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Risk assessment report for the integrity of Prut river's ecosystem





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RISK ASSESSMENT REPORT FOR THE INTEGRITY OF PRUT RIVER'S ECOSYSTEM

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CHAPTER I. GENERAL CONSIDERATIONS REGARDING ENVIRONMENTAL RISK ASSESSMENT

Cross-border cooperation in water resources policy is a proven tool to improve management of water resources in regions that share common resources. In the coming decades, climate change, human impact and increasing demand for water will be an additional problem, increasing the likelihood of conflict at the local level. For instance, unilateral measures regarding the adaptation to water scarcity (generated by hydraulic structures on rivers) and climate change may lead to increased competition for water resources.

In addition, the amendment of farmland productivity can lead to a number of new or modified agricultural systems, including those based on intensive methods. The latter, in turn, can increase the impact on the environment, which will result in biodiversity loss, the formation of deposits of sediments, erosion and soil degradation.

In this respect, protection of water resources is an important strategy, economically efficient in solving any problems related to water quality. Although for some countries, protection of water resources is a common practice, however, worldwide requires new approaches, innovative water quality management. Such an approach consists of the implementation of security plans for water resources, aimed at effective management of resources, beginning with the watershed and ending with the consumer, by **evaluation of the potential risk**.

To implement policy based on the principles of integrated management of water resources requires a better coordination between various state agencies; it is also necessary the legal and institutional framework review.

The change of the status and, especially, of the natural water quality and biodiversity will affect all the stakeholders with responsibilities in the management of water resources, including the final users. For this reason, all stakeholders should be informed about the potential impact on the system, to enable them to take the necessary decisions and to prepare to cover associated costs. For example, for the regulations regarding the wastewater treatment will be necessary to employ a common strategy based on the ability of surface water cleaning, which presents a clear trend for reduction.

Construction and operation of the drinking water and sewage treatment plants should be regularly reviewed, particularly in the vulnerable areas, in order to ensure or enhance their degree of safety and capacity to deal with unspecified modifications of the leakage.

The success of the environmental assessment and prediction of aquatic ecosystems status depends on a suitable database on water quality, biodiversity, risk factors, natural factors of socioeconomic status in the watershed.

Unfortunately, in the recent years there was a tendency to merge the observation networks, reducing the number of physical, chemical and biological parameters investigated. The parameters that, in the opinion of experts, do not refer to indicators of toxicity, but without which it is impossible to assess the processes of aquatic ecosystems functionning, their level of trophicity, the degree of vulnerability, self-restoration and self-purification are excluded from the list. Relatively short strings of data do not provide a complete picture of the variability and make it difficult to detect changes, while rows of data for long periods of time allow highlighting the recent trends and extreme values within a broader context.

Earlier were developed numerous logical and statistical models for evaluation of the stability of aquatic ecosystems, which included hydrological, geomorphological, hydrochemical, hydrobiological parameters and also various integrated indicators, such as pollution index, the selfcleaning index. Aquatic ecosystems stability assessment was based on a broader spectrum of mathematical methods, formulas and procedures for assessing the correspondence degree of models. Currently, the principles of monitoring, assessment and management of aquatic resources are set out in the EU Water Framework Directive. At present there is a range of acute environmental problems generated by water pollution, drainage system changes, greater diversity of human activities, but also some natural phenomena (floods, droughts, landslides, etc.), with implications on the environmental condition and population way of life. Concentrations of pollutants in surface water vary depending on the season, the highest being in the warm seasons.

Water pollution is caused, in most cases, by the communal household sector (insufficiently treated wastewater, discharges of untreated water from the communal system, inadequate solid wastes management), agriculture (manure accumulated, deposits of pesticides, etc.) and energy, such as oil depots, petrol stations and other sources of continue pollution. Along with these indicators for which were found values that could be compared with the regulations, in the river water were also found substances such as fuel oil, industrial reagents, oils, fats, viruses, bacteria, parasites, heavy metals, etc.

Therefore, the first step to take is an assessment of the existing situation. Based on this analysis and security requirements a security policy can be established. Security policy sets the basic rules for the protection against security incidents. Implementation of the security policy is essential to secure the information processing. If the personnel and resources of the organization are not really involved in, the security policy will remain a mere piece of paper before anyone lacks credibility (president of the organization, customers, auditors or the media).

In the present context, hazard is the probability of occurrence in a certain period of a phenomenon potentially harmful to humans and the environment. So the hazard is a natural or anthropogenic phenomenon, harmful for humans, consequences of which are due to exceeding the safety measures that any society imposes. Natural hazards are a form of interaction between man and the environment in which society adaptation thresholds are exceeded.

Vulnerability reveals how much the man and his assets are exposed to various hazards, indicating the level of damages that can be produced by a certain phenomenon and is expressed on a scale between 0 and 1, figure 1 expressing utter destruction of goods and the total loss of human life in the affected area. Environmental destruction increases the vulnerability. For ex., deforestation causes enhanced erosion and landslides, producing faster and stronger floods, and increased vulnerability of settlements and communication routes.

I.1. The logical framework of the risk assessment

Problem formulation

The initial phase is to formulate the problem and consists of the process of planning and evaluation that defines the feasibility, scope and objectives of the risk assessment, also providing stakeholders the opportunity to achieve consensus. This process includes examining of the scientific data, legal regulation issues and factors specific to studied ecosystem. Problem formulation identifies the potential risks of ecosystem, stressors, measurement and final target setting. The information is contained in a conceptual model that simulates how stressors affect the biological components (individuals, populations, communities, concerned ecosystem).



Problem formulation in the risk assessment

The steps of risk assessment in aquatic ecosystems:

The methodology of the environmental risk assessment has three stages:

- 1 screening assessment;
- 2 preventive quantitative risk assessment;

3 - detailed quantitative risk assessment.

An analogous structure was used in present research.

Useful information:

A. Background:

- a) The overview of the history of the studied ecosystem
- b) Presentation of the legislative regulatory acts applying to the studied ecosystem
- c) The ecosystem specificity.

B. Specific items:

a) Management objectives and action planning: management objective is to determine the factors that might generate major imbalances in the Prut River biocoenoses, and actions to restore natural balance

b) Current state of knowledge (previous monitoring reports, existing environmental impact assessments, adjacent land use categories, etc.)

c) Identification of contaminants/potential risk factors

d) Identification of ways of exposure to contaminants/risk factors

e) Identification of the potential receptors at level of ecological detail (habitat of major importance, rare, threatened or endangered species, etc.) - species inventory method

f) Definition and measurement of the limit values for contaminants/risk factors; setting the risk scenarios/hypotheses

g) Development of conceptual model (summary of site ecology - sources of contamination, dominant exposures and pathways of contamination, relevant trophic levels)

h) The strategy for Risk Assessment (choice of assessment methods includes: size of samples, sampling stations, statistical aspects; also contains the sampling and analysis plan based on one of four categories - direct measurements, modeling, interpretation or synthesis).

I.2. Legal protection of water

As a result of various human activities, the water courses undergo quantitative and qualitative changes, which may lead to environmental imbalances. The level of water pollution has greatly increased in recent decades as a result of economic development and rapid population growth. The efforts to prevent pollution and combat its effects are coordinated by the State, based on a legislation designed to protect the water resources.

To meet the requirements of the accession process and therefore in conformity with the *acquis communautaire* in the field of water, Romania adopted a series of laws aimed at regulating this sector:

- Law no. 17 of 7 August 1990 on the legal regime of internal maritime waters, territorial sea, contiguous zone and exclusive economic zone of Romania, republished in the Official Monitor no. 765/2002;
- The Order of the Minister for Waters, Forests and Environmental Protection no. 485 of 22 August 1995 on approval of the Regulation for organization and operation of the Alarm system in case of accidental pollution of waters in Romania, published in the Official Monitor no. 267/1995;
- Water Law 107/1996, published in Official Monitor of Romania, Part I, no. 244 of 8 October 1996 (governing wastewater discharge into surface waters), amended by Law 310 of 28 June 2004, published in the Official Monitor no. 584/2004;
- The Order of the Minister of Waters and Environmental Protection no. 756 of 11 March 1997 on approval of the Regulation on environmental pollution assessment, published in the Official Monitor no. 303/1997;
- The Order of the Minister for Water, Forests and Environmental Protection no. 275 of 11 April 1997 approving the Methodological Norms on the establishment of a special supervisory regime for non-compliance of the measures established to ensure conditions from the water management authorization, published in the Official Monitor no. 100 bis/1997;
- The Order of the Minister for Waters, Forests and Environmental Protection no. 276 of 11 April 1997 approving the Methodology regarding the plans for water restrictions and use during critical periods, published in the Official Monitor no. 100 bis/1997;
- The Order of the Minister for Waters, Forests and Environmental Protection no. 277 of 11 April 1997 approving the Normative on the content of the technical documentation necessary for obtaining water management approval and water management authorization, published in the Official Monitor no. 100 bis/1997;
- The Order of the Minister for Waters, Forests and Environmental Protection no. 278 of 11 April 1997 approving the Framework methodology for drawing up plans to prevent and control accidental pollutions, published in the Official Monitor no. 100 bis/1997;
- The Order of the Minister for Waters, Forests and Environmental Protection no. 279 of 11 April 1997 approving the Methodological norms on the location approval, published in the Official Monitor no. 100 bis/1997;
- The Order of the Minister for Waters, Forests and Environmental Protection no. 281 of 11 April 1997 approving the Procedure on the access mechanism to water management information, published in the Official Monitor no. 100 bis/1997;
- The Order of the Minister for Waters, Forests and Environmental Protection no. 282 of 11 April 1997 approving the Procedure concerning the participation of water users, riverans, and public in the consultation work, published in the Official Monitor no. 100 bis/1997;

- Law no. 171 of 4 November 1997 on the approval of the National Landscaping Plan (NSP) Section II: Water, published in the Official Monitor no. 325/1997;
- The Order of the Minister for Waters, Forests and Environmental Protection no. 1097 of 17 December 1997 approving: Technical rules on the methodology for the management and control of biological treatment process with activated sludge in the treatment plants for municipal, industrial and animal husbandry wastewaters, NTPA-003/1997; Guidelines establishing programs to harvest and analyze samples of wastewater NTPA-004/1997; The methodology for sampling wastewater effluents end NTPA-005/1997, published in the Official Monitor no. 47/1998;
- The Order of the Minister for Waters, Forests and Environmental Protection no. 1098 of 17 December 1997 approving the Normative regarding provision of equipment, materials and glassware of the profiled laboratories within the water management units, NTPA-006/1997, published in the Official Monitor no. 47/1998;
- The Order of the Minister of Waters and Environmental Protection no. 699 of 30 July 1999 approving the Procedure and competences for issuing approvals and authorizations for water management, published in the Official Monitor no. 476/1999;
- The Order of the Minister for Waters, Forests and Environmental Protection no. 811 of 6 September 1999 approving the Procedure for notification, published in the Official Monitor no. 572 bis/1999;
- Government Decision no. 472 of 9 June 2000 on some measures to protect the quality of water resources (sets payments for wastewater discharges, charges and penalties for exceeding the limit values laid down. These values are regularly updated and the list of indicators contains much of the 32 dangerous substances - approx. 67%), published in the Official Monitor no. 272/2000;
- Government Decision no. 964 of 13 October 2000 (updated in by G.D. no. 1.360 of 10 November 2005) approving the Action plan for water protection against pollution caused by nitrates from agricultural sources, published in the Official Monitor no. 526/2000;
- The Order of the Minister for Waters, Forests and Environmental Protection no. 1618 of 24 October 2000 on the approval of representative sections of the National surveillance system for water quality, published in the Official Monitor no. 26/2001;
- Government Decision no. 1212 of 29 November 2000 on approval of the Regulation for organization and functioning of the Basin Committees, published in the Official Monitor no. 44/2000;
- The Order of the Minister of Waters and Environmental Protection no. 325 of 21 March 2001 on the approval of the Technical Instructions for the application of the Government Decision no. 472/2000 on some measures to protect the quality of water resources NTPA 012 and for amending the Order no. 242/1990, published in the Official Monitor no. 152/2001;
- Law no. 192 of 19 April 2001 on fish fund, fishing and aquaculture, published in the Official Monitor no. 200/2001;
- The Order of the Minister of Waters and Environmental Protection no. 452 of 4 May 2001, and of the Ministry of Agriculture, Food and Forestry no. 105.951 of 8 May 2001 for approval of the Organization and functioning of the Commission and Support Group for the implementation of the Action plan for water protection against pollution caused by nitrates from agricultural sources, published in the Official Monitor no. 296/2001;
- The Order of the Minister of Waters and Environmental Protection no. 706 of 27 July 2001 approving the Regulation on the organization of the certification of units specialized in the preparation of studies, projects, construction, consulting in water management and technical documentation for obtaining approvals and permits for water management, published in the Official Monitor no. 565/2001;
- Government Decision no. 100 of 7 February 2002 approving Quality standards that must be met

by surface waters used for drinking, and Normative on methods of measurement and frequency of sampling and analysis of samples of surface water for the production of drinking water, published in the Official Monitor no. 130/2002, amended by Government Decision no. 662 of 7 July 2005, published in the Official Monitor no. 616/2005;

- Government Decision no. 118 of 7 February 2002 approving the Action programme for reducing the aquatic environment and groundwater pollution caused by the discharge of dangerous substances, published in the Official Monitor no. 132/2002;
- Government Decision no. 188 of 28 February 2002 approving the Norms on the conditions for waste water discharge into the aquatic environment, published in the Official Monitor no. 187/2002;
- Government Decision no. 201 of 28 February 2002 approving the Technical norms on the quality of the water for mollusks, published in the Official Monitor no. 196/2002;
- Government Decision no. 202 of 28 February 2002 approving the Technical norms on quality of surface waters that require protection and improvement to support fish life, published in the Official Monitor no. 196/2002, amended by Government Decision no. 563 of 26 April 2006, published in the Official Monitor no. 406/2006;
- Government Decision no. 188 of 20 March 2002 approving the Norms on conditions for waste water discharge into the aquatic environment, amended by G.D. 352/11.05.2005;
- Government Decision no. 459 of 16 May 2002 on the approval of the Norms of water quality of natural areas fit for bathing, published in the Official Monitor no. 350/2002;
- Law no. 458 of 8 July 2002 on Drinking water quality, published in the Official Monitor no. 552/2002, amended by Law no. 311/2004, published in the Official Monitor no. 582/2004;
- The Order of the Minister of Waters and Environmental Protection no. 1141 of 6 December 2002 approving the Procedure and competences for issuing approvals and authorizations for water management, published in the Official Monitor no. 21/2003;
- The Order of the Minister of Waters and Environmental Protection no. 1146 of 10 December 2002 approving the Normative regarding the benchmarks for classification of surface water quality, published in the Official Monitor no. 197/2003;
- Government Emergency Ordinance no. 202 of 18 December 2002 on Integrated management of the coastal zone, published in the Official Monitor no. 965/2002;
- The Order of the Minister of Waters and Environmental Protection no. 1241 of 16 January 2003 approving the Procedure for modification or withdrawing approvals and authorizations for water management, published in the Official Monitor no. 104/2003, replaced by the Order nr. 15/2006, published in Official Monitor no. 108/2006;
- The Order of the Minister of Waters and Environmental Protection no. 1406 of 3 March 2003, and of the Ministry of Health and Family no. 191 of 7 March 2003 approving the Methodology for rapid assessment of risk to the environment and human health, published in the Official Monitor no. 213/2003;
- The Order of the Minister of Waters and Environmental Protection no. 35 of 2 April 2003 approving the Methods of measurement and analysis used to determine the priority/priority hazardous substances from wastewaters and surface waters, published in the Official Monitor no. 305/2003;
- The Order of the Minister of Agriculture, Forestry, Waters and Environment no. 1069 of 18 December 2003 approving the Methodology of activities specific to water management (responsible - National Administration *Romanian Waters*;
- The Order of the Minister of Agriculture, Forestry, Waters and Environment no. 1072 of 19 December 2003 approving organization of the support national integrated monitoring for surveillance, control and decisions to reduce the contribution of pollutants from agricultural sources into groundwaters and surface waters, and for approval of the corresponding supervisory and control Programme, and procedures and instructions for the assessment of monitoring data regarding the pollutants from agricultural sources in surface waters and

groundwater - (National System for Integrated Monitoring, managed by the National Administration *Romanian Waters*) published in the Official Monitor no. 71/2004;

