Project funded by
EUROPEAN UNION

Institute of Zoology Interim scientific report

20 September 2018 – 20 November 2019

1. Introduction

During the first half of Project period LP team was involved in analysis of long-term comprehensive research carried out by the Laboratory of Hydrobiology and Ecotoxicology and Laboratory of Ichthyology and Aquaculture on Dniester and Prut Rivers, in the framework of national and international projects, over the last 40 years. This data along with literature overview allowed am assessment of the impact of HPPs on water and riparian ecosystems in the Dniester basin and selection of important indicators of impact. In this report, Institute of Zoology presents the views of experts (which were published in the proceedings of the international conference "Hydropower impact on river ecosystem functioning") on the impact of hydropower on river ecosystem functionality. The obtained results will allow implementing the next step – that of choosing relevant data sets for description of specific hydropower impact on Dniester and Prut river ecosystems and test them in the field, thus having a broader view on the impacts and jointly analyzing the issues related to the protection and restoration of river ecosystems.

2. Materials and methods

The multiannual research results analyzed included various national and international projects, including those from the last 5 years such as AQUASYS (2015–2018) and the on-going projects BSB 165 "HYDROECONEX" and BSB 27 "MONITOX". Also previously published papers were also overviewed [1-2]. The samples were collected from Dniester river, accumulation reservoir Dubasari, Prut river and accumulation reservoir Costesti-Stinca.

The following Research methods were used:

2.1 Water temperature was measured using a hydrological thermometer, with a division value of 0.1 °C [3]. ***The content of dissolved oxygen*** was determined using titrimetric method in accordance to the national standard SM SR EN 25813, 2011 [4]. Fixation of oxygen was performed in oxygen flasks in the field, at the place of sample collection. Threshold values of the analyzed parameters for 5 classes of the quality of surface waters are represented in Table 1.

Table 1. Classification of surface waters in accordance to temperature, dissolved oxygen and oxygen saturation Source: National Standard SM SR EN 25813, 2011 [4].

Quality indicator	I class	II class	III class	IVth class	Vth class
			The status of thermal regime		
Temperature, °C	Natural temperature fluctuations	Cold waters: 20 °C - summer 5°C - winter Warm waters: 28 °C - summer 8°C - winter	Cold waters: 20 °C - summer 5°C - winter Warm waters: 28 °C - summer 8°C - winter	Cold waters: >20 °C - summer >5°C - winter Warm waters: >28 °C - summer >8°C - winter	Cold waters: >20 °C - summer >5°C - winter Warm waters: >28 °C - summer >8°C - winter
Dissolved oxygen, mg/L	≥8 (BckL)	≥7	≥5.5	≥4	<4
Oxygen saturation, %	≥90 (BckL)	≥80	≥60	≥40	<40

BckL- background level

2.2 Multiannual dynamics of main ions and suspended matter was assessed as described by common research methods [4-5]. The quantity of suspended matter was determined by filtering a certain volume of freshly sampled water onto pre-prepared and weighted filters, followed by drying at 105 ° C in a thermostat to a constant weight, and then burning in a muffle furnace at 600 ° C, cooling it in a desiccator and weighing on an analytical balance [5]. The total quantity of suspended matter, their mineral ($S_{min.}$) and organic constituent (S_{org}) were determined in a selected volume of water (S_{total}), concentrations were expressed in mg/L.

2.3 Bacterioplankton species

Sampling and processing of samples was carried out in accordance to generally accepted hydrobiological methods [8].

2.3 Phytoplankton species

Sampling and processing of samples was carried out in accordance to generally accepted hydrobiological methods [8-10]. The species composition of phytoplankton was determined using a microscope (Lomo "Mikmed-2") and existing determination guides [12]. Based on the types of algae, indicators of saprobity, the water quality of the Dubasari reservoir was determined [6-7, 12]. The definition of water quality classes of the ecosystem under study was carried out in accordance with the Regulation on the requirements for surface water quality of the Republic of Moldova [9-11].

