benthic invertebrates and ichthyofauna of the mouths of the delta branches ($x \pm SD$, Tukey test, n = 3)

Di-ti-	Т	Affected area		Protected area	
Biotic community	Taxa -	Bystryi	Vostochnyi	Tsyhanka	Starostambulskyi
	Cyanophyta	2.07 ± 1.87^{a}	4.11 ± 1.54^{a}	3.13 ± 3.49^{a}	8.31 ± 3.27^{a}
Phytoplankton	Euglenophyta	6.70 ± 6.43^{a}	2.09 ± 1.82^{a}	2.30 ± 3.98^{a}	1.86 ± 1.62^{a}
	Chlorophyta	3.51 ± 3.45^{a}	5.22 ± 3.61^{a}	6.67 ± 3.44^{a}	10.94 ± 0.71^{a}
	Bacillariophyta	86.26 ± 5.98^{a}	85.38 ± 2.08^{a}	81.85 ± 9.12^{a}	77.04 ± 5.74^{a}
	other	1.46 ± 1.32^{a}	1.86 ± 1.62^{a}	6.05 ± 0.96^{a}	3.20 ± 3.34^{a}
	Cyanophyta	1.93 ± 1.86^{a}	4.20 ± 1.44^{a}	5.30 ± 2.14^{a}	0.90 ± 1.56^{a}
	Euglenophyta	4.23 ± 2.41^{a}	3.46 ± 3.00^{a}	1.19 ± 2.06^{a}	4.55 ± 4.55^{a}
Microphytobenthos	Chlorophyta	5.05 ± 1.52^{a}	5.16 ± 4.65^{a}	1.55 ± 2.69^{a}	0.90 ± 1.56^{a}
	Bacillariophyta	86.71 ± 4.76^{a}	84.57 ± 9.85^{a}	89.21 ± 3.13^{a}	89.72 ± 7.07^{a}
	other	2.08 ± 3.61^{a}	2.61 ± 2.63^{a}	2.74 ± 2.43^{a}	3.93 ± 4.67^{a}
	Alismatales	40.74 ± 6.42^{a}	40.24 ± 21.24^{a}	46.67 ± 5.77^{a}	47.35 ± 8.83^{a}
	Poales	33.33 ± 0.00^{a}	36.50 ± 14.57^{a}	16.39 ± 3.76^{a}	23.86 ± 14.24^{a}
Macrophytes	Saxifragales	12.96 ± 3.21^{a}	2.56 ± 4.44^{b}	13.06 ± 3.37^{a}	11.36 ± 1.97^{a}
	Ceratophyllales	12.96 ± 3.21^{a}	1.75 ± 3.04^{b}	13.06 ± 3.37^{a}	7.20 ± 6.46^{ab}
	other	0.00 ± 0.00^{a}	18.94 ± 13.11^{a}	$10.83 \pm 10.10^{\rm a}$	10.23 ± 9.30^{a}
Benthic invertebrates	Gastropoda	9.77 ± 2.93^{a}	11.82 ± 1.50^{a}	5.92 ± 3.75^{a}	9.66 ± 4.09^{a}
	Bivalvia	8.17 ± 2.97^{a}	3.10 ± 1.49^{a}	3.52 ± 4.09^{a}	7.02 ± 3.48^{a}
	Oligochaeta	22.84 ± 0.95^{a}	22.65 ± 5.95^{a}	24.42 ± 3.59^{a}	20.22 ± 3.80^{a}
	Malacostraca	16.56 ± 3.52^{a}	19.51 ± 4.10^{a}	24.24 ± 8.72^{a}	22.89 ± 5.20^{a}
	Insecta	33.79 ± 2.54^{a}	34.57 ± 7.00^{a}	35.08 ± 5.56^{a}	31.91 ± 2.41^{a}
	other	8.88 ± 3.38^{a}	8.34 ± 3.46^{a}	6.82 ± 3.83^{a}	8.30 ± 1.84^{a}
Fishes	Cyprinidae	40.77 ± 17.99^{a}	47.38 ± 19.44^{a}	39.91 ± 23.36^{a}	44.44 ± 50.92^{a}
	Gobiidae	31.28 ± 13.79^{a}	36.19 ± 11.98^{a}	47.53 ± 8.76^{a}	43.52 ± 38.92^{a}
	other	27.95 ± 4.24^{a}	16.43 ± 7.73^{a}	12.55 ± 14.60^{a}	12.04 ± 12.53^{a}

Note: Other phytoplankton = Other microphytoplankton = % Dinophyta + % Cryptophyta + % Chrysophyta; Other macrophytes = % Salviniales + % Asterales + % Myrtales; Other benthic invertebrates = % Hydrozoa + % Gymnolaemata + % Polychaeta + % Hirudinea + % Arachnida + % Nematoda; Other ichthyofauna = % Cobitidae + % Siluridae + % Esocidae + % Mugilidae + % Gasterosteidae + % Syngnathidae + % Percidae; similar letters within each taxonomic group indicate selections that do not vary one from another according to the results of the Tukey test (P > 0.05).



Fig. 1. Impact of hydrotechnical construction on species diversity of biotic groups in the mouths of the branches of the delta: a-phytoplankton, b-microphytobenthos, c-macrophytes, d-benthic invertebrates, e-fishes; grey-Bystryi (affected area), green-Vostochnyi (protected area), blue-Tsyhanka (protected area), orange-Starostambulskyi (protected area); $x \pm SD$, Tukey test, n = 3; similar letters within each biotic group indicate selections that did not significantly differ one from the other according to the results of Tukey test (P > 0.05)