- Government Decision 898 of 10 June 2004 approving the Instructions regarding the exploitation of groundwater and areas of interface between fresh and salty waters, published in the Official Monitor no. 598/2004;
- Government Decision no. 974 of 15 June 2004 for the approval of the Norms on surveillance, sanitary inspection, and monitoring of drinking water quality and Procedure of the sanitary authorization of production and distribution of drinking water, published in the Official Monitor no. 669/2004, amended by G.D. no. 342/2013, published in the Official Monitor no. 351/2013;
- Order no. 15 of 11 January 2016 of the Minister of Environment and Water Management for approval of the Procedure of temporary suspension of the authorization for water management and Procedure for modification or withdrawing approvals and authorizations for water management, published in Official Monitor no. 108/2006;
- The Order no. 161 of 16 February 2006 of the Minister of Environment and Water Management for approval of the Normative on surface water quality classification to determine the ecological status of water bodies, published in the Official Monitor no. 511/2006;
- Law no. 265 of 29 June 2006, published in Official Monitor no. 586/2006 approving the Government Emergency Ordinance no. 195 of 22 December 2005 on Environmental protection (*Environmental Protection Law Chapter IX: Protection of waters and aquatic ecosystems*, published in the Official Monitor no. 1196/2005);
- The Order no. 132 of 29 January 2007 of the Minister of Administration and Interior approving the Methodology to elaborate the Plan of analysis and hedging of risks, and Framework Structure of the Plan of analysis and hedging of risks, published in the Official Monitor no. 79/2007;
- Government Decision 210/2007 of 28 February 2007 amending and supplementing certain normative acts transposing the *acquis communautaire* in the field of environmental protection, published in the Official Monitor no. 187/2007.

In the Republic of Moldova was elaborated the **Environmental Strategy** for the years 2014-2023, and the **Action Plan** for its implementation, approved by Government Decision no. 301 of 24.04.2014.

The overall objective of the Strategy lies in creating an efficient system of environmental management to improve the quality of the environment and ensure the population the right to a clean natural environment, healthy and sustainable, in agreement with EU Directives.

By the Order of the Minister of Environment no. 40 of 27.5.2014 was established the **Group to monitor the implementation of specific objectives**, and set its tasks in order to ensure the monitoring and reporting mechanism for the implementation of the Strategy.

To ensure the good governance conditions and efficiency of institutional and managerial potential in the field of environmental protection, to achieve the environmental objectives, the legislation of the Republic of Moldova was harmonized with EU directives:

According to Government Decision no. 932/11.20.2013 was approved the *Regulation on monitoring the status of surface and groundwaters*, which includes a complex system of multiannual quantitative and qualitative evaluation of waters using standardized methods of sampling, analysis and synthesis, for the sustainable management and capitalization of the aquatic resources.

There were established the types of monitoring (surveillance monitoring, operational monitoring and investigation monitoring) and described the procedures and technical measures for monitoring of the water condition.

By the *Regulation on quality requirements for the surface waters* (Government Decision no. 890 of 11.12.2013) were set out the quality requirements and grade the quality classes of the surface waters.

Surface water classification is based on the results of monitoring water quality and provides a classification system of surface water quality in five categories:

- *Quality class I* (very good): surface water where is no alteration (or there are minor alterations) of the values of physico-chemical elements and biological quality. Synthetic pollutants concentrations have no influence on aquatic ecosystems and do not harm human health. Surface waters corresponding to quality class I are proper for all types of use. For its graphic representation, the blue color is used;

- *Quality class* II (good): surface waters slightly affected by human activity, but which can provide all utilities properly. The functioning of aquatic ecosystems is not affected. The simple treatment methods allow the use of water for drinking purposes. For its graphic representation, the green color is used;

- *Quality class* III (moderate): surface waters whose physicochemical and biological quality values moderately deviate from natural fund of water quality due to human activities. Moderate signs of disturbing the functioning of the ecosystem are recorded, and the conditions required for salmonids can be assured no longer. Simple treatment is not sufficient for the use of water for drinking purposes, normal treatment methods being applied. For its graphic representation, the yellow color is used;

- *Quality class* IV (moderately polluted): surface waters showing evidence of major alterations of the physico-chemical and biological values of quality in the functioning of aquatic ecosystems. The conditions for the cyprinids can be assured no longer. The waters do not meet the drinking water conditions without intensive, advanced treatment. For its graphic representation, the orange color is used;

- *Quality class* V (polluted): surface waters showing evidence of severe alterations of the physicochemical and biological values of quality in the functioning of aquatic ecosystems. Biological components, particularly fish, are damaged and water can not be used for drinking purposes. For its graphic representation, the red color is used.

It was elaborated and adopted the *Law no. 86 of 29.5.2014 on Environmental Impact Assessment* in accordance with Directive 85/337/EEC of 27 June 1985 on the assessment of the effects of certain public and private projects on the environment. The law establishes the principles, procedures and how to conduct the environmental impact assessment in Moldova and neighboring states.

Central public administration authorities have initiated the establishment of the environmental protection functions within their institutions. The Defence Ministry has created the Service for ecology and environmental protection, whose mission is planning, coordination, organization, monitoring and verification of the activities for ecological safety and environmental protection in the National Army.

According to Government Decision no. 775 of 4 October, 2013 "On the districts borders of hydrographic basins and sub-basins, and special maps in wich these are determined", in order to ensure implementation of the mechanism for managing water resources, the borders of two River Basin Districts: Dniester (74% of the population lives in this district), and the Danube-Prut and the Black Sea (26% of the population) were established.

In order to assure the protection and quality of water resources, it was initiated the implementation of the Water Law. Thus, by Government Decision no. 250 of 03.04.2014, the nominal composition of the Committees Dniester, and the Danube-Prut and the Black Sea river basin districts, which are advisory bodies in the management and protection of water resources in the basin Dniester and Prut River was approved. It was drafted the project of the Management Plan of Dniester River Basin District, and Management Plan of Prut River Basin District, that are currently under public consultation.

There were developed and approved the management plans for the Ramsar wetlands "Lower Dniester" and "Unguri Holoșnița".

It was launched the project "East-Avert - prevention and protection against floods in the upper basins of the rivers Siret and Prut, by applying a system of monitoring with autonomous stations", financed by the EU, under which started the works of rehabilitation of the dam at

Hydrotechnical node Stânca-Costești and development of the information system for tracking floods and node buildings behavior. The elaboration of a feasibility study on flood risk and hazard and flood risk maps, and plans for flood risk management are in progress.

It was approved a new *Strategy for water supply and sanitation in the Republic of Moldova* until the years 2028 (Government Decision no. 199 of 03.20.2014). In order to ensure implementation of the Strategy were developed and approved two laws: *Guidelines for the elaboration of the water supply and sanitation in the Republic of Moldova* and *Guidelines for feasibility studies regarding the water supply and sanitation infrastructure*. The Law no. 303 of 12.13.2013 on Public Water Supply and Sewage entered into force on 14 September 2014.

Specific objectives regarding the management of water resources consist of: a) improve the quality of at least 50% of surface waters and the implementation of the watershed management;

b) ensuring the access, by 2023, of about 80% of the population to systems and services for water provision, and about 65% of the population to sewerage systems and services.

The Strategy development was also dictated by the political vector of European integration of the country, the current needs for harmonization of the national legislation with European Union Directives, and the need to implement a framework policy on biodiversity conservation and rational use of natural resources of flora and fauna.

The Republic of Moldova still faces many problems in the field of biodiversity conservation. Interest in natural resources does not decrease, their use is often irrational, and many of the national targets for protecting biodiversity need to be settled. Current conditions (climate change, habitat fragmentation, pollution, extinction of species, etc.) and the socio-economic development paradigm of the Republic of Moldova calls for a more realistic approach to the role of biodiversity for the national economy and boost its protection processes.

In the submission process there is a draft of the Government Decision on the approval of the *Biological Diversity Strategy of the Republic of Moldova* for 2015-2020 and the Action Plan for its implementation which is prepared in accordance with the Programme of the Government of the Republic of Moldova "European Integration: Freedom, Democracy, Welfare "(2013-2014), Chapter "Environmental Protection", and the provisions of the Environment Strategy for the years 2014-2023, and the Action Plan for its implementation and the chapter "Environment" of the *Association Agreement* between the Republic of Moldova, on the one hand, and the European Union and the European Atomic Energy Community and the Member States, on the other hand.

I.3. Glossary of terms

The concepts used in this report have the following meanings:

water status monitoring - evaluation system of the physical, chemical, biological and microbiological parameters of water, depending on the natural conditions and man;

monitoring programs - tool for coherent and comprehensive assessment of the of water resources state, so as to facilitate the prognosis, development and approval of water basins management plans and analysis of the progress in their implementation;

indicators of water quality - requirements for water quality, expressed by the concentration of a particular physicochemical parameter, group of physico-chemical or biological parameters, which will not be exceeded in order to protect human health and the environment;

national monitoring system - the system by which the State permanently supervises the state of water resources and human impact, based on parameters and indices with spatial and temporal covering, which provide the information needed to compile the strategy, measures to prevent the anthropogenetic consequences, natural calamities, and restoration of the ecological situation;

significant upward trend – a statistically and environmentally significant growth of of a pollutant, group of pollutants or indicator of pollution in water concentration, for wich a reversal trend is considered necessary;

water quality requirements - all physico-chemical, biological and bacteriological characteristics, expressed quantified, which allow the classification of a sample in a category, to serve a specific purpose;

water classification system by quality - established requirements for surface waters quality, set in five classes of surface water quality, water quality indicators list, maximum numerical value of the parameters and covered parameters list;

quality class of surface waters - the level of water quality, established for a specific water body, tracking the actual water utilities;

water quality indicator - the physical value, chemical substance, hydrobiological, microbiological, virological or helminthological component, and other indicators of water quality, included in quality classes;

regulated indicator - an indicator of water quality to be obligatorily taken into account in the assessment of surface water quality;

natural background - physico-chemical, hydrobiological and hydro-morphological conditions of surface water bodies, associated with the undisturbed conditions (undistorted by anthropogenic activities) of the category of surface water (river, lake) and basin/sub-basin;

environmental risk - the probability of the ecosystem modifications causing its degradation, destruction or passage into a state which threatens public health, and (or) the loss of its domestic importance;

environmental risk assessment - scientific reasoning based on probability and amplitude of adverse changes in the ecosystem or its components in the circumstances of a particular human impact;

environmental risk probability - the probability of an event that would harm the ecosystem, therefore being classified as undesirable for the given ecosystem. The probability is evaluated for a certain or several time intervals, e.g., 1 year, 3 years, 10 years;

risk indicators - characteristics of ecosystem and its components, based on which the appearance and dimensions of risk are judged.

CHAPTER II. THE COMPLEX MONITORING OF THE PRUT RIVER'S WATER QUALITY AND HYDROBIOCOENOSES STATE

Given the logical framework and conceptual model discussed above, in the project MIS ETC 1150 was carried out a complex monitoring of water quality and river Prut hidrobiocoenoses state. Monitoring and environmental state predicting are essential components of the environmental risk assessment. Water quality assessment has been carried out in accordance with the *Regulation on environmental quality requirements for surface waters* (approved by Government Decision no. 890/11.12.2013, published in the Official Monitor of the Republic of Moldova no. 262-267/22.11.2013, art. no. 1006) and Prut-Bârlad SH Management Plan 2016-2021 under the Water Framework Directive 2000/60/EC (on line).

II.1. Evaluation of physical and chemical parameters of the Prut River's water

Chemical composition and quality of Prut water is determined by natural and anthropogenic factors. Among natural factors, the structure and composition of rocks, soils, topography of watershed, structure and abundance of hydrobionts communities, etc. are included.

Dissolved oxygen. Dynamics of the dissolved oxygen content was relatively satisfactory for hydrobiont development, its concentration ranging within 3.88-13.82 mg/l. In less than 10% of cases, the content of dissolved oxygen was lower than 5 mg/l, which corresponds to the class of quality III (dangerous for planktonic organisms); in most of cases it corresponded to the classes I-II.

Suspensions. The quantity of suspensions in the Prut River varied from 1.2 mg/l to 350.4 mg/l. Its right tributary – Jijia River (by the discharge of waste waters) increases the content of suspensions in the Prut River on the Leuseni-Cîşliţa-Prut sector by ten times in the Prut River. The increase of suspension content up to 80-100 mg/l provokes the suppression of planktonic organisms development in the Prut River.

Biochemical consumption of oxygen (CBO₅). The values of CBO₅ varied within 1-2.72 mg O_2/I . Thus, according to the *Regulation on environment quality requirements for the surface waters,* the content of dissolved oxygen and the values of biochemical consumption of oxygen indicated the classes of water quality I-II.

Chemical consumption of oxygen. According to the values of CCOMn (indicator of the content of easily degradable organic compounds), the water coresponded to classes of quality I-III, and <15% cases coresponded to IV, but according to the values of CCOCr (indicator of more persistent organic compounds) coresponded to classes of quality II-IV, and <1% to class I. The correlation coefficient between the CBO₅ and CCOCr proved a low intensity of self-cleaning processes (in 90% of cases it was below 0.15) was found.

Mineralization and main ions. The mineralization (salinity) of investigated waters has oscillated between 298-940 mg/l, what corresponds to classes of water quality I-III, and had an obvious tendency to increase along the Prut River. In most cases, the Prut river water was classified as bicarbonate-sulfate- magnesian class, pH values ranging from 7.8 to 8.6.

The Prut waters, taking in account the composition of main ions, corresponded to the quality requirements, which must be met by the drinking water, waters for pisciculture and aquaculture. The content of chloride and magnesium ions in the investigated ecosystems corresponded to class I of water quality, of sulfate ions – to classes II-IV. The content of sodium and potassium ions corresponded to classes II-IV, but in about 5% of samples – to class V, which changes the water properties, especially its suitability for irrigation.

In general, the dynamics of the main ions revealed a slow evolution, with some leaps in the lower sector of the Prut River, which presented no obvious risk for the diversity of aquatic organisms. The discharge of waste waters in the Prut floodplain, penetration from unauthorized landfills into the river bed during heavy rains periodically increase the content of some ions, which affects the properties of water as a source of irrigation and drinking water. **Nutrients.** The content of nutrients is one of the most important indicators of water quality, which determines both the development of several aquatic organisms, as well as trophicity level, intensity of production-destruction processes within the aquatic ecosystems. The dynamics of nitrate and nitrite nitrogen corresponded to water quality classes I-II, of ammonium nitrogen – to classes I-III, and of total nitrogen – to classes I-V. The highest values were recorded during the floods.