2.4 Zooplankton species

Sample collection was carried out in accordance to unified methods for hydrobiological sample collection and processing [12-14]. Identification of zooplankton species and quantitative analysis was carried out with the use of Lomo „MICMED 2” microscope and *Axio Imager A.2 (*Zeiss*)* microscope and identification guides [15-16]. Evaluation of the ecological state and quality class of water was determined in accordance to the National Regulation on Monitroring approved by the Government Decision no.932 from 20.11.2013.

2.4 Zoobenthic samples

The study of structural and functional indicators of zoobenthos was carried out on the basis of literature data [17-20], and studies conducted by the Laboratory of Hydrobiology and Ecotoxicology Institute of Zoology, for the period 2015-2018. The samples were collected from the Dniester river within the territory Republic of Moldova, from the left and right banks at stations Valchinet, Soroca, Camenca, Ierjova, Goieni, Cochieri, Vadul-lui-Voda, Varnitsa, Sucleia and Palanca. The collection and processing of samples was carried out in accordance to the methods generally accepted in hydrobiology [21-22]. The biomass was determined by weighing specimens on ABS 80-4 Kern balance with an accuracy of 0.0001 g. The abundance and biomass were converted to ind/m2 and g/m2, respectively. Species identification was done via microscope Axio Imager A.2 (Zeiss) and a SteREO Discovery V8 binocular (Zeiss) to the smallest possible taxa using determination guides [24-29].

2.5 **Determination and analysis of the ichthyological material** was performed using the classical ecological and ichthyological methods [30-31].

3. Main research results and discussion

3.1 Temperature and oxygen regimes.

Seasonal dynamics of water temperature in the Dniester river indicate that the sector Naslavcea-Valcinet (downstream the buffer reservoir of the Dniester hydroenergetic consructions is characterized by a decrease by 4-8 °C during spring, in comparison to the background level and by 10-15 °C - during summer. During the vegetation period 2015-2018, the concentration of dissolved oxygen varied within the range of 3.5 to 14.3 mg/L. During summer the oxygen regime was most unstable. Two critical zones in regard to concentrations of dissolved oxygen were identified: **1st critical zone: Dniester sector at Naslavcea station** – in accordance to oxygen saturation, during summer, water were classified to the IIIrd quality class and during autumn – to the IVth class; **2nd critical zone - Dniester sector downstream Soroca** - increased temperature during summer coupled with the discharge of untreated municipal wastewaters creates an oxygen deficit of <4mg/L, 42.6% saturation, with a water quality class of IVth class. In Dubassary reservoir (Erjovo, Goieni, Cocieri) the oxygen concentration varied very much during summer-autumn time, but had a stable regime during autumn [3].

The impact of construction and functioning of hydropower and pollution from wastewater discharge is exacerbated by high temperature, posing thus an additional pressure on the resilience of river ecosystems and autopurification capacity. Therefore, during August, 2019, a low water level, coupled with high air temperature led to a decrease in the oxygen up to the critical level: at Naslavcea station, the concentration of oxygen was only 4.1 mg/L or 42,6% saturation, at Soroca – 2.55 mg/L or 29,2 %. Evaluating the state of oxygen balance of Dniester river on the territory of Moldova during the whole vegetation period, it can be stated that sudden fluctuations of oxygen concentrations is an indicator of low ecosystem resilience and inability to withstand hydropower impact and significant changes in the water level in the Dniester river.

3.2 Multiannual dynamics of main ions, salinity and the quantity of suspended particles

Water salt composition is the sum of ions contained in dissolved natural waters, among which the main anions are distinguished: bicarbonates and carbonates (HCO_3^{2-} + CO_3^{2-}), sulphates (SO_4^{2-}), chlorides (Cl^-), cations: calcium (Ca^{2+}), magnesium (Mg^{2+}) and sodium + potassium (Na^+ + K^+).

The dynamics of main ions and their concentration in mg/l, or milimoles are used for numerous calculations, not only for assessing the quality of drinking water or its hardness, but also of many types of water equilibrium (carbonate-calcium, sulphate-magnesium, etc.). This allows evaluating the aggressiveness of water in relation to concrete and metal structures, as well as the appropriateness of water for irrigation for various types of soils.