Microphytobenthos contained 118 species of algae, including 83 in the mouth of the Bystryi branch, 64 in the Vostochnyi branch, 56 in the Tsyhanka branch and 57 in the Starostambulskyi branch. Bacillariophyta was in general represented by 94 species, including 69 found in the mouth of the Bystryi branch; their number in the water areas of the protected zone varied 48–55 species. Eight species of Euglenophyta comprised 4 found in the Bystryi branch and 1–3 species in the other mouths. Cyanophyta was represented by 6 species, 3 in each mouth of the Bystryi, Vostochnyi, Tsyhanka branches, and only *Oscillatoria limnetica* Lemm. was found in the mouth of the Starostambulskyi branch. Chlorophyta was

represented by 6 species, including 4 in the mouth of the Bystryi branch, and 1-2 representatives in each of the mouths in the protected zone. Both species of Dinophyta (Peridinium sp. and Gonyaulax spinifera (Clap. et Lanchm.) Dies.) were found in the branches Bystryi and Vostochnyi, the first was also observed in the mouths of the branches Tsyhanka and Starostambulskyi. The only species of Chrysophyta - Stenokalix densata Schmidle - was found in benthos of the mouths of the Bystryi and Starostambulskyi branches. No statistical difference was determined between the mean values of species richness by divisions of microphtobenthos in various areas (Table 3, P = 0.05-0.92). Also, we found no such difference between the values of overall species richness (P = 0.34, Fig. 1). A total of 22 species of benthic algae were shared by all the mouth areas (18.6% of the overall species richness), including 1 representative of Dinophyta, and others belonging to Bacillariophyta. In the mouth of the Bystryi branch, two species were absent in the water area (Cyclotella striata (Kutz.) Grun. and Cymatopleura elliptica (Bréb.) W. Smith were absent.

In the mouth areas of the branches, we found 27 species of macrophytes, including 24 species in the mouth of the Vostochnyi branch, 14 in the Starostambulskyi branch, 13 in the Tsyhanka distibutary, and 11 in the Bystryi branch. The order Alismatales was represented by 6-13 species in the mouths of the branches. The second richest order of macrophytes was Poales, represented by 2 to 6 species in certain mouths. Other orders were represented by 1 species each, occurring in many branches only during some periods of the studies. Therefore, thickets of Myriophyllum spicatum L. (Saxifragales order) were seen in the mouth of the Vostochnyi branch only in autumn of 2021, but were constantly present in macrophytes of other watercourses. Ceratophyllum demersum L. (Ceratophyllales order) constantly occurred in the mouths of the Tsyhanka and Bystryi branches, and was recorded in other watercourses only during some seasons of the studies. No representatives of Salviniales, Asterales, Myrtales were found in the mouth of the Bystryi branch, but they were recorded in water areas of the protected zone, being regionally rare (Salvinia natans (L.) All. and Trapa natans L), and Nymphoides peltatae (S. G. Gmel.) Kuntze, listed in the Red Book of Ukraine. The multiple comparison revealed significant difference (95.0% significant interval) between the mean values of species diversity of Saxifragales order for the mouth of the Vostochnyi branch and the mouths of the branches Bystryi (P = 0.02), Starosambulskyi (P = 0.04) and Tsyhanka (P = 0.02) and Ceratophyllales order in the mouth of the Vostochnyi branch and the mouths of the branches Bystryi (P = 0.04) and the Tsyhanka (P = 0.04) (Table 3). For the overall species richness of macrophytes of different mouths, we determined no significant difference (P = 0.39, Fig. 1). For all four plots, there were 6 (22.2%) shared species of plants (Butomus umbellatus L., Ceratophyllum demersum L., Myriophyllum spicatum L., Najas marina L., Potamogeton nodosus Poir. and Stuckenia pectinata (L.)). All species that were recorded in the mouth of the Bystryi branch were present in at least one of the aquatic objects in the protected area.

Benthic invertebrates were observed to contain 156 species of animals overall, including 113 in the mouth of the Bystryi branch, 106 in the Vostochnyi branch, 75 in the Tsyhanka branch, and 86 in the Starostambulskyi branch. We found 26 species of Mollusca, including 16 Gastropoda and 10 Bivalvia. In the mouth of the Bystryi branch, we found 10 gastropods and 9 species of Bivalvia. In the mouths of the branches in the protected zone, the number of gastropods varied 5 to 13, and the number of Bivalvia - 2 to 5. Annelida type included three classes: we found 6 species of each Polychaeta and Hirudinea, and 25 species of Oligochaeta. Representatives of Polychaeta were recorded in the mouths of the Bystryi and Tsyhanka branches. In the mouth of the Vostochnyi branch, Hirudinea was represented by all the 6 recorded species, and only by Piscicola geometra (Linnaeus) in the other watercourses. Species diversity of Oligochaeta was represented by 25 species, including 22 recorded in the mouth of the Bystryi branch, and varying 13 to 20 in the mouths in the protected zone. The Arthropoda type was observed to include representatives of three classes. Representatives of Arachnida class were not identified to the level of species; they were present in the mouth of the Vostochnyi branch. The number of Insecta species in some mouths varied 13 to 24 during various periods of the studies. In total, we recorded 59 species of insects. In all water areas, there dominated Diptera (in particular Chironomidae) - in total 28 species (15-22 species in some watercourses), other

divisions in the mouths of the branches were represented by 3-8 species. Of the 26 species of Malacostraca class, 16 were recorded in the mouth of the Tsyhanka and 19 in each of the other mouths. Amphipoda (13 species), in general, dominated. Also, in all the mouths, we found such crustaceans as Mysida, Cumacea and Dekapoda, whereas Isopoda were absent in the mouth of the Tsyhanka branch. On the sites, we recorded 8 to 14 species of Malacostraca during some periods. Benthos of all the branches included representatives of the types Cnidaria (2 species in total) and Bryozoa (4 species in total), and also the Nematoda class, which were not identified to the level of species. We determined no significant differences between species richness of some classes of benthic invertebrates (Table 3, P = 0.21 - 0.88). The results of multiple comparison revealed the significant difference (at the level of 95.0% P = 0.02) between the values of the total species richness in the mouths of the Bystryi and Tsyhanka branches, which had the greatest difference in the numbers of species of benthic invertebrates both in general and during certain periods of the study. All the areas had 47 shared species of benthic invertebrates (30.1%), most belonging to Oligochaeta (11 species) and Chironomidae (12 species). The benthic communities of the mouth of the Bystryi branch (the zone of impact of hydrotechnic construction) included no species that were seen all across the sites in the protected area, in particular Chelicorophium robustum (G. O. Sars), Euxinia maeoticus (Sowinsky) and Polypedilum exsectum (Kieffer).