The dynamics of the total nitrogen also dependends on the increase of the content of organic nitrogen: the leaps up to class of water quality V were determined by the huge values of organic nitrogen (in 2013 - up to 15-25 mg/l) in the river lower sector.

The concentrations of mineral phosphorus in the Prut River water corresponded to classes of quality I-II. In most of cases the dynamics of total phosphorus fits to water quality classes I-II, but in about 4% of cases – to the class III, as a result of the increase of the content of organic phosphorus.

II.2. Monitoring the structural and functional characteristics of the main communities of aquatic organisms that inhabit the river Prut

II.2.1. Bacterioplankton

According to the total number of bacterioplancton, the water quality corresponded to quality classes: I-II (45%); III (27%), IV (15%), V (13%).

With regard to the average values of the number of ammonifying bacteria, the water of the Prut River referred to the class "moderately polluted". However, periodically, particularly in the summer time, the number of ammonifying bacteria indicates "polluted" and "very polluted" water, especially downstream of urban waste waters discharge.

The density of phenolytic bacteria indirectly indicates the river pollution with phenols. According to the classification of water quality based on the density of phenolytic bacteria, the water of the reservoir Stânca-Costești and Prut River referred to the class "sufficiently clean" (3a) and "moderately polluted" (4b).

The density of petrolytic bacteria mostly depends on the degree of water pollution with petroleum substances. According to the classification of water quality based on the density of petrolytic bacteria, the water of Stânca-Costești reservoir and Prut River referred to the class of quality 4a ("moderately polluted"). On the Prut River, at Sculeni, Leova, Cahul, and Giurgiulești stations, where spots of petroleum substances often appear at water surface, the density of petrolrezistent bacteria counts up to 1500-1800 cells/ml and the water quality corresponds to the class 5a ("very polluted").

The number and the high production of total bacterioplankton, as well as the number of saprophytic bacteria denote a pollution of the Prut River and Stânca-Costești reservoir, mainly with organic compounds, which represents an evident risk for the water quality and hydrobiont diversity.

II.2.2. Phytoplankton

In the phytoplankton composition from the middle sector of the Prut River in the years 2012-2015 were identified 88 species and interspecific taxa, including: Cyanophyta - 12 Chrysophyta - 1 Bacillariophyta - 39, Euglenophyta - 7 and Chlorophyta - 29.

The diatoms identified mostly belong to Pennatophyceae class, being represented by genera *Navicula, Synedra, Nitzschia* and *Cymbella*, the dominant species being *Cyclotella kuetzingiana, Synedra acus* var. *acus, Melosira granulata* var. *granulata, Nitzschia palea* var. *palea*, and *Nitzschia acicularis* var. *acicularis*.

In the composition of the phyllum Chlorophyta, the main role in forming of taxonomic diversity rests to the genera *Scenedesmus, Monoraphidium, Pediastrum* and *Tetraedron*, and the dominant complex is consisting of species of *Actinastrum hantzschii* var. *hantzschii*,

Monoraphidium komarkovae, Monoraphidium contortum, Tetrastrum triangulation, Scenedesmus quadricauda var. quadricauda and Coelastrum microporum.

In the composition of the phyllum *Cyanophyta*, more representative was the genus *Oscillatoria*, and the dominant complex is consisting of species of *Synechocystis aquatilis*, *Aphanizomenon flos-aquae* f. *flos-aquae*, *Oscillatoria planctonica*, *Merismopedia tenuissima* and *Oscillatoria lacustris*.

The species *Merismopedia tenuissima* is widespread in the summer and autumn. In recent years it is quite common in the phytoplankton composition and regularly develops in large quantities. The species *Synechocystis aquatilis*, by its significant development, sometimes causes "water bloom."

The quantitative parameters of the phytoplankton attest well pronounced seasonal differences, conditioned by the share of certain species of algae in the phytoplankton composition, and by influence of different hydrological, hydrochemical, and hydrobiological factors upon them.

Water transparency of Prut river is higher in the middle sector than in the lower sector, with variations between 100-250 cm in all seasons. Sometimes, in spring and summer, water transparency is low (15-75 cm at Sculeni). The process of photosynthesis occurs with higher intensity at a depth of 25-50 cm, where the highest values of phytoplankton primary production were determined.

So, when phytoplankton biomass reaches high values, the intensity of photosynthesis is often lower than during a weak development, due to different productivity of algae species. Reducing of the photosynthesis intensity, with the increasing phytoplankton biomass, may be subject to a number of factors, among which reducing the concentration of nutrients, reducing transparency, and respectively of the euphotic layer due to higher biomass, the worsening of the algae physiological condition and reducing their production capacity thereof, supersaturation with oxygen due to the intense photosynthesis, metabolites influence upon algal cells.

In all the seasons, the values of organic matter destruction have exceeded those of phytoplancton production, in most cases being lower than 1 (0.504-74.99 g O_2/m^{-2} 24h). In accordance with A/R ratio, the water quality of investigated water bodies varied from class I to V.

According to phytoplankton biomass and primary production values, the middle sector of the Prut River belongs to the trophicity category eutrophic, periodically mesotrophic.

Saprobic index values, calculated based on quantitative parameters of indicator species from phytoplankton composition, which in excess of 56% are typical β -mezosaprobic, ranged 1.36-2.87, in most cases within the β -mezosaprobic area, corresponding to quality classes II-III.

Analysis of multiannual data reveals that, in at least 18% of cases, the phytoplankton biomass corresponded to quality class II, in 23% to III, in 45% to IV, and in 14% to class V.

II.2.3. Zooplankton

The habitat conditions in the river Prut are not favorable for the development of zooplankton due to the water flow in the upper sector and water turbidity in the medial and lower sectors. Since the zooplankton structure in running waters is formed by the species of floodplain tributaries and stagnant water species, the pollution of small rivers and the drainage of floodplains are directly causing loss of zooplankton biodiversity in the Prut river basin. The highest species richness was recorded in the stagnant ecosystems of the basin and in the lower Prut, where the specific diversity is increased by the entry of alien species from the wetlands of the river, and by the reversed flow of the Danube during floods of spring and summer (high density of rotifers at Giurgiuleşti).

In the structural complex of the zooplankton from the Prut River Basin in recent years have been identified 106 taxa: rotifers - 47%, copepods - 30%, and cladocerans - 23%. The highest species diversity in this community has been registered for genera *Brachionus*, *Filinia* and *Asplanchna* of rotifers, *Mesocyclops*, *Eurytemora* and *Acanthocyclops* of copepods, Bosmina, *Daphnia* and *Pleuroxus* of cladocerans. Most constant taxa were the larvae of copepods (nauplii and copepodites), the species *Brachionus quadridentatus*, *Keratella quadrata*, *Notholca squamula*, Filinia longiseta and Asplanchna priodonta of rotifers, Acanthocyclops vernalis, Mesocyclops leuckarti, Mesocyclops crassus, Eurytemora affinis, Metadiaptomus asiaticus and Eucyclops serrulatus of copepods, respectively Chydorus sphaericus, Daphnia cucullata, and Bosmina longirostris of cladocerans.

Representatives of one or two main groups of zooplankton (Rotatoria, Copepoda, Cladocera) are missing from the taxonomic structure of planktonic communities in many of the analyzed samples. Due to their broad ecological valence, juvenile stages of copepods (nauplii and copepodites) were recorded as a constant element of zooplankton in the Prut River. This phenomenon is common for rivers with high contents of suspended particles. The predominance of rotifers in the planktonic community is a sign of ecosystem trophicity increase.

The less representative is the group of crustaceans from the suborder Cladocera. Under favorable conditions, the numbers of different species of cladocerans can reach very high values (Giurgiulești August 2012-65.300 ind./ m^3). The decrease in the cladocerans specific diversity and numerical density (a trend of the last 25-30 years) has among its leading causes the untreated or insufficiently treated wastewater discharge, and also pollution of water bodies with pesticides and heavy metals.

The overall density of zooplankton as a separate parameter is not normally used to assess the trophicity of the aquatic ecosystems. It is always used together with the total biomass of zooplankton. The increase of hydrobionts biomass shows the increased trophicity of the water body.

The analysis of zooplankton community structure from the Prut River consisted of calculation of a range of parameters and biocenotic indices: density, biomass, production, frequency, dominance and saprobic index.

More than 72% of identified taxa are indicator organisms. The analysis of zooplankton species abundance as function of saprobity zone revealed that the $o-\beta$ -mesosaprobic indicators and β -mesosaprobic zones dominated in aquatic ecosystem of the Prut River.

The amplitude of the fluctuations of the saprobic index was highest at Cahul and Cîşliţa-Prut stations, this fact revealing the instability of river ecosystem in the area.

According to the values of saprobic index of zooplankton community, the quality of investigated waters corresponded to classes I-II.

II.2.4. Zoobenthos

Benthic invertebrates (approx. 200 taxa) have been sampled during May 2012-March 2015 from the shallow waters (depths up to 1.2 m) of the Prut River, including the Stânca-Costești reservoir.

Their diversity depends on various ecological conditions: hydromorphological, hydrochemical, type of substrate, and level of anthropogenic load. Taking into account the number of recorded species, the best environmental conditions were found at Braniste and Sculeni stations.

Theodoxus transversalis is a rare species included in the *Red List* of **IUCN** (International Union for Conservation of Nature). Only empty shells of the species were sampled in the previous decade, but in the summer of 2013 ten individuals were found alive. This is an important fact for monitoring and preservation of *Theodoxus transversalis*, since each population of this rare species and, respectively, their habitats need protection.

The highest number of rare species was found at Teţcani, where three of the biggest Ephemeroptera species from Prut: *Ephemera vulgata, Polymitarsis virgo* and *Palingenia longicauda* were also collected.

At the Tetcani and Braniste stations, species characteristic for clean zones of the aquatic ecosystems were registered – 10 species of Ephemeroptera and 11 species of Trichoptera. These stations showed the highest diversity of substrates: stones, gravel, sand, clayey sand, silt, macrophytes, sunken trees, which, together with the absence of the adverse effects of Stânca-Costești reservoir and waste water discharges, contribute to the creation of most favorable environmental conditions for a high diversity of species of benthic animals.

The least favorable sites for the benthic biodiversity are located near the dam of Stânca-Costești reservoir, downstream of the confluence with the Jijia River (Leușeni) and at the mouth of the Prut River (Giurgiulești). In the last case, the impact of Giurgiulești port is a strong one: besides the presence of unfavorable hydrochemical conditions, there is another factor which affects the development of benthic invertebrates, and namely the regular cleaning of the river fairway.

The ecosystem of Prut River is faced at Cîşliţa-Prut station with both chemical and biological pollution. In a range of samples, species such as *Corbicula fluminea* and *Sinanodonta woodiana* constitute a significant part of the macrozoobenthos, according to their density, as well as biomass. *Corbicula fluminea* forms 40-46% (40-200 ind./m²) of total density of molluscs, reaching to 196.44 g/m², and *Sinanodonta woodiana* - more than 70% (260 g/m²) of total biomass of malacofauna per station. The invasive Asian species, *Sinanodonta woodiana*, which was firstly recorded in the lake Beleu, also appeared at Cîşliţa-Prut, Cahul and Goteşti stations. Other often met alien species is *Dreissena bugensis*.

At Criva station, *Unio crassus* – native bivalve reofile mollusk only formed 5% (440 ind/m^2) of the total density. The species *Unio crassus* is assessed as endangered and rare, being included in IUCN (International Union for Conservation of Nature) **Red List**. The decline of the species within Europe over the last 45–60 years is estimated as being higher than 50%, and the threats upon this species are known and will continue to increase.

A special attention was given to the representatives of class *Oligochaeta*, taking in account their role as indicator species of aquatic ecosystems pollution with organic compounds.

The total number of invertebrate taxa has reached 174, including: 20 taxa of Annelida, 40 of Chironomidae, 16 of Crustacea, 13 of Ephemeroptera, 15 of Trichoptera, 21 of Gastropoda, 17 of Bivalvia and 32 taxa of other groups.

The highest number of rare species has been remarked at Teţcani and Branişte stations, among them: *Theodoxus transversalis, Pisidium moitesserianum, Ephemera vulgata, Anabolia furcata, Thienemanniella clavicornis, Anabolia laevis, Caenis macrura, Aphelocheirus aestivalis, species of Simuliidae.*

Also, there have been recorded 7 species of *Ephemeroptera* and 11 species of *Trichoptera* at the Braniște station. At the station Sculeni, only one species of *Plecoptera - Taeniopteryx nebulosa* was recorded. In the model stations, the value of EPT index (*Ephemeroptera, Plecoptera, Trichoptera*) included 13-15 species.

Water quality was expressed using the **Saprobic index** calculated by different methods (Pantle-Buck and Zelinka-Marvan).

Parameter	Phytoplancton	Zooplancton	Zoobenthos
Total number of species and varieties	131	56	174
Share of indicator taxa	56.5%	72%	50%
Saprobic index	1.44 - 3.17	1.15-2.50	1.30 - 3.65
Dominant saprobity	β - mesosaprobic	<i>o</i> –β-mesosaprobic	β -mesosaprobic
zone	β -o and o- β mesosaprobic	β -mesosaprobic	α- mesosaprobic

Application of the saprobity system: phytoplancton, zooplancton and zoobenthos

II.2.5. Prut River ichthyofauna (state and threats)

According to the literature published to date, the **ichthyofaunistic diversity** of indigenous representatives in the Prut River continues to fall dramatically, largely because of the increasing anthropogenic pressure. A significant reduction of the migratory and semi-migratory fish species (acipenserids, clupeidae, salmonids, percidae, anguillidae, some cyprinids), species vital-dependent on the floodplains (crucian carp, tench, umbra, eel, etc.) and those specific for areas with higher

altitudes (grayling, indigenous trout, minnow, afterbarbe, European bullhead, loach, etc.) was noticed.

The investigations carried out on the ichthyofauna in the Prut river basin, over the years 2010-2014, established a componence of 53 fish species assigned to 11 families and 8 orders: Order Acipenseriformes, Family Acipenseridae (1 species); Order Clupeiformes, Family Clupeidae (1 species); Order Esociformes, Family Esocidae (1 species); Order Cypriniformes, Family Cyprinidae (26 species), Family Balitoridae (1 species), Family Cobitidae (5 species); Order Siluriformes, Family Siluridae (1 species); Order Gadiformes, Family Lotidae (1 species); Order Gasterosteiformes, Family Gasterosteidae (2 species); Order Sygnathiformes, Family Sygnathidae (1 species); Order Perciformes, Family Percidae (6 species), Family Gobiidae (5 species), Family Centrarchidae (1 species), Family Odontobutidae (1 species).

In the spring of 2015 it was identified a new species for the river Prut - *Benthophilus nudus* (the Pontic swelling bare).

Prut River still hosts a significant number of species, some endemic, particularly rare and protected at both national and international level.

This shows the great importance of this river macroecosystem for the conservation of the international ichthyofauna diversity and requires urgent protection measures and rebuilding of natural habitats, as an inalienable component of ensuring a high biodiversity.

Now virtually disappeared (or are sporadically met) representatives of the families: Petromyzontidae, Acipenseridae, Salmonidae, and Cottidae. The most important causes of the quasitotal extinction of sturgeons and salmonids is the river hydrobiotope pronounced alteration (by fragmentation, silting and pollution), and contingents decimation by fishing (especially during the reproductive migrations).

Besides the typical reofile species were affected lacustrine and swamp stenobionte species populations, such as crucian carp, tench, umbra, etc. which were vitally dependent on habitat of ponds and small meadow lakes (subject to massive draining in the years 70s of the XXth century).