Research carried out during years 50ies, before the construction of the Dubasari reservoir, demonstrated that along the territory of Moldova, a clear inverse correlation was established between the amount of salinity and water flow of Dniester river [1]. Some elements of this dependency was also observed in the first years after the construction of the Dniester hydropower plant even up to the years 80-90s, when a clear seasonal dependence of the dynamics of the main ions and salinity was traced in the river. In the spring during flood period and summer-autumn floods, the concentrations of main ions were significantly lower than those in the low season. Nowadays, the opposite is true. The salinity value are minimal in the summer and maximum in the spring, with the exception of 2013, when the autumn concentrations were somewhat higher than during spring. Hydrocarbonates and carbonates are the predominant anions in all freshwaters. According to the classification of Alekin [33] the water of Dniester river belong to the class of hydrocarbonate waters. The main ion dynamics affects and at the same time depends upon the pH of water and the content of free dissolved carbon monoxide and bicarbonates. For Dniester ecosystem, quite often the content of hydrocarbonate ions makes water alkaline.

Among the cations, calcium cations predominate. The earlier, pre-hydropower situation was characterized by a strong positive correlation ($r > 0.9$) between the hydrocarbonate-carbonate anions and calcium cations, but this is not observed anymore today, after hydropower functioning. Even when comparing the seasonal averages, the vibrational range is smoothed somewhat; this correlation is close to moderate ($r = 0.7$).

This is also evidenced by the fact that during dry years 2015-2016 in Moldova, there have been cases when water from calcium bicarbonate (C_{Ca}) were metamorphosed into sodium bicarbonate (C_{Na}). Sodium is the most mobile natural water cation characteristic of sodic waters. In the Dniester basin this cation is found in the upper horizons of groundwater (springs, wells) and some small rivers.

In the past, a proportional ratio of sodium to chloride anions was characteristic for the Dniester river. In the last 10 years, an increase in sodium concentrations but not for chloride anions was recorded. Such case indicate that there is a replenishment of river water reserves from the upper horizons of groundwater.

One of the main factors that led to a radical change in the amount and the size of suspended particles in the water of the Dniester in recent years was the hydropower construction and their functioning on the river. The sharp decrease in sand-and-pebble materials entering the river have been noted in the river's ecosystem. If in the 70-80 'years of the last century sand, sand-pebble and sand-silt sediments predominated in the Dniester river and Dubasari reservoir, and black and gray clay silts were noted only in places with wastewater discharge (for example downstream the treatment facilities of Ribnitsa, at Goieni river banks zones with slow flow , near Iagorlik with slow water flow and in a small area downstream the confluence of the Byk tributary), today the gray and black silt sediments are spread all over, starting from the village of Valcinet, on Unguri-

Golosnitsa sector, all along the Dubasari reservoir and in the lower sector of Dniester River Bendery-Palanka. Small patches of sand deposits can be still found only at Camenca - up to Unguri, at Vadu-lui-Vod – upstream of Varnita and near Tiraspol and Slobozia.

The dynamics of suspended particles in the Dniester water was undergoing tremendous changes. Therefore, before the construction of the Dubasari reservoir (1951-1954) the average concentration of suspended particles was approximately 350 mg/l, with an average annual water flow of 6.97 km^3. The annual stock of suspended substances near Bender was 4000-5005 thousand tons in 1951-1953, but it decreased to 2711 thousand tons, which was almost 2 times lower. Construction and exploitation of Dniester hydropower station during 1983-1987 contributed to a reduction of suspended particles in Inferior Dniester river sector up to 17-100 mg/l in more than 90% of cases. At a relatively high water flow (8.11 km^3) in 1983, the annual stock of suspended particles decreased to less than 700 thousand tons, and in 1986-1985 - to less than 267-403 tons/year [34].