The ichtyofauna included 28 species of fish, including 22 in the mouth of the Bystryi branch, 14 in the Vostochnyi branch, 16 in the Tsyhanka branch and 10 species in the Starostambulskyi branch. Nine of the 11 representatives of the Cyprinidae family were captured in the mouth of the Bystryi branch, and 3-8 species of this family in the other mouths (protected area). Gobiidae was represented by 8 species, including 7 in the mouth of the Bystryi branch, 6 in the mouth of the Tsyhanka branch, 5 in the mouth of the Starostambulskyi and 4 in the mouth of the Vostochnyi. The only species of Syngnathidae, Syngnathus nigrolineatus Eichwald, was observed in the mouths of all the branches. Representatives of the families Cobitidae (Cobitis elongatoides Bucescu et Maier), Gasterosteidae (Pungitius platygaster (Kessler)) and Percidae (Sander lucioperca (Linnaeus) and Perca fluviatilis Linnaeus) were caught only in the mouth of the Bystryi branch, Siluridae (Silurus glanis Linnaeus) in the mouth of the Vostochnyi branch, Mugilidae (Mugil cephalus Linnaeus) in the mouth of the Tsyhanka branch, and Esocidae (Esox lucius Linnaeus) in the mouth of the Starostambulskyi branch. The results of Tukey test comparison showed no significant difference in mean values of both representativeness of some families of ichthyofauna (P = 0.29-0.99) and overall species diversity (P = 0.09, Table 3, Fig. 1). All the examined water areas had 6 shared species of fishes (Alburnus alburnus (Linnaeus), Rhodeus amarus (Bloch), Pseudorasbora parva (Temminck et Schlegel), S. nigrolineatus, Neogobius fluviatilis (Pallas), N. syrman (Nordmann) and Proterorhinus semilunaris (Heckel)), which accounted for 21.4% of the overall species richness of ichthyofauna.

In total, biotic groups of the mouth areas of the delta branches were found to comprise 367 species of animals and plants, including 250 species in the mouth of the Bystryi branch, 231 in the mouth of the Vostochnyi, 180 in the mouth of the Tsyhanka and 193 in the Starostambulskyi. In the mouth of the Bystryi branch, the total number of animals and plants was 1.08–1.39 times higher than on the protected sites. Such a tendency was also determined for some botic groups: 1.11–1.29-fold difference for phytoplankton, 1.30–1.48 for micropphytobenthos, 1.07–1.51 for benthic invertebrates and 1.38–2.20 for ichtyofauna. Macrophytes were characteristic of the opposite: the species diversity in the water area of hydrotechnical construction (affected area) was 1.08–2.00-fold lower.

Of all the recorded species, 25.3% (93 species) occurred in all the mouth sites, of which 12.8% (47 species) were benthic invertebrates, 8.7% (32 species) algae, 1.9% (7 species) of each macrophytes and ichthyofauna.

Similarity of species compositions, evaluated using Bray-Curtis distance revealed that the range of indices between the examined water areas varied 47.5% (for phytoplankton of the mouths of the Vostochnyi and Starostambulskyi branches) to 81.5% (for macrophytes of the mouths of the Bystryi and Starostambulskyi branches) (Fig. 2). According to the species composition of phytoplankton, the mouths of the branches are comprised by two clusters: Bystryi – Vostochnyi (the similarity level stambulskyi (75.8%) (Fig. 2d), and such of ichthyofauna in the mouths of the Vostochnyi and Tsyhanka (73.3%, Fig. 2e). According to the species compostion of the biota, similarly to algae flora, the mouths of the branches are characterized by two clusters: Bystryi – Vostochnyi (67.4%) and the Tsyhanka – Starostambulskyi (70.2%, Fig. 2f).



Fig. 2. Similarity of the species compositions of biota of the mouths of delta branches subject to various degrees of hydrotechnical construction: on abscissa axis – similarity (%); a – phytoplankton, b – microphytobenthos, c – macrophytes, d – benthic invertebrates, e – ichthyofauna, f – overall species composition of branch's biota; Italics – protected area; Bray-Curtis distance equation, Simple Average cluster method, without standardization, presence/absence transformation

Ecological status of water areas. Values of O assemblage indices depended on biomass of some genera of algae, which belonged to functional groups with various F-factors. Therefore, algae of TB group were represented in the mouth areas by genera Surirella, Fragilaria, Nitzschia and Navicula, total mean biomasses of which varied 4.43 ± 1.92 in the mouth of the Vostochnyi branch to 8.70 ± 2.17 mg/L in the mouth of the Tsyhanka branch and 3.62 ± 3.29 and 6.82 ± 1.51 mg/10 cm² in the mouths of the Starostambulskyi and the Bystryi branches respectively, accounting for 37.5-63.9% of phytoplankton biomass and 65.7-72.3% of microbenthos (Fig. 3a-4a). In phytoplankton of the mouths of the Bystryi and Vostochnyi branches, algae of TD group were represented by genera Gomphonema, Cocconeis, Gyrosigma, Caloneis, Stauroneis, Amphora, Rhoicosphaenia, Cymatopleura, Diatoma, Epithemia, Eunotia with average biomass measuring 1.63 to 3.03 mg/L (15.9-25.6%, Fig. 3d). In the mouth of the Starostambulskyi branch, representatives of the group D (Cyclotella, Stephanodiscus, Talassiosira, Synedra) were characterized by average biomass of 2.27 ± 1.01 mg/L and 0.66 ± 0.52 mg/10 cm², which corresponded to 19.1% of phytoplankton biomass and 12.0% of microphytobenthos (Fig. 3b-4b). In phytoplankton, average biomasses of algae of group P (Melosira, Aulacoseira) varied 0.89 ± 0.30 mg/L in the mouth of the Starostambulskyi branch to 1.20 ± 0.26 mg/L in the mouth of the Vostochnyi branch, measuring 7.5-10.4% (Fig. 3e). Those same genera of algae in microphytobenthos were characterized by biomass varying 0.26 to 1.25 mg/10 cm² (3.9–14.8%, Fig. 4e). Groups of algae with low F value accounted for 2.2-3.5% of phytoplankton biomass and 1.3-6.3% of microphytobenthos biomass (Fig. 3f-4f). We determined no significant difference between the mean values of biomass of plankton and benthic algae of certain functional groups in different mouths of the branches: P = 0.08-0.95 for phytoplankton and P = 0.13-0.85 for microphytobenthos.