The diversity of fish species in the Prut river basin, thanks to the pronounced hidrobiotopic diversity and anthropogenic fragmentation, is not evenly distributed across sectors. The characteristic fish zoning of a river anthropogenically unaffected, with habitats well defined (such as trout, and grayling zones, broad snout, barbell, and carp zone), has undergone major changes in the current intensification of human pressure. After construction of the dam reservoir Stânca-Costești (1976), the aquatic ecosystem passed in the category of heavily modified water basins. As a result of the considerable anthropogenic interference, following ecological zones can be emphasized with five major habitat types (often fragmented in microzones).

- 1. Thresholds, fords and sand banks area, with fast flow, sand or gravel substrate It has an intermittent spatial distribution, being predominantly located in the medial sector, downstream of the dam Stânca-Costești until town Ungheni region. Typical representatives are common schneider, gold spined loach, bleak, Kessler's gudgeon, broad snout, chub, barbel etc.
- 2. **River bed area with slow flow, deep and less transparent water** It is the most extensive, located in both sectors up to the confluence with the Danube. Typical representatives are: cat fish, bream, rapacious carp, zander, vimba bream, common roach, barbel, chub, etc. White bream, ide and sabrefish are characteristic for the lower sector of the Prut only.
- 3. **Stânca-Costești reservoir area** Typical representatives are bream, roach, perch, rapacious carp, zander, bleak, cultured carp, Asian cyprinidae, etc.
- 4. **The area of natural lakes Manta and Beleu** Typical representatives are: silver crucian carp, roach, white bream, bleak, common and the Danube ruff, stone moroko, cultured carp, Asian cyprinids. In the reproductive and floods period, ichthyofauna picture is strongly influenced by the Danube and Prut ichthyocoenoses (when significant amounts of pike, bream, zander and catfish, ide (few individuals), etc. are entering).

5. The flooded and isolated areas with temporary or permanent water cover (supplied during floods from the river Prut). Typical representatives are: crucian silver carp, pumpkinseed, bitterling, stone moroko, perch, bleak, roach, pike offspring, etc.

Ichthyofauna diversity within an area can change depending on the season, nictemeral period, the hydrological regime, the position within the area etc. Usually, in riverbed ichthyocoenoses, the share of reofile fish species increases; in the holes of gravel pits and slow portions are installing the reophileous-stagnophileous species characteristic for lower altitudes; in the stagnant waters ichthyocoenoses increases the share of limnophileous fish species.

But whatever the habitat characteristic, on the background of active processes of fragmentation, limnification and silting is found the numerical supremacy of the representatives euritopic, generalist, and highly competitive, such as crucian silver carp, white bream, roach, perch, bleak etc., which successfully increased their livestocks everywhere.

Among the species euribiont, generalist, opportunistic and numerous in the macroecosystem of Prut River can be mentioned: bleak - abundant in both sectors of the river Prut (medial and inferior) and in all fish zones (I, II, III, IV, V); perch - becomed especially numerous in the medial sector (II, III) and in some flooded micro-depressions and dead branches isolated from river floodplain (V); roach - species common to all fish zones of river Prut (II, III, IV, V); crucian silver carp - invasive alien species, ubiquitous, particularly numerous in shallow, stagnant and rich in aquatic vegetation (IV, V); white bream and Eurasian ruffe - have sharply risen their livestocks in the river bed hydrobiotopes of lower Prut and in the natural lakes Beleu and Manta, especially in the area of supplying backwaters (II, IV). Some species with short life cycle, such as bitterling, stone moroko, tubenose goby etc. can demonstrate high abundances in some riparian habitats, with varying frequencies even in the same areas.

It is gratifying that some vulnerable fish species (such as chub, golden spined loach, ide, broad snout, roach, common barbell, vimba bream, sabrefish, Black Sea shad, Schraetzer, schneider, Kessler's gudgeon etc.) can still be quite common in some habitats of Prut river.

Threats to the ichtyofauna of Prut river

Obstacles to fish movement (dams, dikes, fences, silt stoppers)

The reservoir Stânca-Costești was formed in 1976 on the middle of the Prut River, at kilometer 576 from the confluence with the Danube. It has a length of 70 km, an area of 5900 ha, average depth of 12.5 m, maximum depth of 34 m, the volume of 772 million m³ of water. Construction of the reservoir Stânca-Costești caused the rupture of longitudinal connectivity of the river, having a highly detrimental effect primarily on the hydromorphological balance, but also on aquatic habitats and their ecological functions.

The construction of the Giurgiulesti port and, from 2014, of a port near the town of Cîşliţa-Prut, strongly affected the reproductive and trophic migrations of fish species between the ecosystems of Danube and Prut River.

Destruction of wetlands (lifting of banks and draining of wetlands)

Wetlands have diverse functions: regulation of the hydrological regime of rivers, amortization of processes of erosion and silting, maintaining groundwater level, biological purge of surface water, preservation of animal and plant diversity, ensuring the continuity of the trophic and reproductive migrations, and many other benefits to ecosystems. Currently, all these important functions are exploited in the lower Prut, ensuring human needs only. There is a large scale poaching during prohibition (using electricity, prodding, chase, obstruction of migration routes etc.), "pseudosanitary" indiscriminate cuttings of trees (with intensification of erosion of the riverbed), burning of reeds, extracting sand from the riverbed (under pretext of deepening and straightening), oil pollution (even within the scientific reserve "Lower Prut" where oil is extracted),

illegal hunting, expansion of land for agricultural purposes up to the river bank (without respecting the integrity of protection strips), waste disposal even in Prut floodplain etc.

In the years 50'-70' of the last century, agrarian policy directed towards increasing agricultural land has led to extended draining of river flooplains and essential reduction of wetland areas. An estimated of 33.000 ha were drained in the lower Prut flooplain.

Extraction of sand and dredging

Currently extremely important damages are brought to litophylous and psamophylous fish species by extracting sand from river Prut, near Ungheni town. Because of the great difficulties in accounting for extractions of sand, official figures are much lower than the real ones.

In the sector of Lower Prut, sand extraction is officially motivated only for navigation purposes that require the deepening and cleaning of the riverbed. These activities condition adverse effects such as: hydraulic - modifying the natural water flow and thus the transport of sediments; morphological - triggering and/or enhancing processes of erosion and/or alluvial deposits in the influence area of the ballast extraction; hydro-geological - modifying the natural level of groundwater in adjacent area; polluting - alteration of surface water quality due to polluting technological discharges from machinery from the ballast extractions; biological - reducing the number of species. Sand and gravel constitute a favorable environment for the development of benthic fauna, important food for fish, because at less than one meter deep the water circulates through the interstices of the gravel and promotes the oxygenation of these areas for which benthos show a particular affinity. Many insect larvae are developing here: Trichoptera, Ephemeroptera, Plecoptera, Diptera, Coleoptera and Turbellaria, molluscs, crustaceans and water mites, this fauna having an essential role in the phenomenon of self-purification of a water course. Sands and gravels also serve as substrate for the reproduction of many migratory and local litophyilous and psamohyilous species, currently in a sharp numerical regression, due to alteration of the characteristic breeding places.

Anthropogenic pollution of the waters of the Prut River

Chemical stress can be expressed by replacing the species "**more competitive**, **but more sensitive**" by the species tolerant to stressors. In some cases there may be a true "**blooming of opportunistic species**" that are normally excluded or marginalized by competition or predation.

The response of fish reproductive system to environmental factors is very varied and largely depends on the particularities of impact factor and those bio-ecological of taxa. The most common **disorders of the reproductive function** in fish subjected to **chemical stress** are:

- asymmetrical development of ovaries and testes, their abnormal shape;
- early sexual maturation and reduction of somatic metabolism intensity (appearance of dwarf forms);
- changes to the duration of oogenesis and spermatogenesis;
- displacement of the calendar terms of reproduction;
- cases of mass resorption of sexual cells in different phases;
- reduction of the deposited portions;
- disorders in the vitelogenesis process;
- reduction of the fecundation capacity;
- reduction of the proportion of individuals capable of reproduction;
- abortion of eggs with follicular membrane lysis etc.

The risk posed by heavy metals

Qualitative and quantitative analysis of contamination with Cd, Pb, Cu and Cr was done for the digestive tract, muscle tissue, skin, skeleton, gonads, liver and gills in specimens of perch (Perca fluviatilis), bream (Abramis brama), vimba bream (Vimba vimba), common rudd (Scardinius erythrophthalmus) from the reservoir Stânca-Costești, Prut River. The main source of these metals is food. Measurement of heavy metals was performed using a GF-HR-CS AAS with platform contrAA600, AnalytikJena.

Cadmium and copper were concentrated in fish tissues. The highest concentrations were found in the digestive tract and skin. At this trophic level, metals do not represent any danger to human consumption because the values in muscle did not exceed the maximum levels for contaminants in food (EC Regulation no. 1881 of 19 December 2006, laying down maximum levels for certain contaminants in foodstuffs). Maximum levels for fish muscle, according to the CE, are: cadmium - 0.05 μ g/g (wet weight) and lead - 0.3 μ g/g (wet weight). Chromium and lead were not detected in the samples analyzed.

Assessment of genetic and physiological biomarkers for monitoring the health of fish stocks in the basin of river Prut

Were analyzed samples of muscle tissue from seven fish species: Zingel streber (streber), Perca fluviatilis (perch), Gymnocephalus schraetser (schraetzer) Squalis cephalus (chub) Alburnus alburnus (common bleak), Carassius gibelio (silver crucian carp), Abramis sapa (white-eye bream), collected from river Prut.

The many genetic markers available allow quick access to genetic intrapopulational and interpopulational variability, the study of population structure, analysis of the degree of relatedness and identification of species.

Captive fish populations and their restocking in the wild can be used as a strategy for conservation of endangered populations. Small populations, threatened, lose most of the genetic variability as consequence of genetic drift and inbreeding, and a low genetic variability may reduce the average robustness of the population, affecting its viability, particularly when parasites or competitors are present. The fish highly homozygous may experience a decrease of fertility rate and growth, compared to populations more heterozygous. Restocking can be done to increase the effective population size and genetic variability of local populations threatened.

The plans for conservation or management, without prior knowledge of the genetic structure, could lead to overexploitation or segmentation of populations and, subsequent, gene pools could be lost or genetic diversity within populations might be reduced.

Genetic analysis of the Prut River fish community was carried out using three different molecular techniques in order to quantify the genetic diversity and phylogenetic relationships: RAPD (Random Amplified Polymorphic DNA) analysis, mitochondrial DNA analysis, and DNA sequencing.

No site showed an evidence of a recent bottleneck or a significant shortage of heterozygosity, as expected in a scenario of inbreeding in small populations.

Phylogeographical studies have provided evidence in support of scenarios of Prut River Basin colonization with invasive species originating from Asia, through lineages mainly from the Amur River.

Physiological biomarkers from the selected fishes represent different functional responses and levels of biological organization, including: a) biochemical and physiological responses and b) indices of general condition and health. Indices of health and general condition included liver somatic index (**LSI**) and spleen somatic index (**SSI**) and condition factor (**CFDC**).

As **biochemical markers** were assessed the activities of superoxide-dismutase (**SOD**), glutathione peroxidase (GPX) and the level of malondialdehyde (**MDA**) (lipid peroxidation) in muscle of fish. As a result, the fish population from Prut has low levels of oxidative stress, a high level of health and a good physiological condition.



Thresholds and fors area - "house" of reophileous-oxyphilous fish species



Prut water transparency in the upper and middle sectors, after the confluence with the River Jijia



Stânca-Costești dam, Cîşlița-Prut port, Giurgiulești terminal



"Trap gorge" - Rotaru backwater

Winch on lake Beleu

Chinese nets

As regards the **biometric data** obtained by calculating the main indices, such as profile index, **Fulton** coefficient of fattening, **Kiselev** index of quality, and index of carnosity, our results showed a corresponding growth, correlated with a good health of the fish population analyzed.

Biological pollution of ichthyocoenoses

An important topic that requires a particular approach is the problem of alien fish species and their invasive potential in receiver ecosystems.

It is well known that many species areal has changed significantly in the last 80-100 years, the human factor being decisive. Important features of invasive species, which ensures biological progress against other taxa, primarily, are: large dispersion capacity and rapid proliferation, followed by a pronounced genetic variability, associated with higher trophic competitiveness and correlated with early sexual maturity.

Other species (most from the marine Ponto-Caspian complex), conditionally assigned to the group of alien species, have proliferated and rapidly expanded their area of spreading in recent decades due to the changes of: climate, hydrobiotopes, thermal, hydrochemical and hydrological gradients, and, not least, thanks to their exceptional adaptive potential (greater pipefish, three-spined stickleback, Ukrainian stickleback, species of gobies etc.).

Significant changes are seen in the share of native species of fish that have become even dangerous for local ichthyocoenoses functional state. These multidominant species, in conditions of active eutrophication of natural ecosystems, have proliferated in excess, being considered true "native invaders" (bleak, white bream, roach, perch, etc.).

In conditions of excessive densities, the intra- and interspecific competition is intensified, and because their territorial niches are mainly in the littoral area, increases the impact on survival of eggs and offspring of large fish species, these being actively consumed. Because of the deplorable state of the predatory trophic level within the ecosystem, the excessive abundance of "**indigenous invaders**" can not be further effectively controlled by the ichthyophagous species.

True invaders of aquatic ecosystems are considered: silver crucian carp - *Carassius gibelio* with 41 points estimated according to the **FISK** (**Fish Invasiveness Screening Kit**) protocol, Chinese sleeper - *Perccotus glenii* with 38 points, respectively stone moroko - *Pseudorasbora parva* and pumpkinseed - *Lepomis gibbosus*, each with 34 points.

Illegal fishing

Since the maintenance of productivity and biodiversity directly correlates with fish age (the larger individual, the more prolific), was found a deplorable condition in most populations of large species. Juvenile individuals entered in full fish exploitation, lack the opportunity to participate in the reproductive process. From the years '50s up to now, the industrial catch weight decreased dozen times, and competition for industrial shares increased in reversed proportion.

The problem of illegal fishing in the Lower Prut sector has become very actual. Illegal fishing with nets with small mesh size (20 mm - 35 mm) occurs more and more often. Juveniles of many fish species of economic value (like catfish, carp, pike, zander, rapacious carp and even rare such as: ide, burbot, apron, etc.) are freely sold on local markets in lower Prut sector and then used as food for domestic animals.

In the Prut riverbed, where is more difficult to install nets, is commonly used the electric and stick fishing, and on the backwaters supplying the lakes Beleu and Manta migration paths are restricted to concentrate the fish in "special points for extraction with winch").

Unstable weather conditions and increasing effect of natural disasters

Besides the anthropogenic factor that clearly altered the state and structure of ichthyocoenoses, lately there has been a significant influence due to unstable weather conditions and increased effect of natural disasters.



Sinanodonta woodiana

Unio crassus

Corbicula fluminea



In a "healthy" lotic ecosystem, stenotope native species have satisfactory abundances (zingel and golden spined loach)



Chinese sleeper, loaches, silver crucian carp and eel - species that can withstand to almost complete lack of dissolved oxygen in the hydrobiotop

For many fish species, especially those with a life cycle short to medium, the floods serve as an important means of expanding into new territories and widening the areal. Among the species of fish that successfully apply the strategy "type of jump expansion", first come the invasive alien species, such as silver crucian carp, stone moroko, pumpkinseed and Chinese sleeper. These taxa, despite the propensity expressed for stagnant waters, in order to spread more rapidly can easily pass over the impediment of fast flow, silt stoppers and small thresholds from riverbed, and floods gives them the perfect opportunity to reduce the time and distance to conquer new territories.

