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Monitoring Protocol

Monitoring Protocol for assessment of the impact of hydropower on river ecosystem functioning

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1. INTRODUCTION

1.1 The impact of hydropower constructions on the state of ecosystems of Dniester and Prut transboundary rivers under a changing climate

The Dniester and its tributaries are a main source of water resources in the region, providing water for agriculture, industry for several localities, including the oblast centers and major cities of Moldova and Ukraine. The average annual flow of the Dniester is about 10 km^3. About 60% of river annual stock is formed during the summer-fall period, 25% – during spring as a result of snow melting; 15% – during winter period from the groundwater supply. Annually, there are up to 5 floods on the Dniester, during which the water level raises up to 3-4 meters or even higher. The greatest amplitude of water level fluctuations (up to 9-10 m) is observed at Zaleshchiki post, located upstream of the Dniester reservoir[1]. The changes in the annual stock of the Dniester river as a result of Novodnestrovk hydropower constructions is not significant - by 1 %[2], but as regarding the monthly stock, a significant decrease by 30-50% occurs from March to July at Hrushka station and from March to April at Bendery station[3]. In all seasons, a gradual increase in the Dniester annual flow was clearly visible from its source to the mouth: from 7.0 km^3 in Zalishchyky and 10.2 km^3 in Bender in 1951-1980 to from 7.3 to 9.1 km^3, respectively, in 1991-2015. The observed decrease of annual streamflow downstream the DHHC by 0.6 km^3 in Mogilev-Podolsky and 1.1 km^3 in Bendery can most probably explained by the synergism of the HPP and climate change influence. Thus, the DHHC impact has changed the contribution of individual parts of the Dniester catchment to its total runoff. For example, if in 1951-1980 about two-third (68.6%) of the Dniester's annual flow was formed in the upper part of its basin, then after the dams construction this share increased by 11.6%, and currently accounts for 4/5 [4].

Construction and exploitation of Dniester hydropower station during 1983-1987 contributed to a reduction of suspended particles in Inferior Dniester river sector up

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[1] Creating a system of innovative transboundary monitoring of the transformations of the Black Sea ecosystems under the impact of hydropower development and climate change – HydroEcoNex. Brochure developed within the Hydroeconex project, Eco-Tiras, Chisnau, 2019.

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[3] Jeleapov, A. The Dniester river: main changes of the past century. In: Proceedings of the International Conference "Hydropower impact on river ecosystem functioning", Eco-Tiras, International Association of River keepers, Tiraspol, 8-9 October, 2019, 95-100.

[4] Corobov R., Trombitsky I., Matygin,A., Onishchenko E. Kolvenko, V. On the issue of annual stock of Dniester river (in Russian) In: Proceedings of the International Conference "Hydropower impact on river ecosystem functioning", Eco-Tiras, International Association of River keepers, Tiraspol, 8-9 October, 2019, 176-182.

to 17-100 mg/l. A sharp decrease in sand-and-pebble materials entering the river have been noted in the river's ecosystem, while black and gray clay silts became more predominant. 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[5]. At the sector Naslavcea-Valcinet (downstream the buffer reservoir of the Dniester hydroenergetic constructions) there is a decrease by 4-8 °C during spring, in comparison to the background level and by 10-15 °C - during summer. The concentration of dissolved oxygen vegetation varies within the range of 3.5 to 14.3 mg/L, with most unstable values during summer[6]. The reductions in the sediment load due to hydropower can have several consequences on the river, including coarsening of substrate and formation of pavement layers or flushing of the sediment load due to flushing of reservoirs, resulting in a surplus of sediments in downstream river sections. High loads of mostly fine sediments cause high concentrations of turbidity and losses and mortality of aquatic organisms[7].