During some periods of the study, assemblage indices Q for phytoplankton varied from 3.73 in the mouth of the Vostochnyi branch to 4.49 in the mouth of the Tsyhanka branch (Fig. 3h), and for microbenthos – from 3.43 in the mouth of the Starostambulskyi branch to 4.85 in the mouth of the Bystryi branch (Fig. 4h). We determined no significant difference between the mean values of the calculated Q indices for the mouths of the branches: P = 0.06 for phytoplankton and P = 0.72 for microbenthos.

In the composition of aquatic macrophytes, we recorded 20 indicator species, including 4 that preferred eutrophic, 10 mesoeutrophic, and 6 mesotrophic waters. Eutrophic species (CSi = 1–5) contained *C. demersum*, *N. marina*, *S. pectinata* and *P. nodosus*, which were recorded in all

the watercourses, with projective cover ranging 0.35 ± 0.27 in the mouth of the Bystryi branch to 3.05 ± 2.64 in the mouth of the Tsyhanka branch (Fig. 5a). The dominating mesoeutrophic species (CSi = 6-9) were Phragmites australis (Cav.) Trin. ex Steud., Typha angustifolia L. and B. umbellatus; projective cover of this group varied on average $2.6 \pm 1.1\%$ in the mouth of the Tsyhanka branch up to $9.1 \pm 3.5\%$ in the mouth of the Vostochnyi branch (Fig. 5b). Dominating mesotrophic species (CSi = 10-13) was Sparganium erectum L. The total areas of projective cover for mesotrophic species ranged $0.22 \pm 0.22\%$ in the mouth of the Bystryi branch, where only S. erectum was recorded, to $6.2 \pm 7.8\%$ in the mouth of the Vostochnyi branch, where five mesotrophic species were found (N. peltata, S. erectum, T. natans, Potamogeton gramineus L. and Lemna minor L., Fig. 5c). We determined no significant difference between mean values of projective cover of eutrophic and mesotrophc species (Fig. 5a, 5c): P = 0.13 and P = 0.24 respectively. Analysis of the comparison of mean values of projective cover for mesoeutrophic species (Fig. 5b) revealed significant difference at the level of 95.0% for the mouths of the branches Vostochnyi and Tsyhanka (P = 0.03) and the mouths of the branches Vostochnyi and Starostambulskyi (P = 0.02). Mean values of Macrophyte Biological Index for Rivers (IBMR) ranged 6.59 ± 0.25 in the mouth of the Bystryi branch to 7.47 ± 1.21 in the mouth of the Tsyhanka branch; no significant difference was found between values of the index for the examined mouths (P = 0.04, Fig. 5d).

Species composition of benthic invertebrates included 79 indicators of saprobity, which for certain areas equaled 59.4-63.6% of their total species composition. According to composition, there dominated β-mesosaprobic species (17-34 species in some seasons), which on average was 24.5 ± 5.82 species (Fig. 6a). The number of α -mesosaprobic species varied 5 to 14 in the mouth of individual branch in a particular period of studies equaled on average 7.83 \pm 2.92 (Fig. 6c). Mean number of β mesosaprobic species varied 0.90 ± 0.42 thou spec./m² in the mouth of the Tsyhanka branch to 2.85 ± 1.75 thou spec./m² in the mouth of the Starostambulskyi branch, and such of α -mesosaprobic – within 0.56–2.55 thou spec./m². The benthic communitis of the mouths of the branches contained one polysaprobic species Tubifex tubifex (Müller) with average number of 0.06 ± 0.13 thou spec/m². According to the results of multiple comparison, we have seen significant difference between the number of β mesosaprobic species in the mouths of the branches Bystryi and Starostambulskyi (P = 0.05) and the Bystryi and Tsarychanka branches (P =0.02, Fig. 6a). The Tukey test revealed no significant difference according to species richness of other indicator groups (P = 0.11-0.36, Fig. 6b-6d).

The calculated saprobity indices ranged within 2.28–2.64, and mostly corresponded to β -mesosaprobic zone, maximum values of the index were determined for the mouth of the Tsyhanka branch in autumn of 2020 and 2021, which – according to SZ&M – was α -mesosaprobic zone.

Multiple comparison of mean values of SZ&M saprobic index revealed significant difference according to those indicators (at the level of 95.0%) between the mouths of the Tsyhanka and Starostambulskyi branches (P = 0.03, Fig. 6e).