Also, some native and opportunistic species successfully apply the method to spread through floods, for example, pike, perch, roach, bleak, European bitterling, greater pipefish, Ukrainian stickleback.

After major floods from 2008 and 2010, the whole hydrographic network of the Republic of Moldova was "invaded" by culture alien species (silver carp, bighead carp, grass carp) and carp penetrated from adjacent fish farms.

Major floods also caused the interpenetration of fish zones from the lotic ecosystems. Representatives from trout, grayling, broad snout, barbel, and chub **zones** arrived in the carp, perch and bream **zones**, and taxa with limited spread areals have suddenly changed their boundaries. There is an expansion through floods of endemic species of fish from the Danube (Danube ruffe, Danube spined loach, golden spined loach, and some species of gudgeons). Similarly, after major floods some previously heavily clogged substrates were cleaned in some areas of the river Prut. Unclogging reaction was not long expected: significantly increased the livestocks of young groups of reophylous litho-psamophylous species of fish (vimba bream, broad snout, chub, barbel, gudgeons, apron, golden spined loach).

CHAPTER III. THE RISK CHARACTERIZATION (ASSESSMENT OF MAGNITUDE AND PROBABILITY), ADVERSE EFFECTS AND ANALYSYS OF THE STRENGTHS, LIMITATIONS AND UNCERTAINTIES OF THE MODEL USED

III.1. Risks description (negligible, acceptable, unacceptable)

The risk assessment is based and developed on current knowledge where the risk level is constantly updated based on demographic, population and phylogenetic specific data on effects of alien species on ecosystems. Risk limitation and management is essential and **the risk is not evenly distributed among species and ecosystems**. Modern research on genetic and ecological implications of alien species should be deepened, in particular in the causal relationship between the introduction of species and loss of biodiversity. This is particularly relevant in the context of climate change when the balance of ecosystems will be altered and, consequently, the potential effect of fish introduction.

Threats are **external risks**, sudden and nonspecific environmental changes, acting negatively by restructuring and reducing the numbers of population individuals or species; **vulnerabilities** are **endogenous risks**: genetic, ecological, biochemical, ethological, reproductive, inability to adapt to environmental changes, destruction or modification of habitat to which stenotope species are generally sensitive and only answer through contingents downsizing or disappearance of all individuals up to **extinction**.

The **risk of extinction** of a species is composed of the sum or total of threats and vulnerabilities or the species.



(T1+T2+...+Tn) + (V1+V2+....+Vn) = Total, cumulated or general risk

From a quantitative perspective, any risk is very difficult to calculate because it is very difficult to put into a mathematical formula an ecological risk given by a certain intensity of threat, or what effect has a genetic vulnerability across the entire species population of Prut River, not from a local habitat.

Assessment of the ecological risk of pollution sources or different construction, for example, a wastewater treatment plant for the river sector where wastewater discharge occurs, is made based on operational monitoring performed directly at the confluence area. For example, evaluation of different types of environmental risks at the confluence of tributary streams on Jijia River with Prut River, considering the research data obtained within MIS ETC 1150 project.

Risk index	Type of ecological risk	Risk level	The degree of risk acceptance
HWR	The risk of water pollution of river Prut	high	Unacceptable
HWS	The risk of water pollution by deposits	high	Very unacceptable
HBP	The risk of macrofite communities degradation	medium	Unacceptable
HBSF	The risk of benthic communities degradation	high	Unacceptable
HBPHPL	The risk of phytoplankton communities degradation	high	Unacceptable
HBZPL	The risk of zooplankton communities degradation	high	Unacceptable
HBZBT	The risk of zoobenthos degradation	high	Unacceptable
HFish	The risk of fish communities degradation	medium	Unacceptable in prohibition season
HWM	The risk of increasing water mineralization	low	Unacceptable
HWO	The risk of increasing the organic matter content	medium	Unacceptable

List of ecological risk types at the confluence of River Jijia with River Prut

Risk scale

In the circumstances of criteria substantiation in order to assess severity of the consequences of Prut river pollution, three specific areas of risk can be defined:

- negligible risk, typically associated to pollutions with minor consequences, rare and very rare;

- **acceptable risk**, afferent to frequent minor pollutions or rare and very rare major pollutions; - **unacceptable risk**, afferent to major pollutions with high probability or frequency of occurrence,

which can not be neglected.



Specific risk areas, defined according to the criteria of evaluation/assessment of the severity of the consequences and limits of their acceptability ($\Gamma 1$ - upper limit of risk acceptability; $\Gamma 2$ - the lower limit for the consideration of risks.

III.2 influences upon water quality in the Prut River watershed

În accordance with Order no. 161 of 16 February 2006 of the Minister of Environment and Waters Management (in Romania) approving the Normative concerning the classification of surface water quality in order to determine the ecological status of water bodies, and with the *Regulation regarding the quality requirements for surface waters* (Government Decision no. 890 of 12.11.2013 – Republic of Moldova) five ecological states of the water bodies are defined: very good (I), good (II), moderate (III), poor (IV) and bad (V) (in accordance with Directive 2006/44/EC of the European Parliament and of the Council of 6 September 2006 on the quality of freshwaters needing protection or improvement in order to support fish life). For lakes, one will also take into account the trophic level, five trophic levels: ultra-oligotrophic, oligotrophic, mesotrophic, eutrophic and hypertrophic being correspondent to the five ecological states.

The establishing of ecological status of continental aquatic ecosystems must be based on biological quality elements, taking also into account the hydromorphological, chemical, physicochemical and specific pollutants that influence the biological indicators. Evaluation of these elements can show the presence of natural conditions, their minor alterations or the extent of human impact, respectively, the quality status of water bodies within a certain period of time.

Biological elements underlying the assessment of ecological status for Danube and large rivers will be considered according to the following hierarchy:

- 1. phytoplankton
- 2. phytobenthos
- 3. macrozoobenthos
- 4. macrophytes/angiosperms

5. fish.

Currently, in the assessment of the status of aquatic ecosystems are excluded the maximum permissible concentrations of various chemicals in natural waters, established for different types of use, and included parameters concentration limits for different classes of water quality: from very good to polluted.

List of major pollutants

1. Organohalogenated compounds and substances which may form such compounds in the aquatic environment

- 2. Organophosphorus compounds
- 3. Organotin compounds

4. Substances and preparations, or their degradation products, which have been shown to have carcinogenic or mutagenic properties or properties which may steroidogenically affect the thyroid, reproduction or other endocrine functions in or via the aquatic environment

- 5. Persistent hydrocarbons and persistent or bioaccumulable organic toxic substances
- 6. Cyanides
- 7. Heavy metals and their compounds
- 8. Arsenic and its compounds
- 9. Biocides and plant protection products
- 10. Materials in suspension
- 11. Substances which contribute to eutrophication (in particular, nitrates and phosphates)

12. Substances which have an unfavorable influence on the oxygen balance (which can be measured using parameters such as BOD₅, COD, etc.).

a) The point pressures

23 pollution sources from the basin Prut (17% of the total sources from the Prut watershed) concern the Bahlui River-Jijia River Basin.

b) Diffuse pressures

- Chemical fertilizers. In the adjacent area of Iaşi city, the amount used exceeds the maximum permissible norms of 1.5 kg N/ha.year and, respectively 0.15 kg P/ha.year.
- Pesticides. In the same area it is used an average of 0.39 kg/ha. year, above the average of Prut watershed (0.14 kg/ha.year), but below the European average (1.39 kg/ha.year)
- Domestic animals have a density of 0.73 cow equivalents/ha, over the Prut river basin average (0.65) and over the average of the Danube river basin (approx. 0.5).
- Human agglomerations not connected to sewerage (15%), below the average of Prut basin (50%).
- Erosion of land and minor riverbed.

III.3. Sources of uncertainty

III.3.1. Ecological state assessment model for Prut River, based on risk indicators analysis Introduction and objectives

Aquatic ecosystems are acknowledged to be complex, involving a multiplicity of interacting processes among a myriad of species, each simultaneously responding to near-stochastic variations in their environments. Yet the requirement to be able to recognize, classify and predict the responses of aquatic ecosystems to environmental influences is long standing, paramount and increasingly urgent. Ecosystems comprise numerous organisms interacting among themselves and with the characteristic features of a defined environment. For understanding how simulations of such complex systems can be made, components and functions have been conceptually minimalized by Ripl and Wolter (2002) to those of a single **dissipative ecological unit**, within which entities are able to increase thermodynamic efficiency in ways that internalize material exchanges and closes resource loss. The components are obligatory interlinked by pathways of energy, traded primarily in the currency of high-energy organic carbon bonds. The dissipative ecological unit, necessarily comprises: (1) primary producers, which harvest solar energy and synthesize the high-energy bonds in the manufacture of organic material; (2) water, as a moderating feedback to control of production; (3) a reservoir of organic detritus; (4) decomposers, drawing energy by oxidizing detrital stores and (5) a web of consuming heterotrophs, whose activities are fueled by the oxidation of organic carbon (herein referred to as Corg) and, through which, the original energetic input is progressively unlocked and dissipated. Aquatic ecosystems are typically organized around food webs that involve herbivorous, detritivorous and carnivorous heterotrophs. They are energized, in part, by the primary production of macrophytic plants, rooted in shallow areas, together with an abundance of associated epiphytes, and by the microphytic algal and bacterial photoautotrophs of the phytoplankton, suspended in open, deeper water. The development of habitat templates and their adaptation to aquatic locations is presented in detail in Reynolds (2003).

The **aim** of this study is to **detail the Prut river ecosystem model** for **enabling simulation** of its operation in terms of fisheries and simulating possible risk situations directly or indirectly. This model is designed using the latest software implementations patterns theorized in literature.

2. Material and method

In the field of complex systems simulations, in general, and the aquatic ecosystems, in particular, many efforts have been made over the past 40 years, which materialized in the development of more complex applications able to simulate the state and evolution of such ecosystems based on limited field data and measurements. In this purpose, for the aquatic ecosystem model of the river Prut, **AQUATOX** application is best suited.

2.1. AQUATOX, software for aquatic ecosystems simulation

AQUATOX is a simulation model for aquatic systems. AQUATOX predicts the fate of various pollutants and their effects on the ecosystem (aquatic plants, invertebrates, and fish). AQUATOX is a valuable tool for ecologists, biologists, water quality modelers, and anyone involved in performing ecological risk assessments for aquatic ecosystems. AQUATOX simulates the transfer of biomass, energy and chemicals from one compartment of the ecosystem to another. It does this by simultaneously computing each of the most important chemical or biological processes for each day of the simulation period; therefore, it is known as a process-based or mechanistic model. AQUATOX can predict not only the environmental fate of chemicals in aquatic ecosystems, but also their direct and indirect effects on the resident organisms. Therefore, it has the potential to establish causal links between chemical water quality and biological response and aquatic life uses.

AQUATOX is the only general ecological risk model that represents the combined environmental fate and effects of conventional pollutants, such as nutrients and sediments, and toxic chemicals in aquatic ecosystems. It considers several trophic levels, including attached and planktonic algae and submerged aquatic vegetation, invertebrates, and forage, bottom-feeding, and game fish; it also represents associated organic toxicants.

The fate portion of the model, which is applicable especially to organic toxicants, includes: partitioning among organisms, suspended and sedimented detritus, suspended and sedimented inorganic sediments, and water; volatilization; hydrolysis; photolysis; ionization; and microbial degradation. The effects portion of the model includes: acute toxicity to the various organisms modeled; and indirect effects such as release of grazing and predation pressure, increase in detritus and recycling of nutrients from killed organisms, dissolved oxygen sag due to increased decomposition, and loss of food base for animals.

AQUATOX combined algorithms from these models with ecotoxicological constructs; and additional code was written as required for a truly integrative fate and effects model. The model was linked to Microsoft Windows interfaces to provide greater flexibility, capacity for additional compartments, and user friendliness. The U.S. Environmental Protection Agency (US EPA) issued Release 1 in 2000. The program has evolved steadily and in May 2014 reached version 3.1 plus.

AQUATOX is the latest in a long series of models, starting with the aquatic ecosystem model CLEAN and subsequently improved in consultation with numerous researchers at various European hydro-biological laboratories, resulting in the CLEANER series and LAKETRACE. The MACROPHYTE model, developed for the U.S. Army Corps of Engineers, provided additional capability for representing submersed aquatic vegetation. Another series started with the toxic fate model PEST, developed to complement CLEANER, and continued with the TOXTRACE model and the spreadsheet equilibrium fugacity PART model.

AQUATOX provides Latin hypercube uncertainty analysis, nominal range sensitivity analysis, and time-varying process rates and limitations to photosynthesis for detailed analyses. Results are given in tabular and graphical forms, including biomass, chemical concentrations, process rates, limitations to photosynthesis, nutrient and toxicant mass balances, sensitivityanalytical tornado diagrams, and probabilistic risk graphs; it also computes community similarity indices comparing perturbed and control simulations. The model is perhaps the most comprehensive model available.

State variables and processes	AQUAT OX	AQUAT OX CATS CASM		Qual2K	WASP7	EFDC- HEM3D	QEAFdC hn	BASS	QSim
Nutrients	٠	•	•	•	•	•			•
Sediment diagenesis	•			•	•	•			
Detritus	•	•	•	•	•	•			•
Dissolved oxygen	•		•	•	•	•			•
DO effects on biota	•								•
pН	•			•					•
NH ₄ toxicity	•								
Sand/silt/clay	•				•	•			
Sediment effects	•								
Hydraulics						•			•
Heat budget				•	•	•			•
Salinity	•				•	•			
Phytoplankton	•	•	•	•	•	•			•
Periphyton	•	•	•	•	•				•
Macrophytes	•	•	•						•
Zâooplankton	•	•	•						•
Zoobenthos	•	•	•						•
Fish	•	•	•					•	•
Bacteria			•						•
Pathogens				•		•			
Organic toxicant fate	•	•			•			•	
Organic toxicants in : - Sediments	•	•			•	•			
- Stratified sediments	•				•	•			
- Phytoplankton	•	•							
- Periphyton	•	•							
- Macrophytes	•	•							
- Zooplankton	•	•					•		
- Zoobenthos	•	•					•		
- Fish	•	•					•	•	
- Birds or other animals	•	•							
- Ecotoxicity	•	•	•					•	
- Linked segments	•			•	•	•	•		•

AQUATOX is designed to be a comprehensive, general, mechanistic model of the fate and effects of pollutants in aquatic ecosystems with the simplest spatial and temporal resolutions consistent with this objective. It is designed to represent average daily conditions for well-mixed and stratified systems, and linked well-mixed segments.

AQUATOX uses a very efficient fourth- and fifth-order Runge-Kutta integration routine with adaptive step size to solve the differential equations. The optional hourly time step is especially useful for simulating diel dissolved oxygen (DO) fluctuations, ammonia toxicity (ignored by most models), and calcium carbonate precipitation. The depth distributions of ponds, lakes,

reservoirs, streams, and estuaries are represented in the model by idealized geometrical approximations, following the topological treatment of Junge; differences between waterbodies are determined by the mean and maximum depths. Based on these relationships, fractions of volumes and areas can be determined for any given depth.

Unlike many risk assessment models, AQUATOX represents a complete aquatic ecosystem, including the capacity to model a variable number of biotic groups, each is represented by a set of process-level equations encoded in object-oriented Pascal. Biomass is expressed as ash-free dry weight (AFDW). Algal and moss biomass can be converted to chlorophyll a for comparison with monitoring data.