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[8]. The decrease in the proportion of Rotatoria occurred after the construction of the reservoir, which is 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. The siltation of the rocky-sandy bottom area coupled with the rapid development of the thickets of macrophytes and filamentous algae contributed to a decrease in mactobenthos diversity[9]. As regarding the structure of macrozoobenthos the share of lymno-rheophilic forms increased (from 0 to 40 ± 12% of total abundance), while the proportion of rheo-lymnophilic forms decreased.

Hydropeaking and the absence of normal spring floods, especially in the last 4-7 years, raised barriers for 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. The current state of fish stocks in the Dniester river is critical, despite the annual restocking of the Dubasari reservoir and the prohibition of industrial

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[8] Iaroshenko M. F. Hyrofauna of Dniester river. Moskow, Publishing House of Academy of Sciences of SSSR (in Russian) М. Ярошенко М.Ф. Гидрофауна Днестра. М.: Изд.АН СССР, 1957.169 с.

[9]

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. In addition, the unnatural thermal conditions extending until October-November at the Naslavcea-Camenca station, led to resorption of the gonads in 75% of European roach females and 80% of perch females, already at the 3rd stage of development[10], [11]. An interesting finding is also that in Dubassary reservoir there is a high richness of species of ichthyoparasites, conditioned probably by conditions of because of long-term regulation of water flow[12]-[13], which might have led to decreased dissolved oxygen, increased temperature and transparence.

The economic valuation of wetland ecosystems of Dniester river basin on the territory of Moldova showed a very high fragmentation, which adversely affects the level of their biodiversity and, consequently, the economic value of services provided by this ecosystem component[14]

On the Prut river, the dam and Stanca - Costesti reservoir modified the distribution in time and space of the annual average flow rates of suspended solids (Qs, kg/s). Suspension alluvial flows differ greatly upstream and downstream from 55 kg/s to 2.28 kg/s[15].

Ihthyofauna of the Prut River also suffered from the construction of the dam, with loss of rheophilous species (barbell, chub or other species) and development of species with a mixed profile[16].The Stânca-Costeşti dam generated transformation of the typical habitats in the humid areas at the border of Prut river. The fish quantities in Prut river have decreased from 37 species in 1947 to 26 found nowadays. In addition to the native species present at the time of the dam construction (*Squalius cephalus*, *Barbus barbus*, *Silurus glani*s, etc.), a number of species of high economic value such as *Hypophthalmichtys molitrix* and *Hypophthalmichtys nobilis* have been introduced, but also of gradual introduction of invasive species such as *Neogobius fluviatilis* and *Perccottus glenii* that benefited from the conditions created by human intervention[17].

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[10] Bulat Dm. Ichtyofauna of the Republic of Moldova: threats, trends and recommendations for its rehabilitation. Monography. Chisinau, 2017, 343 p. (in Romanian) [Ihtiofauna Republicii Moldova: ameninţări, tendinţe şi recomandări de reabilitate. Monografie. Chişinău: S.n., 2017. 343 p. ISBN 978-9975-89-070-0].

[11] Bulat, Denis Bulat,Dumitru Usatîi,Marin Zubcov, Elena Şaptefraţi, Nicolae Fulga, Nina The state of ecological reproductive fish species of Dniester and Prut rivers under actual ecological conditions (in Romanian), Starea grupelor ecologice reproductive de peşti din fluvial Nistru şi râul Prut în condiţiile ecologice actuale, In: Proceedings of the International Conference, Eco-Tiras, Tiraspol, 2019, 30-35.

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[16] Vartolomei F., Andrei M.T., Dumitraşcu M., Dumitraşcu C., 2011: Environment protection measurements in Prut basin by declaring Natural Protected Areas. Recent Advances in Fluid Mechanics and Heat & Mass Transfer, WSEAS Press, pp. 408-413, ISBN: 978-1-61804-026-8.