Fig. 3. Impact of hydrotechnical construction on biomass of functional groups of phytoplankton and Q assemblage index in the mouths of the branches of the delta: a – Bacillariophyta, confined to lotic conditions (TB), b – Bacillariophyta, confined to shallow-water turbid biotopes (D), c – representatives of eutrophic lakes and rivers (C); d – benthic and epiphytic Bacillariophyta and filamentous Chlorophyta (TD), e – Bacillariophyta and Chlorophyta, which develop in the conditions of eutrophic epilimnion (P), f – representatives of shallow-water mesotrophic limnophilous biotopes (W2) + epiphytic Cyanophyta in places of occurrence of macrophytes (TC) + Chlorophyta of shallow-water eutrophic zones (J) + Euglenophyta in places of organogenic contamination (W1) + diazotrophs tolerant to low contents of nitrogen and carbon (H1) + representatives of standing water with high concentration of organic substances (W0), g – total biomass, h–Q assemblage index; grey – Bystryi (affected area), green – Vostochnyi (protected area), blue – Tsyhanka (protected area), orange – Starostambulskyi (protected area); x ± SD, Tukey test, n = 3; according to the results of comparisons of selections within each group of group of algae, the Tukey test revealed no significant differences for biomass and assemblage index Q (P > 0.05)



Fig. 4. Impact of hydrotechnical construction on biomass of functional groups of microbenthos and assemblage index Q in the mouths of the delta branches: a – Bacillariophyta, confined to lotic conditions (TB), b – Bacillariophyta, confined to shallow-water turbid biotopes (D), c – representatives of eutrophic lakes and rivers (C); d – benthic and epiphytic Bacillariophyta and filamentous Chlorophyta (TD), e – Bacillariophyta and Chlorophyta, which develop in the conditions of eutrophic epilimnion (P), f – representatives of shallow-water mesotrophic limnophilous biotopes (W2) + epiphytic Cyanophyta in places of occurrence of macrophytes (TC) + Chlorophyta of shallow-water eutrophic zones (J) + Euglenophyta in places of organic contamination (W1) + diazotrophs that are tolerant to low content of nitrogen and carbon (H1) + representatives of standing water with high concentration of organic substances (W0), g – total biomass, h – assemblage index Q; grey – Bystryi (affected area), green – Vostochnyi (protected area), blue – Tsyhanka (protected area), orange – Starostambulskyi (protected area); x ± SD, Tukey test, n = 3; the results of comparisons of selections within each group of algae according to biomass and assemblage index Q revealed no significant differences using the Tukey test (P > 0.05)

The benthos of the mouths of the branches contained representatives of seven indicator groups of the Biological Monitoring Working Party (BMWP) Index. The first group, which includes Ephemeroptera and Trichoptera families with high indicator properties (7 points according to the BMWP scale), was represented by 1–2 taxa (Fig. 7a). From the other group (6 points according to the BMWP scale), which is comprised by mollusks and crustaceans which are sensitive to water quality, and also less sensitive indicatory families of insects, we found 2–9 indicatory taxa of each (Fig. 7b). The third group (5 points according to the BMWP) is composed of various families of Hemiptera and Coleoptera, joined by the Diptera representatives that are the most tolerant to water quality; the total representativeness of this group in the locations varied 1 to 4 taxa (Fig. 7c). The fourth group (4 points according to the BMWP scale) contains families that are more tolerant to contaminations, for example Piscicolidae

leeches and Ephemeroptera larvae of the Baetidae family, which were periodically present in the benthic communities (Fig. 7d). Another indicatory group (3 points according to the BMWP scape) includes families of mollusks, crustaceans and leeches with low sensitivity to water quality; in some mouths, it was represented by 2–6 indicatory taxa (Fig. 7e). The two other groups comprise larvae of Chironomidae and Oligochaeta, characterized by the lowest sensitivity to water quality (2 to 1 points according to the BMWP respectively); they were a constant component of benthos in all the mouths of the branches. The multiple analysis of the similarity of species richness of indicatory groups revealed no significant difference between the mouths of the branches (P=0.15–0.90, Fig. 7a-7g). Values of BMWP index varied during different periods of the studies within 49–92 points in the mouths in the protected zone to 72–104 points in the mouth of the Bystryi branch. According to mean values of the indices, water in the mouths of the Bystryi, Vostochnyi and Starostambulskyi branches corresponded to the class II "slightly polluted water", and the mouth of the Tsyhanka branch – class III "moderately impacted water". The results of the multiple comparison revealed no significant difference between mean BMWP values in the mouths of the delta branches (P = 0.39, Fig. 7f).



Fig. 5. Impact of hydrotechnical construction on projective cover of trophic groups of macrophytes and values of Macrophyte Biological Index for Rivers in the mouths of delta branches: a – eutrophic species, b – mesoeutrophic species, c – mesotrophic species, d – Macrophyte Biological Index for Rivers (IBMR); grey – Bystryi (affected area), green – Vostochnyi (protected area), blue – Tsyhanka (protected area), orange – Starostambulskyi (protected area); x ± SD, Tukey test, n = 3; according to IBMR, similar letters within each trophic groups and the mouths of the branches indicate selections that have no significant difference one from another, as revealed by the Tukey test (P > 0.05)



Fig. 6. Impact of hydrotechnical works on the representativeness of groups of benthic invertebrates in relation to saprobity of water and saprobity index, as revealed by SZ&M: a – species richness of β -mesosaprobic species, b – species richness of α -mesosaprobic species, c – number of β -mesosaprobic species, d – number of α -mesosaprobic species, c – saprobity index according to SZ&M; gray – Bystryi (affected area), green – Vostochnyi (protected area), blue – Tsyhanka (protected area), orange – Starostambulskyi (protected area); x ± SD, Tukey test, n = 3; similar letters within each group according to saprobity and the mouths of the branches, as revealed using SZ&M, indicate selections that had no significant difference between each other according to the results of the Tukey test (P > 0.05)



Fig. 7. Impact of hydrotechnical works on the representativeness of indicatory groups according to the results of the Biological Monitoring Working Party Index (BMWP) in the mouths of the delta branches: a – number of taxa of Caenidae, Limnephilidae, Polycentropodidae, b – number of taxa of Neretidae, Viviparidae, Unionidae, Nereididae, Palaemonidae, Corophildae, Gammaridae, Coenagrionidae, Hydroptilidae; c – number of taxa of Gerridae, Naucoridae, Mesovelidae, Pleidae, Halyplydae, Dytiscidae, Hydropsychidae, Simmulidae; d – number of taxa of Piscicolidae, Baetidae; e – number of taxa of Lymnaeidae, Planorbidae, Physidae, Valvatidae, Hydrobiidae, Sphaeriidae, Erpobdellidae, Glossiphonidae, Asellidae; f – biological monitoring working party index (BMWP); grey – Bystryi (affected area), green – Vostochnyi (protected area), blue – Tsyhanka (protected area), orange – Starostambulskyi (protected area); x ± SD, Tukey test, n = 3; the comparison of the selection within each group of indicators and mouths of the branches according to BMWP revealed no significant differences by the Tukey test (P > 0.05)