2.2. Setting up and particularization of the aquatic ecosystem model for Prut River according to AQUATOX principles of simulation

Any ecosystem model consists of multiple components requiring input data. These are the abiotic and biotic **state variables** or compartments being simulated. In AQUATOX the biotic state variables may represent trophic levels, guilds, and/or species. The model can represent a food web with both detrital- and algal-based trophic linkages. Closely related are **driving variables**, such as temperature, light, and nutrient loadings, which force the system to behave in certain ways. In AQUATOX state variables and driving variables are treated similarly in the code. This provides flexibility because external loadings of state variables, such as phytoplankton carried into a reach from upstream, may function as driving variables; and driving variables, such as temperature, could be treated as dynamic state variables. Constant, dynamic, and multiplicative loadings can be specified for atmospheric, point- and nonpoint sources. Loadings of pollutants can be turned off at the click of a button to obtain a control simulation for comparison with the perturbed simulation.



Conceptual model of ecosystem represented by AQUATOX (Park R. A. and Clough J. S., 2014b)

AQUATOX utilizes differential equations to represent changing values of state variables, normally with a reporting time step of one day. These equations require starting values or **initial conditions** for the beginning of the simulation. If the first day of a simulation is changed, then the initial conditions may need to be changed. A simulation can begin with any date and may be for any

length of time from a few days, corresponding to a microcosm experiment, to decades, corresponding to an extreme event followed by long-term recovery.

The aquatic ecosystem of the River Prut involves initializing variables that define the baseline. This involves 9 principal steps necessary to set up a new simulation. However, after those steps, the model should be reconsidered and made fine adjustments of these elements to customize the functionality of the ecosystem modeled in detail.

The first step is to establish the **aquatic model** to be implemented and simulated. From this point of view River Prut is a Stream type model.

The second step is to set a **period of time** during which it will conduct simulation. This parameter can be changed whenever a new simulation is running. This may include periods from a few days to several years.

The third step requires the introduction of initial conditions for dissolved nutrients (ammonia, nitrate, phosphate, oxygen, and carbon dioxide). Because the nitrite (NO_2) occurs in very low concentrations and is rapidly transformed through nitrification and denitrification therefore, it is modeled with nitrate. Un-ionized ammonia (NH_3) is not modeled as a separate state variable but is estimated as a fraction of ammonia. In the sediment bed, if the optional sediment diagenesis model is included, nitrogen is explicitly modeled; otherwise inorganic nitrogen in the sediment bed is ignored, but organic nitrogen is implicitly modeled as a component of sedimented detritus.



Nitrogen Cycle in AQUATOX (Park R. A. and Clough J. S., 2014b)

The phosphorus cycle is simpler than the nitrogen cycle. Decomposition, excretion, and assimilation are important processes that are similar to those described above. As was the case with ammonia and nitrate, if the optional sediment diagenesis model is included, flux of phosphate (PO₄) from the sediment bed may be added to the water column, especially under anoxic conditions. Additionally, sorption to calcite (the most stable form of CaCO₃) may have a significant effect on phosphate predictions in high pH systems due to precipitation of calcium carbonate. This optional formulation is important to adequately simulate marl lakes. As was the case with ammonia,

remineralization includes all processes by which phosphate is produced from animal, plants, and detritus, including decomposition, excretion, and other processes required to maintain mass balance given variable stoichiometry.

Oxygen is an important regulatory endpoint; very low levels can result in mass mortality for fish and other organisms, mobilization of nutrients and metals, and decreased degradation of toxic organic materials. Dissolved oxygen is usually simulated as a daily average and does not account for diurnal fluctuations. It is a function of reaeration, photosynthesis, respiration, decomposition, and nitrification. AQUATOX also represents both lethal and non-lethal effects from low concentrations of dissolved oxygen.

Carbon dioxide is also a very important limiting nutrient. Similar to other nutrients, it is produced by decomposition and is assimilated by plants; it also is respired by organisms.



Phosphorus Cycle in AQUATOX (Park R. A. and Clough J. S., 2014b)

Carbon dioxide also is exchanged with the atmosphere; this process is important, but is not instantaneous: significant undersaturation and oversaturation are possible. The treatment of atmospheric exchange is similar to that for oxygen.

Except the initial conditions, a major impact in making a realistic simulation is the use of field measurements made during the set time period that wants to be simulated. Thus our field teams have done systematic measurements of these indicators into the river Prut on several sections of it to be able to simulate the whole dynamic ecosystem. *Use Dynamic Loading* option instead *Use Constant Loading* creates a more precise model which can be used in future predictions. The stream simulations are not sensitive to nutrient initial conditions but rather to loadings. This allows the simulation to run several times for calibration so that it can be subsequently tested for a number of possible disturbing factors.

The fourth step is the **Detritus** section. In AQUATOX, the term detritus includes all nonliving organic material and associated decomposers (bacteria and fungi). It allows to specify initial conditions of detritus in the **sediment bed** (I) and in the **water column** (II). The sediment bed has in turn two compartments: the I.1 labile detritus which is readily decomposed and assimilated and the I.2 refractory detritus which is resistant to decomposition. In the water column, the initial conditions and loadings are represented in a global variable, the total suspended and dissolved detritus (II.T). It can be input as Organic Matter (dry weight), Organic Carbon or Carbonaceous Biochemical Oxygen Demand (CBOD) and the model will make the necessary conversions. The total suspended and dissolved detritus initial conditions and loadings are divided into four compartments: II.T.1 particulate refractory detritus and II.T.2 particulate labile detritus, and II.T.3 dissolved refractory detritus and II.T.4 dissolved labile detritus. Initial conditions and loadings are parsed by specifying % particulate and % refractory.



An example for Use Dynamic Loading option for nutrients

AQUATOX also includes a representation of the sediment bed as presented in *Sediment Flux Modeling* (Di Toro, 2001). This optional sediment submodel tracks the effects of organic matter decomposition on pore-water nutrients, and predicts the flux of nutrients from the pore waters to the overlying water column based on this decomposition. The model assumes a small aerobic layer above a larger anaerobic layer. For this reason, it is best to apply this optional submodel in eutrophic sites where anaerobic sediments are prevalent. This model involves the initializing of two supplementary variables (compartments): refractory buried detritus and labile buried detritus.

Step five is the **Plant** section. The Plant section is divided in five compartments: diatoms, green, blue-green, other algae (dinoflagellate), and macrophytes. Initial conditions for the plant and any external loadings must then be specified. A "seed" loading may be specified (generally as a constant load) to prevent permanent extinction from occurring. Seed loadings are generally very small loadings (e.g. 1-5 mg/l) that allow for reintroduction of an organism after environmental conditions have improved to allow that organism to be viable in the system again. Algal biomass is a function of the loading (especially phytoplankton from upstream), photosynthesis, respiration, excretion, non-predatory mortality, grazing, and washout. Phytoplankton also are subject to sinking, and periphyton to sloughing. AQUATOX can represent as many as 24 different algal groups, providing realistic simulations of competition, succession, and varying tolerances to herbicides, detail unmatched by other models.



Simplified schematic of the AQUATOX sediment diagenesis model (without Silica, Sulfide or COD-Chemical Oxygen Demand). Particulate organic matter in the sediment bed (POC - Particulate Organic Carbon, PON - Particulate Organic Nitrate and POP - Particulate Organic Phosphate) is divided into three reactivity classes as follows: G1 (reactivity class 1) equivalent to labile organic matter, G2 (reactivity class 2) equivalent to refractory organic matter, and G3 (reactivity class 3) nonreactive. SOD represent the Sediment Oxygen Demand. (Park R. A. and Clough J. S., 2014b)

Submersed aquatic vegetation or macrophytes can be an important component of shallow aquatic ecosystems, although they are ignored by many models. Seasonal macrophyte growth, death, and decomposition can affect nutrient cycling, and detritus and oxygen concentrations. By forming dense cover, they can modify habitat and provide protection from predation for invertebrates and smaller fish. AQUATOX simulates these functions. To enable such a highly complex simulation, organisms are parameterized. The program has many parameterized components combined in five databases: *Animal Library, Chemical Library, Plant Library, Site Library* and *Remineralization Library*. It is evident that in this databases the researcher can add new parameterized components or modify existing ones to suit the conditions and particularities of simulated ecosystem.

Step six, the Invertebrate section. Within AQUATOX, invertebrates are classified as *shredders*, *sediment feeders*, *suspension feeders*, *clams*, *grazers*, *snails*, and *predatory invertebrates*. The change in animal biomass is a function of a number of physiological and ecological processes similar to many models; however, AQUATOX includes additional mechanistic relationships. For example, ingestion is reduced for sublethal toxicant effects, suspended sediments and limitations due to habitat preferences. The maximum consumption rate is sensitive to body size, so AQUATOX provides an alternative for fish by using an allometric equation and parameters from the *Wisconsin Bioenergetics Model*.

Relative food preferences are represented by a matrix of preference parameters. The preference factors are normalized so that if a potential food source is not modeled or is below the minimum feeding level, the other preference factors are modified accordingly, representing adaptive preferences or prey switching.

	P	lant Da	nta: <u>N</u> ew	Internal Nutrients:					
Plant Cladoph	nora CR	Scientific	Cladophora	N Half-saturation Internal 0.009 gN / gAFDW WASP7, Ambrose et al. 2006					
, [Search Names		Search Scientific Names	P Half-saturation Internal 0.0013 gP / gAFDW WASP7, Ambrose et al. 2006					
Plant Type:	Periphyton	•	Toxicity Record: Greens	N Max Uptake Rate 0.72 gN / gAFDW-d WASP7, Ambrose et al. 2006					
		1	Taxonomic Type: Greens	P Max Uptake Rate 0.05 gP / gAFDW-d WASP7, Ambrose et al. 2006					
			References	Min N Ratio 0.0072 gN / gAFDW WASP7, Ambrose et al. 2006					
Saturating Light	135	Ly/d Convert	Graham, Auer, Canale, & Hoffman 1982 = 135	Min P Ratio 0.001 gP / gAFDW WASP7, Ambrose et al. 2006					
Use Adaptive Light									
Max. Saturating Light	300	Ly/d Convert	Default	Phytoplankton Only:					
Min. Saturating Light	80	Ly/d Convert	Default	Sedimentation Rate (KSed) 0 m/d					
P Half-saturation External	0.04	mg/L	0.04,Auer and Canale 1982 = 0.0025 to 0.03	Temperature of Obs. KSed 0 °C placeholder					
N Half-saturation External	0.1	mg/L	0.1 Borchardt 1996	Salinity of Obs. KSed 0 % placeholder					
Inorg. C Half-saturation	0.054	mg/L	Collins & Wlosinski '83	Exp. Sedimentation Coeff 0					
Temp. Response Slope	2		DeNicola 1996	Periphyton and Macrophytes Only:					
Optimum Temperature	30	°c	30=Michalski 2003 Ontario Municipal Board	Carrying Capacity 0 g/m ²					
Maximum Tomporaturo	42		Colling & Wlogingki 193	VelMax (macrophytes)					
Min Adaptation Temp	42	с 1	Contrast Avec Concle & Uniferran 4000	Reduction in Still Water 0.6 fraction					
win Adaptation Temp.	0	°C	Granam, Auer, Canale, & Homman 1982	(periphyton) Critical Force (FCrit for					
Max. Photosynthetic Rate	1.4	1/d	calibr.; Auer and Canale 1982 = 1.08	periphyton only) 0.008 newtons					
Photorespiration Coefficient	0.03	1/d	Collins & Wlosinski '83	Event (periphyton) 90 percent 90% lost in sloughing event as default					
Resp Rate at 20 deg. C	0.1	g/g-d	Laws and Wong, 1978, per Collins and WI. 1983	If in Stream:					
Mortality Coefficient	0.001	g/g-d	prof. judgment	Percent in Riffle 50 % Michalski 2003. Ontario Municipal Board					
Exponential Mort. Coeff.	0.05	g/g-d	calibrated	Porcent in Rend					
P : Organics, Initial	0.0044	ratio	Sterner & Elser 2002	Decrease in Pure 0.00 % (All Biomass pot in Riffle or Pool)					
N : Organics, Initial	0.04	ratio	Sterner & Elser 2002						
Light Extinction	0.05	1/m-g/m ³	calibrated	Salinity Effects (Estuary only):					
Wet to Dry	5	ratio	default	Minimum _{0/00} Maximum _{0/00} Coeff1 Coeff2 Reference: Tolerance /00					
Fraction that is lipid		(wet wt.)		Photosynth. 0 0 0 placeholder					
r raction that is lipid	1	(werwic)		Mortality 0 0 0 0 placeholder					

An example for Cladophora genus parameterization

Different prey types have different potentials for assimilation; egestion coefficients indicate the fraction of ingested prey that is defecated or discarded. As in all other cases, the *Use Dynamic Loading* option helps to generate an accurate model for river Prut aquatic ecosystem.

Step seven, Fish section. In AQUATOX fish are classified as *forage fish, bottom fish and gamefish*. At least two species can be modeled for each general class. Furthermore, two size classes can be modeled for each species, and one species can be modeled as multiple year classes. For each trophic guild (*forage fish, bottom fish and gamefish*) it is possible to choose from a list of appropriate species in the database (AQUATOX Animal Library). For the Prut River *Cyprinus carpio* is a species that is particularly well suited for complex simulation because it has a very detailed parameter and thus it can be created a multiple year classes. The average age of the spawners is estimated to be between 20-25 years, as they are a long lived species.



An example for Use Dynamic Loading option for Invertebrates section.

	Ani	mal D	ata: <u>N</u> ew Help	Sensitivity to Sediment (lethal effects) Zero Sensitivity Default no sediment effect
Animal Carp		Scientific	Cyprinus carnio	Organism is Sensitive to Percent Embeddedness:
	arch Names	Name	Search Scientific Names	Percent Embeddedness 100 percent No effect
			Trophic Interactions	Carrying Capacity 16.7 g/sq.m calc. from Leidy & Jenkins 1977
Animal Type:	Fish	•	Toxicity Record: Catfish	
Taxonomic Type or Guild:	Bottom Fish	•	Benthic Metric Designation:	
			References:	Frac. in Water Column 1 fraction Default for this Animal Type
Half Saturation Feeding	0.5	mg / L	Collins & Wlosinski 1983	VelMax 400 cm / s Default
Maximum Consumption	0.05	g/g·d	Collins & Wlosinski 1983	Removal due to Fishing 0 fraction / d Convert Placeholder
Min Prey for Feeding	0.25	g/sq.m	prof. judgment	Ricacoumulation Data:
Sorting: degree to which there is selective feeding	1	unitless	Default no sediment effect	Bioaccumulation Data.
Successful Cadiments Aff	at Eastines E		Default no sodiment effect	Mean lifespan 1460 days
Slope for Sed. Response		unitless	Default no sediment effect	Fraction that is lipid 0.1 (wet wt.) Niimi 1983 (4.5-10.5%)
Intercept for Sed. Resp.	0	unitless	Default no sediment effect	
				Low Oxygen Effects:
Temp. Response Slope	2.3			Lethal Conc. Percentage Killed Reference:
Optimum Temperature	22	°C	Leidy & Jenkins 1977	0 mg/L (24-hour) 25 (1-99)
Maximum Temperature	33	°C	"	EC50 Crowth 2 and rot have Default
Min Adaptation Temp.	2.5	°C	II	
Mean wet weight	3319	g wet	obs BW	EC50 Reproduction 3 mg/L (24-hour) Default
Endogenous Respiration	0.005	1/d	Hewett & Johnson 1992 Tilapia	Ammonia Toxicity:
Specific Dynamic Action	0.1	(unitless)	Hewett & Johnson 1992 Tilapia	LC50, Total
Excretion : Respiration	0.05	ratio		Ammonia (ph=8)
N to Organics	0.1	frac. dry	Sterner and George 2000	Califati Effectes
P to Organics	0.031	frac. dry	Sterner and George 2000	Salinity Enercis:
Wet to Dry	5	ratio	default	Tolerance Tolerance
Gametes : Biomass	0.09	ratio		Ingestion 0 0 0 Placeholder
Gamete Mortality	0.9	1/d	,	Gamete Loss 0 0 0 0 Placeholder
Mortality Coefficient	0.0005	1/d	calibrated	Mortality 0 0 0 Placeholder

An example for Cyprinus carpio parameterization.