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1.2 Purpose and intent of the Monitoring Protocol

The primary objective of the monitoring described in this protocol is to evaluate the state of aquatic ecosystems as a result of the operation hydropower facilities in the context of climate change. **A secondary objective** of monitoring protocol is to promote standardized monitoring methodologies that can be used by scientific institutions, NGOs and managers of existing hydropower plants for assessing the transboundary ecosystem impact (Moldova, Ukraine and Romania) and propose mitigation measures.

1.3 Types of Monitoring

In accordance to the Water Framework Directive there are three types of monitoring of surface water quality (Table 1), namely surveillance monitoring, operational monitoring and investigative monitoring. As regarding assessment of the hydropower impact, it seems it can be more likey to be referred to the first type of monitoring - the surveillance monitoring, which validates the caracterization of pressure and impact assessment, detects long-term trends and provides an assessment of the overall status.

Table 1. Types of monitoring in accordance to WFD

Type of Monitoring	Aim
Surveillance monitoring	Validates the characterisation pressure and impact assessments Detects long-term trends Provides an assessment of overall status
Operational monitoring	Helps classify the status of water bodies identified as 'at risk' Assess change in status of 'at risk' water bodies that may result from the Programme of Measures.
Investigative monitoring	Ascertains the cause and effects of a failure to meet 'Good Status' where it is not clear Assessment of accidental pollution

1.4 . Baseline data requirements, frequency of monitoring

1.4.1 Baseline data requirements

WFD identifies the following options for describing the reference conditions:

1. **Historical data and information**. Reference conditions are described by the historical information, if they are of assured quality. If reference conditions are derived from historical conditions, these should be based upon the condition of water bodies at times of no or very minor anthropogenic influence.

2. **Modeling** A number of different modelling techniques may be used to derive reference conditions. Annex V 1.3 (v) of WFD states that "type-specific biological reference conditions based on modelling may be derived using either predictive models or hindcasting methods. The methods shall use historical, palaeological and other available data and shall provide a sufficient level of confidence about the values for the reference conditions to ensure that the conditions so derived are consistent and valid for each surface water body type".

3. **Expert Judgment**. In order to define reference conditions, expert views are used for deciding which data are appropriate. In addition, robust predictive models can be developed based on monitoring data additionally to the expert judgment. Information from previous similar hydropower projects may be useful, especially if quantitative predictions were made and have been monitored in operation. Expert opinion and judgment can be obtained from previous experience and consultations on similar projects, and from local experts with experience and knowledge of the site.

1.4.2 The statement of Habitats Directive (5) regarding the assessment of the impacts of Hydropower

EU nature legislation requires that any plan or project likely to have a significant effect on one or more Natura 2000 sites undergo appropriate assessment (AA) under Article 6(3) of the Habitats Directive (4). Commonly used methods for predicting the impacts are:

- ***Direct measurements*** may be taken, for example of areas of habitat lost or affected, proportionate losses from species populations, habitats and communities.

- ***Flow charts, networks and systems diagrams*** can identify chains of effects resulting from direct effects; indirect effects are termed secondary, tertiary, etc.

effects in line with how they are caused. Systems diagrams are more flexible than networks in illustrating interrelationships.

- ***Quantitative predictive models*** can provide mathematically derived predictions based on data and assumptions about the force and direction of effects. Models may extrapolate predictions that are consistent with past and present data (trend analysis, scenarios, analogies which transfer information from other relevant locations) and intuitive forecasting. Normative approaches to modeling work backwards from a desired outcome to assess whether the proposed project will achieve these aims. Predictive modeling often plays an important role as the main effects often follow from changes in hydro-morphological structures, resulting in changes in sedimentation regime with serious consequences for underwater biota.

- **Geographical information systems (GISs)** are used to produce models of spatial relationships, such as constraint overlays, or to map sensitive areas and locations of habitat loss. GISs are a combination of computerized cartography, stored map data, and a database-management system storing attributes such as land use or slope. GISs enable the variables stored to be displayed, combined, and analyzed speedily.