During the period of the studies, ichthyofauna contained 6 tolerant species of fish, particularly *Rutilus rutilus* (Linnaeus), *Pseudorasbora parva* (Temminck et Schlegel), *A. alburnus*, *B. bjoerkna*, *P. fluviatilis*, and *C. gibelio*, and only one representative of intolerant species of fish – European bitterling *R. amarus*, the vitality (reproduction) of which depends on presence of mollusks of Unionidae family in the biotope. All tolerant species of fish were captured in different periods of the studies in the mouth of the Bystryi branch; their representativeness varied 20.0% to 38.5% (Fig. 8a). Five species of tolerant fish (except *P. fluviatilis*) were found in each of the mouths of the branches Vostochnyi and Tsyhanka,

depending on the value of species diversity; their relative number ranged 25.0% to 42.9% for the first watercourse and within 14.3–40.0% for the second. In the mouth of the Starostambulskyi branch, we caught two representatives of this group (*A. alburnus* and *P. parva*), which occurred only in certain periods of the study, when their representativeness varied 22.2% to 50.0% of the overall number of fishes.

The only intolerant fish, *R. amarus*, occurred in the mouth of the Bystryi branch throughout the studies, and was periodically absent in the mouths in the protected zone: in November of 2020 – in the mouths of the Vostochnyi and Starostambulskyi branches, and in November of 2020

and June of 2021 - in the mouth of the Tsyhanka branch. The representativeness of intolerant species of fish varied 3.03 ± 5.25 in the mouth of the Tsyhanka branch to 20.37 ± 26.25 in the mouth of the Starostambulskyi branch (Fig. 7b).



Fig. 8. Impact of hydrotechnical construction on the representativeness of groups of fish according to sensitivity to the environmental factors: on y axis – relative number of species of fish in the mouth of branch (%); a – tolerant fish species, b – intolerant fish species; grey – Bystryi (affected area), green – Vostochnyi (protected area), blue – Tsyhanka (protected area), orange – Starostambulskyi (protected area); x ± SD, Tukey test, n= 3; Tukey test revealed no significant differences according to the results of comparison of the selection within each group of fish (P > 0.05)

In general, multiple comparisons of mean values of relative numbers of tolerant and intolerant species revealed no significant differences between the mouths of the branches: P = 0.92 for tolerant and P = 0.51 for intolerant species of fish (Fig. 7a–7b).

In order to evaluate the ecological status, for each descriptor, we calculated EQR values, which depended on reference values of the descriptors and had varying range of changes in the mouths of the branches (Table 4). The broadest ranges were characteristic for EQR according to relative number of intolerant species of fish, and the least number of changes in the EQR occurred according to the SZ&M index of benthic invertebrates and EQR according to the Q assemblage index for microphytobenthos.

The status of all mouth areas corresponded to the "high" category According to the calculated EQR values for phytoplankton and microphytobenthos, "good" according to EQR of benthic invertebrates and EQR of macrophytes, "high" according to EQR of ichtyofauna of the mouths of the Bystryi, Vostochnyi and Starostambulskyi branches, and was "good" for the Tsyhanka branch (Table 5). By the worst value among biological elements of quality in all mouths of the branches, as indicated in the Water Framework Directive of the EU, the overall ecological condition corresponded to "good" category.

Table 4

Values of indicators of ecological quality, calculated by mean values of the descriptors of biological elements of quality in the mouths of delta branches subject to and unimpacted by the hydrotechnical works ($x \pm SD$)

Description of historical elements of quality	Affected area Protected area			rea	On average
Description of biological elements of quality	Bystryi	Vostochnyi	Tsyhanka	Starostambulskyi	for the mouths
Q of phytoplankton	0.81	0.80	0.89	0.85	0.84 ± 0.04
Q of microphytobenthos	0.91	0.88	0.88	0.84	0.88 ± 0.03
IBMR of macrophytes	0.65	0.74	0.69	0.70	0.70 ± 0.04
SZ&M index of benthic invertebrates	0.73	0.74	0,71	0.77	0.75 ± 0.02
BMWP of benthic invertebrates	0.95	0.87	0.73	0.82	0.84 ± 0.09
Relative number of intolerant species of fishes	0.74	0.61	0.74	0.83	0.73 ± 0.09
Relative number of intolerant species of fishes	0.85	0.81	0.30	0.49	0.61 ± 0.26

Note: ecological quality ratio - EQR - ratio of obtained and reference values of the descriptor of biological quality element; reference value Q - assemblage index of phytoplank-ton and microphytobenthos + 5, reference value of IBMR - Macrophyte Biological Index for Rivers = 10.09, reference value of SZ&M - saprobity index of Zelinka & Marvan = 1.80, reference value of BMWP - Biological Monitoring Working Party Index = 86, reference value% of tolerant fishes = 20.0%, reference value% of intolerant species = 10.0%.

Table 5

Ecological status of the mouths of the delta branches subject to and beyond the impact of hydrotechnical works, determined according to certain biological elements of quality

Biological	Affected area	Protected area			
quality element	Bystryi	Vostochnyi	Tsyhanka	Starostambulskyi	
Phytoplankton	high	high	high	high	
Microphytobenthos	high	high	high	high	
Macrophytes	good	good	good	good	
Benthic invertebrates	good	good	good	good	
Ichthyofauna	high	high	good	high	

Note: the given categories of the ecological status correspond to the Water Framework Directive of the EU.