Cyprinus carpio appears in the *IUCN Red List of Threatened Species* of International Union for Conservation of Nature with the *vulnerable* status, this is an additional argument for assessment. In the Prut River simulation were used all possible categories for fish compartment.

Cyprinus carpio covered two sections: *small bottom fish* and *large bottom fish*, because there were two distinct generations parameterized, one with the young and less individuals and the other with mature and more individuals.

The *small forage fish* and *large forage fish* compartments were populated with *Lepomis gibbosus* (pumpkinseed) a species of the Order *Perciformes*, which also has a very good parameterization in the AQUATOX animal database. The species in this order, means *perch-like*, are close related to *Perca fluviatilis*, species of interest identified in Prut River. It also appears in the IUCN Red List of Threatened Species of International Union for Conservation of Nature with the *least concern* status. The last two compartments, *small* and *large gamefish* were stocked with *rainbow trout* and *northern pike* respectively, as both species have been identified in very few exemplars and are subject to IUCN Red List of Threatened Species of International Union for Conservation of Nature with the *least concern* status.

Step eight is for site **physical** characteristics: water temperature, water pH, wind loading, light loading and its seasonal photoperiodicity. Temperature is an important controlling factor in the model.



An example for Use Dynamic Loading option for temperature, pH and light.

Virtually all processes are temperature-dependent. They include stratification; biotic processes such as decomposition, photosynthesis, consumption, respiration, reproduction, and mortality; and chemical fate processes such as microbial degradation, volatilization, hydrolysis, and bioaccumulation. On the other hand, temperature rarely fluctuates rapidly in aquatic systems. Default water temperature loadings for the epilimnion and hypolimnion are represented through a simple sine approximation for seasonal variations based on user-supplied observed means and ranges. Wind is not so important for a stream, so it was kept constant at 1 m/s. For light, the photoperiod was selected to be computed from latitude. pH dynamics is important in simulations for several reasons: pH affects the ionization of ammonia and potential resulting toxicity; pH affects the hydrolysis and ionization of organic chemicals which potentially have effects on chemical fate and the degree of toxicity; pH also affects the decay of organic matter and denitrification can take place which has a significant effect on the food-web. In AQUATOX, the computation is good for the pH range of 3.75 to 8.25, where the carbonate ion is negligible and can thus be ignored. Any predictions above 8.25 are truncated to 8.25 and any predictions below 3.75 are set to 3.75. The

derivation is given by Small and Sutton (1986), with a correction for dissolved organic carbon. In Prut river's case, one cannot speak of situations that lie outside the indicated range.

Ρ	reference percentages a	are initially r	ormalized to	100% based on s	species in t	the simula	ition.	Renormalize		PREDA	TORS				
	Show Pref	erences	Show	Egestion Co	efficient	s C	Show	Comments							
		Crayfish	Chironomid	Tubifex tubifex	Copepod	Daphnia	Mussel	Mayfly (Baetis)	Gastropod	Pumpkinseed	Pumpkinseed22	Carp	Carp, Lg	Rainbow Trout	Northern Pike
	R detr sed	0.3	0.0												
	L detr sed	31.5	100.0	100.0				5.3	1.5			18.2	9.5		
	R detr part														
	L detr part				21.1	7.6	14.3					18.2			
	Peri Low-Nut Diatom				31.6			15.8	16.9						
	Peri High-Nut Diatom							15.8	16.9						
	Phyt High-Nut DiatJC	1				22.7	14.3								
P	Phyt Low-Nut DiatoJC					22.7	14.3								
R	Phyto, NaviculaJC	1				22.7	14.3								
E	Peri, Nitzschia	1						15.8	16.9						
Y	Cladophora				21.1			15.8	12.3			4.5	4.8		
	Peri, Green	1						15.8	16.9						
	Phyto, Green					22.7	14.3								
	Phyt, Blue-Greens JC				21.1	0.8	14.3					4.5	4.8		
	Peri, Blue-Greens							15.8	16.9						
	Cryptomonas	1			5.3	0.8	14.3								
	Fontinalis	1							1.5			18.2	9.5		
	Crayfish														
	Chironomid	10.5								10.0	10.0	9.1	28.6	21.4	
	Tubifex tubifex	10.5										9.1	28.6	21.4	
	Copepod											9.1	4.8	10.7	
	Daphnia											9.1	9.5	10.7	
	Mussel	26.2													
	Mayfly (Baetis)	1													
	Gastropod	21.0								90.0	90.0				
	Pumpkinseed	1													
	Pumpkinseed22													17.0	5.6
	Carp														27.8
	Carp, Lg	1												8.0	5.6
	Rainbow Trout													10.7	5.6
	Northern Pike														55.6

Matrix trophic interaction used for Prut River aquatic ecosystem simulation.

The last step is for edit the trophic interactions. AQUATOX is a food-web model including prey switching based on prey availability. It provides a feeding preference matrix that must be specified to describe the food web in the simulation. Using the ICE (Interspecies Correlation Estimation) database from US EPA and regression equations, the model can be parameterized to represent a complete food web. In Prut river's case, there were a number of adjustments within the matrix to be consistent with data obtained from the field.

Results and discussions

This section includes diagrams showing the physico-chemical and biological parameters evolution over time, from which it will be interpreted the state of Prut aquatic ecosystem. Each color column represented the simulated value for one day.

The results were represented as graphs for each sampling site in order to measure the peak values for each day of the monitoring interval. Multiple comparisons with the legislation from Romania and Republic of Moldova were done in order to measure any possible risk. In case of Prut River it has been published a Romanian **Management plan** based on multiple years of monitoring of this sector in accordance with the Water Framework Directive 2000/60/EC. This management plan was used to compare the results obtained from the simulation in order to show the ecological state of Prut River. The second comparison instrument was the legislation of Republic of Moldova (**H.G. Nr. 890/12.11.2013**).

The simulations were based on the real measurements data for the monitoring interval (Aprilie 2013-April 2015), at eight sampling sites: S1-Costesti-Stanca, S2-Braniste, S3-Sculeni, S4-Leuseni, S5-Leova, S6-Cahul, S7-Cislita and S8-Giur S1- Stânca-Costești, S2-Braniște, S3-Sculeni, S4-Leușeni, S5-Leova, S6-Cahul, S7-Cîşlița-Prut and S8-Giurgiulești.

Phytoplankton total biomass

This ecological state parameter had the best distribution at S1- Stânca-Costești with three peak values with seasonal trend. During the period of spring-summer were recorded the highest values of phytoplankton total biomass for the three years of monitoring. According to H.G. Nr. 890/12.11.2013, the water quality is separated in 5 classes based on the phytoplankton total biomass:

Class I < 0.5 mg/L (highest quality)

Class II < 1.5 mg/L

Class III < 2.5 mg/L

Class IV < 5 mg/L

Class V < 10 mg/L (lowest quality).

Using this classification of water quality, at S1-Stânca-Costești this parameter indicated different quality class variations during the monitoring period with the best quality during the cold seasons and the lowest in the hot seasons. Fast peaks of phytoplankton biomass, increasing during August-September 2013, were observed at this site. This can be explained by a short term input of a high mass of nutrients and their rapid consumption, but this interpretation cannot be sustained for this parameter only, because it is possible to occur errors in sampling and analyses, and the trends were not compared with the peaks of other parameters. This site can be used as a reference one for monitoring because it had constant seasonal variations of this parameter based on a natural cycle which can be compared with the other sites.

At sampling site S2-Braniste was observed the same natural pattern, with the same natural trend, but with high fluctuations during February-May 2014, which suggests a high input of nutrients that increased the biomass suggesting that there are possible anthropogenic causes that might modify the natural equilibrium.

Sampling site S3-Sculeni had natural peaks that do not suggest the possibility of a source of nutrients. This site was also characterized by a higher quantity of biomass during March-April 2015.

At sampling site S4-Leuşeni was observed the existence of a **possible risk** of pollution with nutrients that are responsible for algae biomass increasing.

At sampling site S5-Leova were recorded the highest peaks of the simulated values, but only for the interval August-October 2013. At this station there are many errors in simulation, caused by many factors as sampling quality, samples processing, analysis and not evenly distributed effort. However, this site must be target for future investigations. According to these values the situation can reach critical levels with the impossibility of life supporting which can not be true. The highest peaks are not accepted as presenting the reality but they prove the presence of a **possible risk**.

For sampling sites S6-Cahul, S7-Cîşliţa-Prut and S8-Giurgiuleşti, the simulations did not show any regular trend of the data during the seasons. However, a significant decrease of this parameter values was observed after S5-Leova, but simulation results were inconclusive to estimate the real risk for these monitoring sites. **Not all the models resulted can be accepted!**

Copepods total biomass

The copepods total biomass is not used by the legislation for the evaluation of the ecological state. It is important to estimate their productivity in the water because these organisms are essential components of the food webs. More than this they are predators as well with important tasks in the equilibrium of the aquatic ecosystems.

Site S1-Stânca-Costești: the highest copepod biomass was recorded in March-April with the highest peak at the end of April for the entire monitoring interval. It was observed a constant biomass cycle which can be correlated with the phytoplankton biomass that had the same pattern. The highest value obtained by simulation was around 12 mg/L and the lowest below 1 mg/L.

Site S2-Braniște: two peaks were observed in July-September 2013 and 2014 which are out of the graphs, not close to the reality. They did not follow any pattern compared to site Stânca-Costești. With these errors out of the graph resulted the same biomass in most seasons but this **cannot be validated**.

For sites: S3-Sculeni, S4-Leuşeni, S5-Leova, S7-Cîşliţa-Prut and S8-Giurgiuleşti the simulation provided results with extremely high peaks; **no suitable interpretation** was possible.

Site S6-Cahul: the highest copepod biomass was recorded during March-April for all the years, with the highest peak at the end of April for the entire monitoring period. It was observed a constant cycle of biomass which can be correlated with the phytoplankton biomass that had same pattern. The highest simulated value was around 14 mg/L and the lowest was under 1 mg/L.

The copepods total biomass was simulated close to the reality only for S1 and S6. The errors are multiple for this way of data interpretation, but the results of tye accepted simulations explain how the aquatic ecosystem works.

Dissolved oxygen (DO)

According to the Romanian **Management Plan for Prut River**, the ecological states based on dissolved oxygen concentrations in water are:

10-8 mg/L –very good/good

8-6 mg/L - good/medium.

According to **H.G. Nr. 890/12.11.2013** (Republic of Moldova), the water quality is separated in 5 classes based on the dissolved oxygen concentrations:

Class I > 8 mg/L (highest quality)

Class II > 7 mg/L

Class III > 5.5 mg/L

Class IV > 4 mg/L

Class V < 4 mg/L (lowest quality).

Site S1-Stânca-Costești: the highest concentrations were recorded during December-January-February for 2014 and 2015. This parameter followed similar trends from the beginning of the monitoring till the end. According to simulation, the highest peaks were around 13 mg/L in January-February 2014, and this parameter decreased until July, when reached the lowest level: 8-9 mg/L. The **model is accepted** and offers the entire daily variations for all the studied period, suggesting the **ecological state: very good-good-medium**.

Site S2-Branişte: the highest concentrations were recorded during December-January-February for 2014 and 2015. This parameter partially followed similar trends from the beginning of the monitoring till the end. According to the simulation, the highest peaks were around 13 mg/L in January-February 2014 and this parameter decreased till July, when reached the lowest level: 6-7 mg/L. The **model is partially accepted** and offers the daily variations for the entire period studied, suggesting the **ecological state: very good-good-medium**.

For the sites: S3-Sculeni, S4-Leuşeni, S5-Leova, S6-Cahul, S7-Cîşliţa-Prut and S8-Giurgiuleşti, the simulation provided results with low and high peaks but there are long periods with concentration 0 mg/L - impossible for life supporting (anoxic accident), with no suitable interpretation.

Without these simulation peaks, the **model is partially accepted** and the **ecological state: very good-good-medium**.

Biochemical oxygen demand in 5 days (BOD₅)

According to the Romanian **Management Plan for Prut River**, the ecological states based on biochemical oxygen demand in 5 days are:

2-3 mg/L - very good/good

4-6 mg/L – good/medium.

According to **H.G. Nr. 890/12.11.2013** (Republic of Moldova), the water quality is separated in 5 classes based on biochemical oxygen demand in 5 days:

Class I < 3 mg/L (highest quality)

Class II < 5 mg/L

Class III < 6 mg/L

Class IV < 7 mg/L $\,$

Class V > 7 mg/L (lowest quality).

Site S1- Stânca-Costești: the highest concentrations were recorded during August-September for 2013 and 2014. This parameter followed similar trends from the beginning until the end of monitoring. According to the simulation, the highest peaks were around 3 mg/L in August-September 2013, and decreased till winter, when reached the lowest level: 1.5 mg/L. The **model is accepted** and offers the daily variations for entire period studied, suggesting the **ecological state:** very good.

For the sites: S2-Branişte, S3-Sculeni, S4-Leuşeni, S5-Leova, S6-Cahul, S7-Cîşliţa-Prut and S8-Giurgiuleşti, the simulation provided results with extremely high peaks; **no suitable interpretation** was possible.

Nitrates (NO₃⁻)

According to the Romanian Management Plan for Prut River, the ecological states based on nitrates (NO_3) concentrations in water are:

0.7-2.4 mg/L N – very good/good

2.4 - 5.5 mg/L N - good/medium.

According to **H.G. Nr. 890/12.11.2013** (Republic of Moldova), the water quality is separated in 5 classes based on nitrates (NO_3^-) concentrations in water:

Class I < 1 mg/L N (highest quality)

Class II < 3 mg/L N $\,$

Class III < 5.6 mg/L N

Class IV < 11.3 mg/L N

Class V > 11.3 mg/L N (lowest quality).

Site S1-Stânca-Costești: the highest concentrations were recorded during April-May for 2013, 2014 and 2015. This parameter followed similar trends from the beginning until the end of monitoring. According to the simulation, the highest peaks were around 0.8 mg/L N in spring, and decreased till winter, when reached the lowest level: 0.3 mg/L N. The **model is accepted** and it offers the daily variations for the entire period studied, suggesting the **ecological state: very good**.

Site S2-Braniște: the highest concentrations were recorded during April-May for 2013, 2014 and 2015. This parameter followed similar trends from the beginning until the end of monitoring. According to the simulation, the highest peaks were around 0.7 mg mg/L N in spring and decreased till winter, when reached the lowest level: 0.4 mg/L N. The **model is accepted** and offers the daily variations for the entire period studied, suggesting the **ecological state: very good**.

Site S3-Sculeni: the highest concentrations were recorded during July for 2013 and 2014. This parameter followed similar trends from the beginning until the end of the monitoring. According to the simulation, the highest peaks were around 0.8 mg/L N in summer and decreased till autumn, when reached the lowest level: 0.3 mg/L N. The model offers the daily variations for the entire period studied, suggesting the **ecological state: very good**. The **model can not be accepted** because it generates too many errors (e.g., 0 mg/l N).