- ***Description and correlation***: physical factors (e.g. the water regime, current, substrate) may be directly related to the distribution and abundance of species. Based on the predictions of the changes in physical conditions it may be possible to predict the future development of habitats and populations or the responses of species and habitats on this basis.

- ***Capacity analyses*** involve identifying the threshold of stress below which populations and ecosystem functions can be sustained. It involves identifying potentially limiting factors, and devising mathematical equations to describe the capacity of the resource or system in terms of the threshold imposed by each limiting factor.

1.4.3 Frequency of monitoring

The frequency of sampling depends on ecological needs and economic possibilities. In routine monitoring of river ecosystems affected by hydropower constructions, the samples shall be taken several times per year. The most important seasons are when the water is stratified. The most important monitoring period is the summer stratification time, when the the primary production processes are at at their highest, and also the decomposition of organic matter is most active. Especially in eutrophied or polluted reservoirs several samplings should be organized during the summer period.

Frequencies for monitoring variables indicative of physico-chemical quality elements would be justified on the basis of technical knowledge and expert judgement. Continous monitoring is applied to those indicators, which change frequently with the time (e.g water flow, water temperature, oxygen regime etc.).A longer time interval is required for indicators that remain generally stable within time (e.g. biological indicators) and sampling/identification is time consuming. For example, river geomorphology changes slowly and surveys are particularly expensive so thus this parameter is surveyed once per 3 years[18]. The indicative recommendations about the required frequences for different parameters are presented in Table 2.

Table 2. Frequency of monitoring of rivers in according to Annex 5 of the Water Framework Directive

Quality parameter	**Frequency of monitoring**
Biological	
1. Phytoplankton	Every 6 months
2. Other aquatic species	Every 3 months
3. Mactoinvertebrates	Every 3 months
4. Fish	Every 3 months
II.Hydromorphological	
5. Hydrology	continous
6. Morphology	Every 6 years
III. Physico-chemical	
Thermal conditions	Every 3 months
Oxygen regime	Every 3 months
Nutrient status	Every 3 months
Other pollutants	Every 3 months
Priority substances	Every month

1.4 Types of ecosystem changes important to monitor under hydropower impact

As reflected by Table 1, in accordance to literature study, the hydropower impacts could be classified into two major categories: hydro-morphological and ecological changes.

[18] Yu, Xuezhong, He Daning, Phouving, Phousavanh Balancing river health and hydropower requirements in th Lancng river basin, The Water Framework Directive, Ecological and Chemical Status Monitoring, John Wiley and Sons, 2008

Table 1. Overview of the major types of impacts and indicators of change for hydropower[19], [20]

N/cat	Types of ecosystem changes	Main indicators to be monitored
1.	Hydromorphological changes	- Flow regime - The quantity of suspended particles - Water course, status of beds and banks
2.	Ecological changes	**Chemical and physio-chemical elements supporting the biological elements** • Transparency • Water temperature • Salinity • Oxygen regime • Nutrient loads • Specific Pollutants: priority substances discharged into the body of water **Change in biological communities:** • Bacterioplankton, phytoplankton, zooplankton and zoobenthos; • Macrophyte diversity; • Fish species; • Composition of invertebrate species or guild structure (e.g. from lenthic to lotic specialist species or guilds); • changes in flow-dependent invertebrate life-stage metrics (e.g. mussel recruitment to 2nd year class); changes in recruitment of invertebrate (e.g. *Corbicula*) and vertebrate species (e.g. fish)

2 Hydromorphological changes

2.1 Flow regime

For the normal functioning of the ecological system of the river, it is necessary that the volume of river water runoff ensure the preservation of the ecological balance on the catchment, and exclude the occurrence of processes that can lead to the degradation of water and other ecosystems that interact with the river.