3.3 Impact of hydropower on bacterioplankton

The Dubossary reservoir was created in 1954, and its formation introduced serious changes into both abiotic and biotic factors of the Dniester river ecosystem. Data on bacterioplankton status until reservoir formation is not available. According to the report data of the Laboratory of Ecology of Hydrobionts, Institute of Zoology, comparing data from the 1965-1972 with that from 1986-1990 a tendency of an increase in the saprophytic bacterial groups was observed, related to organic pollution of the Dubasari reservoir [35]. More detailed studies were conducted in 1986, when, in addition to quantifying bacterioplankton numeric indices, their productive and destructive potential was also evaluated. Differences between the studied parameters: the production (P) and the destruction (R) of the organic matter, the total number of bacterioplankton (Ntot) and its saprophytic part (Nsapr) from the beginning of the studies exist, but they are not always statistically significant.

Analysis of bacterioplankton status in Dniester river, downstream the dam of Dubasari hydroelectric station (Vadul lui Voda station) shows that during the period 1991 - 2019, compared to the period 1981-2004 (st. Dubăsari), most of the indices regarding the status of bacterioplankton have increased. The experts microbiologists indicate that such an increase is related to the intense growth of aquatic vegetation, which decays and led to an increase in the concentration of slightly degradable dissolved organic substances, which are the main trophic substrate of bacteria in the Dubasari reservoir. At the same time, compared to the period since the beginning of the research within the project (from 2018 to 2019), most of the indices studied have been reduced by more than half, indicating the need for further studies. Also, it is important to mention, that as bacteria are organisms prone to various environmental variations, their production and destruction indices might be unsuitable indicators for reflecting of the long term changes, a wider statistical analysis shall be considered. These indicators can serve for estimation of the productional and destructional processes, evaluation of the trophic level of ecosystems and auto-epuration capacity as well as secondary microbial pollution, which characterize functionality of freshwater ecosystems.

3.3 Impact of hydropower on Phytoplankton

The low water level and water speed lead to intensification of eutrophication in Medial Dniester and Dubasari reservoir. Investigations carried out on phytoplankton revealed that in Dubasari reservoir, depending on the season, the phytoplankton biomass varies within 1.6-15.8 g/m3, which in most cases characterizes the reservoir as an eutrophic (periodically polytrophic) reservoir. In the period 2017-2018, in Dubsari reservoir, out of total number of identified algae species, ß-mesosaprobic species predominated in the reservoir: *Cocconeis placentula, Cymatopleura solea, Cyclotella Kuetzingiana, Gomphonema olivaceum, Nitzschia sigmoidea, Rhoicosphenia curvata, Synedra acus, Synedra ulona Trachelomonas hispida, Scenedesmus quadricauda*, which account for 59 %[36].

3.3 Impact of hydropower on zooplankton

In accordance to the studies carried out before the construction of hydropower station for Dniester river, the number of zooplancton was formed mainly of Rotifers (over 70%), the rest was represented by microcrustaceans [18]. The decrease in the proportion of Rotatoria could be related to the low water level and accumulation of the silt that became unsuitable for this group, as their filtration apparatus is becoming filled with silt particles and they cannot move further and die.

Between 1955-1959, after the creation of the Dubassari accumulation lake in the medial sector of the fl. Dniester, as before it, was dominated by rotifers (accounting approximately 60 %), but the role of microcrustaceans became more pronounced. The density values of zooplankton in the first years of operation of the Dubasari accumulation lake were recorded at the maximum values of 212.4 thousand ind / m3 (CLIMENCO, 2003, Toderaș et al., 2005), which is typical for newly built reservoirs, however a continuous decrease took place, during the years 2000-2002, when the development of zooplankton constituted 3.7 thousand ind/m3 [37-38]. In the distribution of zooplankton along the Dubasari accumulation lake, a regularity of density and biomass increase was determined towards the lower sector where a significant role was played by microcrustaceans.

During the last years of data analysis [39], the development of zooplankton communities is slowed down, so according to the data for the year 2018, the numerical number of zooplankton was 2.2 thousand ind/m3, which was much lower compared to the period 2000-2002, when a density of 16.1 thousand ind./m3 was recorded.