Discussion

Overall, all the studied biotic groups were characterized by species richness that has formed by organisms that are typical for the mouth water areas of the Kiliia delta (Alexandrov et al., 1999). The highest number of species of algae, invertebrates and fishes was seen in the mouth of the Bystryi branch, but at the same time this area was observed to have the lowest number of macrophyte species. Species diversity of algae communities and benthic invertebrates in the mouth of the Bystryi branch was the most different from the groups in the mouth of the Tsyhanka branch, such of macrophytes – from the mouth of the Vostochnyi branch, and ichtyofauna – from the mouth of the Starostambulskyi branch. However, the Tukey test revealed significant difference only between benthic invertebrates in the mouths of the Bystryi and Tsyhanka branches (Fig. 1), which was related to the fact that those areas are characterized by the largest differences in the overall number of species during all the periods of the studies. At the same time, the significant number of shared species caused the similarity of > 50.0% of the general species composition, as well as species composition of some biotic groups of mouths of the branches both in the protected water areas and zone affected by the hydrotechnical construction.

Similarity of taxonomic structures of phytoplankton and microphybenthos of the mouth areas of the branches is associated with the dominance of Bacillariophyta, and such of benthic invertebrates – with dominance of Insecta, Oligochaeta and Malacostraca. Similarity of the ichthyofauna is caused by representativeness of Cyprinidae and Gobiidae, which had the dominating and subdominating positions in some mouths. In macrophytes of the mouths of all the branches, a high number of species was seen for orders Alismatales and Poales, and significantly different from others was the mouth of the Vostochnyi branch because of sporadic discoveries of representatives of orders Saxifragales and Ceratophyllales. For all other above the rank of species taxa of macrophytes, algae flora, benthic groups and ichthyofauna, we determined no significant difference in the range of changes in their species richness according to the Tukey test (P > 0.05, Table 3).

The selected descriptors of the ecological status are based on determining organic contamination (Zelinka & Marvan's saprobity index, Biological Monitoring Working Party Index), trophic status (Macrophyte Biological Index for Rivers) and overall changes in the structure of communities in aquatic objects (algae assemblage index) and relative number of species of ecological groups of ichthyofauna. The results of calculating the assemblage index for groups of phytoplankton and microphytobenthos depended on representatives and ratio of some functional groups of algae, obtained for certain biotopes. The highest biomass in both plankton and benthos of the mouths of all the branches was seen for TB groups of algae – Bacillariophyta, confined to lotic conditions, which is logical and natural for the delta branches. No significant differences were revealed by the Tukey test between the mean values of biomass of functional groups of plankton and benthic algae of the examined mouths of the delta (Fig. 3–4), which in general caused similar results of the evaluation – "high" status.

In the ecological structure of macrophytes, we determined significant difference between mean values of projective cover of mesoeutrophic species of the mouths of the examined branches in the protected zone (Fig. 5) which are beyond the borders of deepwater navigable channel, which is explained by the broad range of action of natural factors in the delta. The results of assessment of Macrophyte Biological Index for Rivers have generally confirmed the natural status of all the sites, while the decrease in the values of the index in the mouth of the Bystryi branch was not statistically significant, indicating insignificant increase in trophicity in this watercourse, which does not affect its ecological status (Table 5).

The structure of groups of benthic invertebrates according to the number of indicator species of β-mesosaprobic zone was significantly different from such in the mouths of the Bystryi, Tsyhanka and Starostambulskyi (Fig. 6a), but according to the number of indicatory groups of invertebrates in the watercourses, the statistical difference was absent (Fig. 6c-6d). Mean values of saprobic indices according to the Tukey test were significantly different only for the mouths of the branches Tsyhanka and Starostambulskyi, which are in the protected zone outside the borders of the deepwater shipping channel (Fig. 6e). Mean indices of saprobity and water quality revealed the correspondence to B"-mesosaprobic zone (eutrophic water) (\beta"-mesosaprobic (eutrophic waters)) and classes II-III (quite clean - moderately impacted) (Armitage et al., 1983; Zhukinskyi, 2006). Those results correspond to the assessment of the branches of the Romanian part of the Danube Delta, according to which, the benthic invertebrates of the water of the Sfântu Gheorghe branch belonged to the β -mesosaprobic zone composition-wise as well (Stoica et al., 2013). The obtained values of saprobity indices and water quality indicate absence of organic contamination in places of the bed-deepening, as well as outside the borders of the deepwater shipping channel, and varied from the reference values (B'-mesosaprobic waters (mesoeutrophic waters) by only one class or one quality category, corresponding to "good" ecological status.

Ichtyofauna descriptors (relative number of tolerant and intolerant species) were characterized by the highest range of changes both between concrete mouths of branches and within one watercourse throughout the period of the studies (Fig. 8). The tolerant fishes included species that dominated only in the conditions of extreme action of environmental factors (after storms, floods and other disasters). They were the natural part of ichthyofauna, but did not dominate in the groups in regular conditions. In disturbed (by people or natural cataclysms) conditions, those species feel better than others (Mihov, 2014). We should note that a high percentage (20.0%) of tolerant species of fish in the reference conditions is related specifically to the natural instability of hydrological and hydrochemical characteristics of the mouth areas, whereas presence of intolerant species that have their special vital needs indicates the presence of certain "specific", or "unique" conditions in the examined water areas. The representativeness of tolerant species was the closest to the reference values in the mouths of the Bystryi and Tsyhanka branches, insignificant decrease (the mouth of the Starostambulskyi branches) or increase (the mouth of the Vostochnvi branch) of this indicator did not worsen the results of the assessment (Fig 8a, Table 5). The representativeness of the only recorded intolerant fish, R. amarus, was related to presence of large Bivalvia mollusks (Anodonta, Unio), for which the living conditions, specifically absence of a large shallow-water zone, were least favourable in the mouth of the Tsyhanka branch. Unio pictorum (Linnaeus) and therefore R. amarus, which needs mollusks for reproduction were recorded in this area in limited quantity only in spring of 2021, causing low representativeness of intolerant fishes on average for the entire period of the studies and had an effect on the results of the assessment of the ecological condition. Values of the integral indicator of ecological quality of ihtyofauna indicate that the living conditions for young fish in most of the mouths of the branches were close to the reference values and corresponded to the "high" ecological status and only in the mouth of the Tsyhanka branch, insignificant

deterioration of this parameter is a consequence of natural specifics of the water area.