[19] WFD and Hydromorphological pressures technical report, 2- European Commission, 2018 Guidance on the requirement for hydropower in relation to EU nature legislation

[20] Higgins, J. Konrad, Ch., Warner, A., Hickey, J. (2011). A framework for monitoring, reporting and managing dam operations for environmental flows at sustainable river project sites).

Ecological runoff in this case is defined as such a minimal river runoff that allows the river and the adjacent catchment to function stably in the form of a holistic ecological system.

In the annual cycle of the water regime of the river there are several characteristic periods, which are called phases of the water regime. The main phases of the river's water regime are high water, low water and floods. Therefore, monitoring of the hydrological parameters of the river flow regime should be carried out continuously throughout the year. When studying the hydrological regime of a river, one of the main types of observation is the measurement of water flow.

The water flow is the volume of water that flows through a given cross section ("live section") of a stream per unit time. As a rule, the flow rate of large streams is expressed in cubic meters per 1 second (m3 / s).

The water flow Q (m3 / s) through a given section of the river (hydrological target) is determined by the following methods:

1) the "speed - area" method; the average velocity V (m / s) of the flow is determined by various instrumental methods (using hydrometric turntables, various floats, etc.) and also is determined area of the "live section" - F (m2). The water flow find according to the formula: Q (m3 / c) = V (m / s) * F (m2) ;

2) the calculation method "slope-area". With this method, the longitudinal slopes of the water surface of the flow in the selected area are determined, the area of the "live section" of the flow in this place, and the water flow rate is calculated by the Shesi formula or by the equation of movement;

3) at hydraulic structures such as hydroelectric power plants, waterworks, pumping stations. This is the most accurate method for large rivers.

Also, water flow can be quite easily estimated from the curve of water flow - a graph that describes the dependence of the volume of runoff on the value of the river water level. This curve is calculated annually for a given hydrological post, and then used to estimate runoff and other parameters of the hydrological regime of the river.

Thus, with continuous monitoring of the hydrological regime of the river, continuous observations are made over the level of river waters at a hydrological post. This is a fairly simple type of observation, which does not require special expenses. At the same time, monitoring the water level allows you to determine water flow, both instantaneous and integral - for a particular season or year. Assessment of variability and prediction of water flow is the physical basis for solving hydrological, hydrochemical and hydrobiological problems.

2.2 The quantity of suspended particles

The quantity of suspended particles is measured in laboratories by filtering a known volume of freshly sampled water onto pre-prepared and weighted filters, drying the filters 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 then weighing the filter to determine the weight of the captured suspended solids in the sample. The quantity of suspended particles is calculated as follows:

TSS (mg/L) = (Wfsp – Wf)/Vs where:

Wfss: weight of filter with suspended particles

Wf: weight of the filter

Vs: volume of sample

The entire process takes about 2 hours and does not lend itself to instantaneous, continuous measurement.

3. Physico-chemical changes

3.1 Temperature

The changes in water flow and levels have the ability to change water temperature, especially when water is released or retained by the hydro-dam. For example, 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 of water temperature during spring and an increase during summer, in comparison to the background level. ***Water temperature*** is measured using a hydrological thermometer, with a division value of 0.1 °C. For measuring water temperature the thermometer is submerged by two-thirds below the surface of the water. The measurement is taken in a central flowing location. The thermometer shall be let to adjust for at least 1 minute to the water temperature before reading the data and removing it from the water.

In addition, the modern scientific approaches recommend also to use water infrared data from satellite images, that allows to build various maps on water temperature near the dams, downstream of the dams and in the reservoirs.