The data on the development of zooplankton communities of Lake Costesti-Stanca was collected quite sporadically and need further investigations. However, the impact of hydroelectric plant can be still observed. The medial sector of r.Prut is characterized by a specific diversity greater than the Costești-Stânca lake [40], but the values of the quantitative parameters are much lower. Thus, as a comparison in the period of 2016 in the medial sector of the reservoir, the density of zooplankton recorded the values - 20.5 thousand ind/m^3 which is 6 times lower than that found near the dam. During 2004, the zooplankton community of Costești-Stinca Lake registered 24 species, with the main contribution of the rotifers and an increase in the specific diversity from the upper sector of the lake to the dam. The density achieved by zooplankton during the respective period showed a decrease of approximatively 2 times compared to the period 1996-1998. The dominant group in the Prut river belongs to Rotifers.

3.4 The impact of hydropower on zoobenthos

During 1946-1954, 193 taxa of macrobenthos were found near Camenca city [31] mainly these being representatives of reophilic types, which live under the conditions of rapid water flow, which was characteristic during that time. Later, during 1955–1959, a slight decrease in the diversity of zoobenthos was observed at this site — 179 taxa of benthic invertebrates [41].During actual time, 2015-2018, only 92 taxa were recorded, that is 2 times less than during before the construction of hydropower plant. Most probable, the rapid development of the thickets of macrophytes and filamentous algae, as well as siltation of the rocky-sandy bottom area contributed to a decrease in macrobenthos diversity.

Before the construction of the Dubasari reservoir, the presence of *Dreissena polymorpha* (Pallas, 1771) was recorded rarely in the Dniester river, after it, in 1955-1958 the average number of zebra mussel in the reservoir increased to 252 ind./m2 [42]. During 2015-2018, the number of zebra mussel was particularly high, due to the emerged invasive species *Dreissena rostriformis bugensis* (Andrusov, 1897), with a density of 40-640 ind./m2 and a biomass of 4-334 g / m2, respectively.

In the Lower Dniester sector, during 1946–47, 218 macroinvertebrate taxa were recorded [43], during 1981–1985, this number decreased to 142 [42], but it remained unchanged until recent years, 2015–2018, when 139 taxa were collected at Vadul-lui-Vody – Palanca section [44]. The most significant changes in the structure of macrobenthos were noted upstream of reservoir and in the reservoir itself. First of all, it should be noted the disappearance or a significant decrease in numbers of the groups sensitive to negative environmental changes: *Plecoptera, Ephemeroptera and Trichoptera*. It is worth noting the probable extinction o*f Theodoxus transversalis (Gastropoda),* a rare species of mollusk (IUCN). Also should be noted the almost complete disappearance of *Unio crassus* (Crassiana crassa) (Bivalvia), a rare species of bivalve mollusks (IUCN), which is currently found only in the upper part of the Dubassary reservoir. At the same time, it could be noted appearance and spread of invasive species such as *B.sowerbyi, D. rostriformis bugensis, F. fragilis*. Also an increase in abundance and biomass of some gastropods, for example V. viviparus was observed. The density of this species reached 1000 ind./m2 and biomass 2555 g/ m2, most probably being related to intensive development of a muddy bottom and accumulation of gray and black silts in this area [34].

3.5 The impact of hydropower on ichthyofauna

The research work carried out during years 50ies of the last century, before the construction of Dubassary hydropower plant in the Dniester river 49 to 75 species of fish were found [35-36]. Analysis of the research results conducted during 1996-2000 showed that on the river sector Naslavcea-Camenca 42 species of fish were identified, among which 25 – belonged to Cyprinidae family, 5 species to *Percidae* and *Gobiidae*, 2 species each from *Cobitidae and Gasterosteidae* and one each from the families *Acipenseridae, Esocidae and Siluridae*. Even though at the first sight, a high diversity was observed, a cardinal change in the species composition was observed in comparison to 1950-1959. On this sector of Dniester river, all sturgeon and rare fish species practically disappeared*:* *Beluga Huso huso*, sturgeon *Acipenser gueldenstaedti*, stellate sturgeon *Acipenser stellatus*, herring *Alosa kessleri pontica*, brook- European trout *Salmo trutta fario*, grayling *Thymallus thymallus*, dace *Leuciscus borysthenicus*, tench *Tinca tinca*, burbot Lota lota, *Abramis ballerus bluefin*, gudgeon of the river Gobio kessleri [45-47].