In general, all biological components are in the conditions close to the natural conditions, indicating the results of correspondence of their characteristics to the categories of the ecological status (Table 5), and also correlate with the results obtained by other researchers for various areas of the Danube delta, according to which the ecological status – depending on the biological element of the quality – was determined as "good-moderate" (Graf et al., 2015; Očadlík et al., 2021; Năstase et al., 2022).

Thus, we determined a high similarity of the species composition, and in general determined no significant difference in the taxonomic and ecologic structures of biotic groups of the mouth of the Bystryi branch, where the deepwater shipping channel is functioning, and other undisturbed mouth areas. This could be associated with the fact that construction of the protective levee of the channel for deepwater shipping has to a certain degree limited the sea influence and stabilized the hydrodynamic conditions, created additional biotopes (stoney mound on one side, a broad shallow-water zone on the other side) and provided the development of both plant and animal groups, which in general promoted increase in their biodiversity. Furthermore, a significant part of the water effluent of the Danube runs in this channel (Mykhailov et al., 2004) and therefore there occurs a strong drift of hydrobionts (Lyashenko & Zorina-Sakharova, 2014; Lyashenko & Zorina-Sakharova, 2015), which find shelter in the stabilized conditions of the shipping channel. We should note that isolation of the mouth areas is a regular phenomenon for the development of the delta (Mykhailov et al., 2004), and therefore the construction of levee and creation of a protected water area to a certain degree repeats the natural processes, which further reflect in the results of the assessment of the ecological status. The impact of bed-deepening works on the biota is a local and short-term phenomenon, as indicated by the studies carried out in other estuaries, which demonstrated absence of significant changes in the water composition in the zone of bed-deepening and outside its borders (Adekunbi et al., 2018), and the structure of benthic and plankton groups has a tendency toward fast recovery (Bemvenuti et al., 2005; Donázar-Aramendía et al., 2018; Miro et al., 2022). Location of the areas of dredging below the mouth of the Bystryi branch, when turbid water currents are mostly driven out to the sea, and do not fall into the branch, minimizes the impact of this factor on adjacent freshwater ecosystems even more.

Water areas of the front margin of the delta were characterized by significant fluctuations in abiotic factors, in particular force and directions of the current, intensity of wavy dynamics, changes in water salinity, etc., i.e. this area is a specific zone with natural stress (Elliott & Quintino 2007; Facca, 2020), ecological pressure (Lyashenko & Zorina-Sakharova, 2015) and vary by specific groups (Zorina-Sakharova et al., 2014; Lyashenko & Zorina-Sakharova, 2015). Complexities of the assessment of anthropogenic pressure in the ecosystems under natural stress are explained by the Estuarine Quality Paradox (Elliott & Quintino, 2007), when significant disorders in the structure of the communities - due to the negative impact - are a part of the amplitude of natural reactions of hydrobionts to variable environmental conditions. Assessment employing the descriptors of biological elements of quality in transitional water can be especially complicated: various indicators give contrasting results through unclear reaction to natural and anthropogenic stress, and also through complicated identification of reference conditions, which was observed in attempts to assess the activity of people in some estuaries of the world (Monti et al., 2021; Miro et al., 2022). We determined no negative impact of hydrotechnical construction and functioning of the deepwater shipping channel on the groups in the mouth of the Bystryi branch, and the construction of the protective levee caused increase in the species diversity of animal and plant communities, and bed-deepening works inhibit (for a short period of time) plankton and benthic biota. Both processes, as seen by the Water Framework Directive of the EU, are negative, but compensate one another, leading to absence of difference in species compositions and the structures of the biotic groups between the disturbed area of the delta and water areas outside the effect of deepwater shipping channel.

Conclusions

In general, in the mouth areas of the branches of the Kiliia branch of the Danube, there were recorded 367 species of plants and animals, including 122 species of phytoplankton, 118 microbenthos, 27 macrophytes, 156 benthic invertebrates and 28 fishes. Of the four mouth areas, the highest biodiversities of algae flora and animal groups and the lowest number of macrophytes were seen in the mouth of the Bystryi branch, where in 2004, there were performed works oriented at provision of the functioning of deepwater navigable channel the Danube-Black Sea. The general peculiarity of biotic groups in all the mouth water areas was high similarity of their species compositions, taxonomic and ecological structures, which is understandable regarding the confinement to the same hydrological system of the delta and closeness of the branches to one another, comparatively small sizes of the watercourses and distances the water travels from the place of their ramification, which to a significant extent neutralizes the processes inside water bodies and guaranties similar hydrochemical values of water, and also if there is no significant anthropogenic pressure in most watercourses.

Ecological status of all examined sites corresponded to "high" category according to the values of the descriptors of phytoplankton and microphytobenthos, "good" according to benthic invertebrates and macrophytes, and varied "high" to "good" according to ichtyofauna. The overall ecological status, which – in accordance with the Water Framework Directive of the EU – was determined according to the worst indicator of the biological quality elements, corresponded to the "good" category for all mouth areas, which is coherent with the results of the International studies of the Danube.

Absence of significant difference in the structure of biotic groups of the mouth of the Bystryi branch, where hydrotechnical works are regularly carried out to provide the functioning of deepwater shipping channel, and other mouths located in the protected zone, may be related to relatively small – compared with natural stress-causing conditions of transitional water of the Danube delta – anthropogenic impact, i.e. prevalence of natural factors over the local-scaled anthropogenic activity in the delta, and also the biopositive effect of the protective levee that levels out the negative impact of dredging on the structure of the biota in the mouth area of the delta. All the differences in the species composition and the structure of biotic groups, recorded in the mouths of the branches, are related to the specifics of their existence in the naturally unstable conditions of the delta.

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The authors declare no conflict of interests.

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