3.2 The content of dissolved oxygen

The determination of the content of dissolved oxygen and biochemical oxygen demand are important to assess the impact of hydropower as an increase in temperature and algal abundance can contribute to a decrease in dissolved oxygen and BOD values. The dissolved oxygen (DO) can be determined either with a portable oxygen meter or by traditional iodometric Winkler-method. This method is also used for calibration of different portable DO Meters, and as a reliable laboratory method. The most important step of this method is the collection of the sample into the narrow-mouthed glass flasks with stoppers (Winkler flasks, or any other suitable flasks) of 130 to 350 ml capacity, calibrated to the nearest 1 ml. Each flask and its stopper bears the same identification number. During sampling, precautions must be taken to ensure that no bubble is left under the stopper. Flasks should be filled up to overflowing, taking care to avoid any change in the concentration of dissolved oxygen. After filling, the dissolved oxygen is immediately fixed, by an addition of manganese (II) sulphate solution and alkaline reagent. The flask is inverted several times to mix the contents thoroughly. The formed precipitate is allowed to settle for at least 5 min and then mixed by several inversions of the bottle to ensure a homogeneous mixture. In the presence of oxygen a brown precipitate is formed, and in its absence the precipitate remains white. After complete addition of the precipitate, 1-2 ml of hydrochloric acid or H_2SO_4 1: 1 is added and the bottles are well mixed until complete dissolving. With a light protection, the samples may be stored for up to 24 h. All measurements taken in the field (the weather, growth of algae, the presence of dead fish floating in the water or oil spills etc.) are important to be recorded in the field notebook for a broader conclusion after oxygen analysis. The quantitative content is poured into an Erlenmayer flask and titrated with a 0.01N thiosulphate solution until an yellow color (straw color) is obtained, then 1 ml of starch is added, which gives a blue coloration. Titration is continued until the color is completely discolored and passed into a starch blue. The quantity of oxygen is calculated in according to the formula:

O_2=nN*8*1000/V-2,

where,

n – l solution of sodium thyosulphate used during titration, 8 – equivalent mass of oxygen

1000- recalculation of the volume to 1L

V – volume of titrated sampe

The sampled values are compared to threshold values for 5 classes of the quality of surface waters as those represented in Table 1.

Biochemical oxygen demand is the amount of oxygen consumed by microorganisms within a period of 5 days for the biochemical decomposition of the organic substances contained in water at a temperature of 20 ° C. In two bottles of known volume, the water to be analyzed is collected under similar conditions as those for the determination of dissolved oxygen. In one of the bottles the oxygen is fixed (see dissolved oxygen determination), and the second is kept in the dark at about 200° C for 5 days. In the bottle in which the oxygen was fixed, the determination is made as shown in the determination of dissolved oxygen. After 5 days the oxygen dissolved in the second bottle is determined, under the same conditions as for the first bottle.

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

4.Ecological changes

4.1 Transformations of the riverine habitats caused by hydropower reservoirs and dams

Transformation of riverine habitats is calculated as the percent ratio (%) between the reservoir surface area and surface area of all aquatic ecosystems of a given sub-basin before the construction of the reservoir (%). The transformation of the habitats is also assessed by the degree of fragmentation which is the percentage of the basin area cutt off from the sea by the constructed hydropower dams. Fragmentation of habitats contribute to a decrease in diadromous fish species which suffer from disruption of migratory routes to the sea.

4.2 Assessing the status of bacterioplankton, production and destruction rates

Microbiological sampling are collected in glass bottles of at least 300 ml, previously washed with a non-ionic detergent and rinsed at least three times with distilled or deionized water and then autoclaved. The sterilized bottles are opened only prior to sampling, water is collected at a depth of 15 - 30 cm. Analysis of samples is carried out within 6 hours after their collection. If it is not possible to perform analysis within this period, the samples can be kept in the fridge, but not longer than 24 hours. For determination of total number of bacteria, a known volume of water is filtered via a membrane filter, with pore size of 0.23-40 μ (for removing phyto and zooplankton), which are installed in Zeits device fixed in Bunsen balloon. The membrane filters are usually changed 2-3 times. After filtration, the membrane filters are placed on a Petri dish, lined with filter paper soaked in formalin and dried. After that filters are placed in vessels with erythrosine solution of 3-5% for 4-24 hours for dying and then on wet filters for discoloration. Subsequently, the filters are examined under fluorescent microscope, for enumeration using up to 20 viewing fields. The determination of the total bacteria number (mln.cel/ml) is carried out according to the formula: $X=SN/sV$, where S – filtration surface of the filter (μ^2), s – surface of the examined viewing field, V – the filtered water volume (ml).