It was found that at the Naslavcea-Camenca station, 75% of European roach females and 80% of perch females, already at the 3rd stage of gonad development are not able to reproduce due to a deep and prolonged resorption of the gonads, caused by unnatural thermal conditions extending until October-November [18]. In addition, hydropeaking and the absence of normal spring floods, especially in the last 4-7 years, do not allow the passage of fish for their successful spawning, not only on this section of the river, but also in the Dubasari reservoir and in the lower part of the river. As a result, the current state of fish stocks in the river is critical, despite the annual restocking of the Dubasari reservoir and the prohibition of industrial fishing on the territory of the Republic of Moldova.

Commercially valuable fish species were almost completely replaced by low valuable, short-cycled or invasive fish species. Therefore, the middle river sector from Naslavcea to Camenca is dominated by such species as three-spined stickleback, Rhodeus and common bleak. The lower sector of the river (downstream of Dubassary reservoir) is dominated by such species as *Clupeonella (tyulka)*, *Atherina and Common bleak*. During 2000 in the Dniester river between Dubasari reservoir and dam of hydroelectric power station - 2 at Naslavcea station economic valuable species and Red Data Book species accounted for 29.3%, while lower-value and short-cycled species for - 71.7%. A similar situation is characterized for Dubasari reservoir, where Red Data Book species and economically valuable species account for only 32%, while low valuable and short-cycles species - for approximately 70%.

In spite of the fact that as a whole the hydrological regime in ecosystems of Dniester and Prut rivers during 2018 were favorable for fish reproduction, owing to high amount of precipitation however, there were cases of hydro-peaking (sudden short-term variations in the water level) caused by the activity of the hydroelectric plants located along these rivers: Dubassary, Novodnestrovsk, Costesti-Stânca. The highest impact occurred on the species of fish with one time reproduction mode, of which the entire generation was compromised. Therefore, such species of fish with early reproduction (March) as the pike perch, suffered from hydropeaking and the low level of water in Dniester and Prut Rivers which had practically no access to their places of reproduction [48]. A major problem of Dniester river became the increase in the number and dominance of invasive species of fish, which affect indigenous species.

4. Knowledge gaps, future research needs

One of the most difficult problem in assessing the impact of hydropower on river ecosystems functioning and auto-purification capacity is insufficiency of statistical data of reliable hydrological indicators on river ecosystem status after the construction and functioning of hydropower. For bacterioplankton no data is available on their status before the construction of hydropower facilities. At the moment additional research is required to know if the production and destruction indices are suitable indicators for reflecting long term ecosystem changes under the impact of hydropower and climate change. Additional research would be required for comparing these indices across various years. Data on the dynamics and migration of metals, trace elements, nutrients and toxic substances are still under the process of systematization and will be published in the near future. The methodology of assessing the impact of hydropower need to include on the compulsory basis the parameters which reflect the change of ecosystems from river state to lake state, the change of the circuit of substances and energy and the processes of degradation of freshwater ecosystems, such indicators will be described in the protocol for monitoring the impact of hydropower on freshwater ecosystems that will be developed within the Hydroeconex project.

5. Conclusions

The available current data revealed significant change at ecosystem functionality level due to hydropower impact, including deterioration of physico-chemical parameters (temperature, oxygen, main ions, total dissolved solids and quantity of suspended solids) and biological parameters (phytoplankton, zooplankton and fish).
The initial period of the project implementation allowed selecting important ecosystem indicators of impact. The developed set of indicators will be tested during the subsequent joint field works during the following project period.

6. References

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