The production of bacteria (P) is performed by filtering the sample through a membrane filter with pore size of 1.5-2.5 μ to remove zoo- and phytoplankton. After that, the water is poured into well-sealed bottles with a polished cap and installed in the water basin for 12-24 hours. For the determination of production, the total number of bacteria is determined at the beginning (N0) and at the end (Nt) of incubation period. The following formula is used to calculate the speed of bacterial production (P): Cw=ln (Nt/N0)/t, where N0 and Nt – total number of bacteria before and after inbubation, t – inclubation time.

P=Cw^B, where Cw – the speed of bacterian specific production and B – the biomass of bacteria in water.

To determine bacterial destruction, the water sample is filtered through a membrane filter with 1.3 μm pore size, incubated in the aquatic basin for 24 hours in well-closed bottles, covered with black bags. At the same time, an unfiltered sample is incubated. Preliminarily the dissolved oxygen content is determined by Winkler's method and the total number of bacteria. Over 24 hours in both bottles the total number of bacteria and dissolved oxygen are determined. After the oxygen difference in the filtered sample and the unfiltered one the amount of oxygen used in the respiration by the microflora (R) is determined. It is known that part of the food used by bacteria is consumed for energy requirements (R), part – for cell formation (P), another part of the food remains unused (K). Thus, the consumption (ration) of bacteria (C) is formed from the sum of three parameters:

C = P + R + K.

4.2 Phytoplankton and zooplankton

Mesoplankton and microplankton are collected by filtration of water through various models of plankton nets made of fine nylon mesh that is pulled through the water either vertically or horizontally at a known distance. The material collected in the bottom bucket is transferred to sample storage bottles.

Collected samples of phytoplankton are fixed with buffered formaldehyde up to the final concentration of 2%. Then the samples are allowed to settle for two weeks and then slowly decanted to the volume of 20-30 ml. The parameters to be investigated for assessing the hydropower impact are: number of phyla present, phyla dominance - top five ranked phyla in terms of % contribution to total biomass. Identification of species and cell counting is carried out under a light microscope Mikmed-5 (600x) in the drop of 0.05 ml and existing determination guides. Based on the types of algae, which are indicators of saprobity, the water quality is determined. The definition of water quality classes of the ecosystem under study is carried out in accordance with the Regulation on the requirements for surface water quality existing at the national level. The biomass of phytoplankton is calculated by the method of geometric similarity equating shapes of cells to corresponding geometrical shapes and assuming that the cell volume of 1 mm^3 is equal to 1pg.

The zooplankton samples are concentrated to 100-150 cm^3 in the laboratory, before being divided into sub-samples. A Bogorov's chamber is used for quantitative assessment (abundance and biomass calculation, using species individual weight) and qualitative (taxonomic structure) processing of subsamples.

Identification of zooplankton species and quantitative analysis is carried out with the use of microscopes (e.g. Lomo „MICMED 2" microscope or *Axio Imager A.2* Zeiss) as well as identification guide. Evaluation of the ecological state and quality class of water is determined in accordance to the existing national regulations.

4.4 Macrophytes

Macrophytes are usually sampled at peak vegetation period. A number of 10 random points at each site in the area of highest biomass (details may vary with study design). Drop the quadrat in a random manner at each marked site.

Photographs are taken to illustrate the sites and macrophyte growth. Species abundance is estimated as a relative plant biomass using a five-degree scale: 1 = very rare; 2 = rare; 3 = common; 4 = frequent; 5 = abundant, predominant. To determine percent coverage of macrophytes, the following formula is used: Coverage% = (Lm/Lt) X 100, where Lt is the length of the transect and Lm is the cumulative length of the transect occupied by macrophytes. For taxonomic identification, plants are collected in plastic bags and brought to the laboratory, where identification is done.

4.5 Benthic invertebrates

For quantitative and qualitative sampling of benthic invertebrates the following sampling devices are used: Petersen and Ekman grabs, with a capture area of 0.025 m^2, the rectangular dredge with a capture area of 8 m^2 , frames, silk or nylon nets with mesh size of 333 μm). Also, the method of "mowing" is used, when a net is dragged for three minutes opposite the water stream or on a one meter distance. The procedure is repeated three times. In such case and if a net with square opening of 25x25 cm is used, than the total picked up area is equal to 0.75 m^2. Bottom scrapers (mesh cloth of 500 – 1000 μm) are used for sampling on hard substrates, including from the hydrotechnical constructions.

The main parameters to be collected for benthic invertebrates are # of phyla present, phyla dominance - top five ranked phyla in terms of % contribution to total biomass), rate of reophilous to limnophylous species, guild structure.

Taxonomic determination of hydrobiont species and quantitative analysis is carried out with the use of modern microscopes and identification guides.

3.5 Fish and ichthyoparasitological indicators

A variety of sampling methods are available, including: gill/trammel, seine fyke (or bongo), and cast nets. Gill and trammel nets are appropriate for slow and moderate currents without significant amounts of floating debris, which can foul and damage the net. Cast nets are useful for almost all types of flows except for fastest currents. They are particularly useful for sampling isolated pools and eddies in otherwise swiftly flowing habitats that would be difficult to sample with other methods. Also, cast nets are useful for sampling the upper to mid-water column or smooth bottom areas and cannot be used over rough bottoms or areas with large amounts of debris. Seines can be used effectively in almost any habitats that are shallow enough to wade safely, except habitats with large amounts of debris or large rough rocks on the bottom. Consultants are encouraged to use the most appropriate method of sampling technique based on initial assessment of baseline instream flow conditions, review of secondary data, and key informant interviews with local fishermen.

Diversity of fish species is a valuable indicator for assessing the health of river ecosystem. In order to evaluate the change in the number of fish species, a comparison is made with the number of fish species before the construction of hydropower dams using the following formula: $C = (n_{fb} - n_{fa})/n_{fb}$, where n_{fb} and n_{fa} are number of fish species before and after construction of hydropower facilities. The status of health of river ecosystem is evaluated in accordance to the change in he number of species (see Table 2), but also in accordance to the dominance of specific species (economically and ecologically valuable species, invasive species etc).

Table 2. Assessment criteria for fish species change under hydropower impact

Change of fish species	Very good	Good	Fair	Poor	Critical
	≤5%	5-10%	11-15%	16-20%	>20%

For parasitological investigations samples are taken from the gills, surface and the inner opercula of the living fish. The skin, fins, eyes, gills, mouth- and gill cavity are studied for ectoparasites. The inner organs such as the digestive tract, liver, gall bladder, spleen, kidneys, gonads, heart and swim bladder are separated and transferred into saline solution and examined for endoparasites under a stereomicroscope. Isolated parasites were fixed in 4% formalin and preserved in 70% ethanol. The smears from the gills, surface and opercula were stained by using silver nitrate impregnation. The slides are rinsed and covered with 5% silver nitrate solution and impregnated for 30 minutes in the dark. The $AgNO_3$ is removed and the slides are covered with distilled water and exposed to ultraviolet light for 40-50 minutes. The health of fish populations isappreciated in accordance to parasitoloical parameters (prevalence, intensity and mean intensity). The Berger Parker Index characterizes the dominance of a respective parasite species within the sample BP=Nmax/N, with Nmax being the number of specimens of the most dominant species and N the total number of collected parasites within the sample.