For the sites: S4-Leuşeni, S5-Leova, S6-Cahul, S7-Cîşliţa-Prut and S8-Giurgiuleşti, the model offers the daily variations for the entire period studied, suggesting the **ecological status:** very good/good. The model can not be accepted because it generates too many errors.

Ammonia (NH₄⁺)

According to the Romanian Management Plan for Prut River, the ecological states based on ammonia (NH_4^+) concentrations in water are:

0.09-0.62 mg/L N - very good/good

0.62–1.4 mg/L N – good/medium.

According to **H.G. Nr. 890/12.11.2013** (Republic of Moldova), the water quality is separated in 5 classes based on ammonia (NH_4^+) concentrations in water:

Class I < 0.2 mg/L N (highest quality)

Class II < 0.4 mg/L N

Class III < 0.8 mg/L N

Class IV < 3.1 mg/L N

Class V > 3.1 mg/L N (lowest quality).

Site S1-Stânca-Costești: the highest concentrations were recorded during April-May for 2013, 2014 and 2015. The parameter followed similar trends from the beginning until the end of monitoring. According to the simulations, the highest peaks were around 0.15 mg/L N in spring and decreased till winter, when reached the lowest level: 0.05 mg/L N. The **model is accepted** and offers the daily variations for the entire period studied, suggesting the **ecological status: very good**.

For the sites: S2-Braniște, S3-Sculeni, S4-Leușeni, S5-Leova, S6-Cahul, S7-Cîşlița-Prut, S8-Giurgiulești, the simulation provided results with extremely high peaks; **no suitable interpretation** was possible.

Conclusions and perspectives

Modeling aquatic ecosystem of the river Prut is a major benefit for both the current assessment of its condition, and for its exploitation in the future, in order to test the impact of various pollutants, to simulate how an aquatic ecosystem tolerates a degree of pollution and, especially, to establish appropriate measures to maintain sources of pollution within bearable limits of the ecosystem.



Phytoplankton total biomass at S1- Stânca-Costești with real predicted results for each day of sampling

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Copepods total biomass at S1- Stânca-Costești with real predicted results for each day of sampling



S1_Costesti-Stanca

Dissolved oxygen at S1- Stânca-Costești with real predicted results for each day of sampling



CBO5 at S1- Stânca-Costești with real predicted results for each day of sampling



Nitrate NO3⁻ at S1- Stânca-Costești with real predicted results for each day of sampling



Ammonia NH₄⁺ at S1- Stânca-Costești with real predicted results for each day of sampling

CHAPTER IV. THE PLAN/MEASURES OF RISK MANAGEMENT

On 29 January 2007 was issued **Order no. 132** of Administration and Interior Minister of Romania, approving the Methodology for elaboration the *Plan for analysis and hedging of risks*, and *Framework-Structure of the Plan for analysis and hedging of risks*.

Section 1 "Natural risk analysis" contains references on:

- Dangerous weather phenomena - considering the areas where such phenomena occurred and their likelihood to occur in new places;

- Floods – considering whether floods are predictable and how long before, dynamic effects and technical condition and of maintenance of water works, areas planned for controlled flood, etc.;

- Storms, tornadoes, drought, frost - considering whether these phenomena are predictable, how long before, the settlements/land/objectives that may be affected.

Section 2 "**Technological risks analysis**" includes references to the industrial risks: analyzes the activities of major-accident hazards involving dangerous substances, the types of hazardous chemicals used in production. For the risks of transportation and storage of dangerous goods there are analyzed the possible accidents that may occur on the road, rail and maritime network for transportation of hazardous materials, the componence and destination of transports.

As regards water pollution risks are inventoried places where such events have occurred and possible new places of their occurrence, areas that may be affected. Avoiding risk event, reduction of the frequency of production or limitation of their consequences is achieved through constant monitoring of environmental parameters and data transmission to the competent authorities. To cover the **cross-border risks** are concluded cooperation agreements with similar institutions from bordering countries, which provide ways for information on probable hazards, warning/alarm if they are occuring, ways of common intervention upon cross-border risks, and also exercises and applications with international participation.

According to Framework Directive 2000/60, transposed in Romania by Law 107/1996 (amended by Law 310/2004), the **environmental objective** is the class good (Class II) of quality, from which the **environmental risk** starts. The water quality in the metropolitan area of Iasi shows risk of failing environmental conditions. Water quality does not meet the requirements of Romanian legislation adopted by implementation. In the top of pollution, the section Prut-Iaşi is on the 3rd place, and in the top of impact, on the 1st place.

Risk acceptance criteria, adopted by the regulatory authorities, show that risks and hazard must be "to the limit of possible reasonableness" (ALARP program) or "to the limit of tangible reasonability" (ALARA program). Prut river pollution can be assigned to the unacceptable risk, given that the probability of occurrence is high. Thus, special attention must be given by resorting to means more diverse, able to reduce the probability of a new pollution and/or reduce the impact of accidental pollution. In this way, the risk becomes negligible or acceptable, in accordance with the criteria of evaluation of the consequences severity, and their acceptance limits. Current policy provides no guarantee that if risks must indeed be assumed, they are at least socially acceptable. According to EU policy on environmental risk management, if there is a credible risk but still unproven scientifically, of the potential health effects to humans or the environment, the precautionary principle should prevail. This means identifying and assessing risks and appropriate measures (balancing the risk by the social utility). If the risks are difficult to estimate, the maximum imaginable risk will be assumed.



Risk levels and ALARP

To decrease the risk, several steps are required:

- 1. Assessment of the current situation is required as an immediate measure to appreciate the risk degree and accelerate the implementation of safety rules.
- 2. Elimination of vulnerabilities, when existing problems are solved, thus is minimizing the risk of success of future attacks.
- 3. Establishment of security requirements, step specifying exactly what we want to protect.
- 4. Development of security policy, stage detailing what and how we protect.
- 5. Implementation of security policy, when are effectively implemented work and procedures to achieve the objectives set by the security policy.
- 6. Implementation of ISO standard for security management. The organization that succeds to implement this standard can anytime demonstrate to its customers the quality of services renders.
- 7. Maintaining security system, a mandatory step in the context of extraordinary dynamics of problems diverse and complex that may arise.

IV.1. Recommendations regarding the protection and preservation of indigenous ichthyofauna diversity

Pollution reduction

Activities to protect, conserve, and improve water quality, as life environment inalienable for fish habitation and maintaining high ichthyofauna diversity, are based on two main groups of activities: structural and nonstructural.

Among the *structural* ones:

- 1. construction of new wastewater treatment plants and upgrade of old ones;
- 2. improvement of ecological and agricultural technologies and practices;
- 3. improvement of landfills;
- 4. development of the sewerage system, etc.

Among the *nonstructural* ones:

- 1. development and implementation of strict standards;
- 2. training of highly qualified personnel;
- 3. conducting information and ecological education campaigns;
- 4. amendment of national legislation on management of waters and aquatic biological resources.

Ecological restoration of Prut River habitats and expanding of protected areas

Protection of biodiversity must be manifested through the conservation of natural biocoenoses in their entirety and not of some species taken part. Therefore, one effective measure for diversity ichthyofauna conservation consists of establishment of protected areas, which must operate not only at declarative level.

In order to protect and preserve the ichthyofauna diversity, an objective of particular importance is the establishment and broadening of integral nature reserves on the Lower Prut, as wetlands of importance in spontaneous flora and fauna "preservation and protection".

One of the most important measures for ichthyofauna diversity conservation, especially in lotic ecosystems, is the counteracting of the effects of river fragmentation and ensuring fish migration, which can be achieved by: construction of fish jumpers or ladders, development of secondary branches along the dam lakes, keeping dead branches in the old riverbed of rivers hydrotechnically arranged, etc.

Ecological restoration consists of actions to ensure the continuity of flow, by destruction of dams, cutting of the steep banks (which would immediately reduce erosion) or their extending as smooth slopes, stabilization of the river bottom in the places with high speed of water current by introducing in the riverbed of stones and large boulders, and also by planting of vegetation or grass, shrubs and trees of the hydrophilous species, which fix the banks through their root system.

Provision of favorable conditions for breeding during the period of prohibition

To maintain ichthyofauna biodiversity and an optimal state of populations it is necessary to provide favorable breeding conditions during the prohibition period. Only four months of adequate protection of the natural reproduction (early March - end of June), jointly with maintaining optimal hydrological regime can provide a high fish productivity for many years.

For the reservoir Stânca-Costești is recommended to maintain the water level as high and constant as possible during the period of prohibition (for flooding the laying areas of roe), reduction by one meter in July and a repeated reduction before setting the ice bridge. This annual dynamic of the hydrological regime will ensure the reproductive success in various species of fish, juvenile growth and development in optimum conditions, mineralization and disinfection of the reproductive substrate, and the covering with aquatic vegetation of the laying areas of roe, thus preparing them for future reproductive year.

In riverbed sectors, water level should be maintained at maximum for at least a month, which will allow flooding of floodplains (mid-April - mid-May usually, when occurs most of the phytofilous fish species reproduction); the maximum hydrological has to be repeated after 2-3 weeks of the completion of the reproductive period, thus facilitating the exit of reproducers and offspring from flooded areas.

A total ban or suspension for a limited period of the industrial fishing, and development of sport fishing infrastructure, accompanied by appropriate environmental education

These measures are extremely important to restore livestocks of rare species with different status. Their implementation will have a positive effect both on the population structure of large-sized species, decreasing the pressure on the elder ages and on ubiquitous small-sized

species, avoiding cases of epizootic deseases and a reduction of the trophic base of the ecosystem (biomass being more actively exploited by the predatory large-sized fish and by sport fishermen).

The ban of fishing of certain species with rarity status, and protection of their characteristic habitats

For the species to be taken under adequate and effective legal protection, it is necessary to know the factors causing its decline. For rare small-sized species (eel, golden loach, European Mudminnow, Eurasian minnow, Schneider, loach etc.), fishing limitation or prohibition will not have any expected effect because they strain through the mesh of the nets, and the small mouth does not allow them to be frequently fished with the angle. Thus, the measure of prohibiting fishing of certain species can be a disadvantage, giving to executive bodies the illusion that species protection is achieved, diverting their attention from the threatening factor. For these species, the most appropriate measure is the protection of habitats where they were identified.

In many cases, however, to highlight the particular importance of certain taxa (for maintaining biodiversity and biocoenoses functionality, economy, etc.), especially those with international protection status, requires a particular approach, with in-depth studies on the taxon biology, its populational state within the research area and whole area of spreading, the study of current international practices, and only then will be possible the development of effective scientific-practical programs of species protection and increase of the population livestocks.

Based on data obtained, an **ecological file** is created **for each species of Community interest**, including specific recommendations and maximum efficiency in taxon rehabilitation.

Usually, taxa with international protection status have discontinuous spreading and the causal factors can be distinguished from one country to another. In many cases, restoration of the entire ecosystem is very difficult and almost impossible, and the decline of the species can be caused by a single limiting factor (for example, a certain source of pollution, the effect of illegal fishing during migration, the dam built on the migration route, etc.). In these circumstances, "removal of this destructive influence" is much easier and cost effective than a full ecosystem protection.

Ecological-industrial reproduction of native species with major regulatory potential

Such species are: zander, pike, European catfish, burbot, sterlet, tench, wild form of European carp, etc. An effective measure is building and installing of artificial nests, especially for zander, catfish, bream, vimba bream and other native species, in the deficitary places for laying of roe, or in the years with unsatisfactory water level.

Currently, there is a possibility of reproduction in captivity conditions, virtually for any species of fish, using recirculation growth systems. This modern method offers several advantages: 1) simulates the optimal conditions for growth and reproduction that are characteristic for each taxon, regardless of external environment influences and bio-ecological demands; 2) the integral control of quality and exact planning of the quantity of aquatic organisms grown; 3) limited water consumption and surface area required by the technological process; 4) producing a minimal negative impact on the environment compared with other methods of semi-intensive and intensive breeding (fragmentation of biotopes, release into the environment of excessive amounts of fertilizers, drugs, anabolism substances, disinfectants, etc.).

To revive the livestocks of species with different status of rarity through ecologicalindustrial breeding methods, the genetic diversity should be considered as well, the use of a small number of spawners being inacceptable.

Populating of ecosystems with rare species must be done with well developed offspring, with active feeding, to ensure a high survival of the future breeding stock. Populating with small offspring with mixed feeding endogenous-exogenous will lead to mass mortality, straining

trophic relations with other planktonophagous species and ultimately compromise the actions of fish stocks recovery.

More active use of the method of "*bio-manipulation and bio-improvement*" in the prevention of the phenomena of bio-invasion and eutrophication

In the actual conditions is necessary to adjust the livestocks of multi-dominant species with short and medium life cycle, by increasing the share of largesized ichthyophagous species such as zander, rapacious carp, catfish, pike, etc.

By its quality of "key-predator", the ichthyophagous fish maintains an optimal balance between the livestocks of fish species from the lower trophic levels that have similar ecological niches, so preventing monopolizing trophic resources by opportunistic taxa (thus serves as a biological regulator and allows the cohabitation of a large species variety).

Increasing the share of ichthyophagous species (zander, pike, catfish, rapacious carp, etc.) should not be reduced to their reproductive measures in ecological-industrial conditions (with later release in ecosystem), but it is also necessary the optimization of the age structure of existing populations, being forbidden the excessive extraction of average and higher age groups (as the most important reproducers and consumers of species with short life cycle).

Depending on the hydro-biotope characteristics and the particularities of the species subject to numerical regulation, it should be populated with the right ichthyophagous (for silver crucian carp, Chinese sleeper, pumpkinseed: pike or catfish; for stone moroko, the opportunistic species: zander or perch; in some small rivers would be appropriate the populating with pike and perch, and for riverbed lakes is recommended populating with European catfish, pike and zander).

Regardless the ecosystem, if possible, is recommended to populate with more ichthyophagous species (e.g. in lakes: catfish, zander, pike and rapacious carp), their diversity being a regulating factor not only for the abundance of prey species, but also serving as guarantor for maintaining the balance in relations between predators being forbidden the "numerical explosion" of one of them.

Many lakes and ponds with fish and recreational destination are located on the course of small rivers and serve as important sources and direct vectors for the spread of invasive fish species in all directions (stone moroko, silver crucian carp, pumpkinseed and others), including the Prut.

When in these aquatic basins intended for fisheries are used the methods of biomanipulation and bio-improving with predatory fish species is recommended to populate these ecosytems with densities well established and preferably with ages at least one year younger compared with the cyprinidae species economically valuable.

In large lakes, such as Stânca-Costești, zander, catfish, pike and rapacious carp in addition to their unquestionable importance as bio-improvers, are now becoming "a popular and fashionable object" in the sport and amateur fishing, amounts collected from such activity may be later used for the revival of environmental status of Prut River Basin.

As ameliorative fish species in the struggle with the phenomenon of "water blooming" are recognized the Asian cyprinids introduced in the 60s of last century. Thanks to different nutrition way (silver carp - phytoplanktonophagous, bighead carp – zooplanktonophagous, and grass carp - macrophytophagous) in the biocoenosis are created only 2-3 trophic links (phytoplankton \rightarrow silver carp, phytoplankton \rightarrow zooplankton \rightarrow bighead carp, or higher aquatic plants - grass carp). In these circumstances, in the simplified ichthyocoenosis (formed by few species) occur minimum energy losses and maximum accumulation of fish biomass (without causing trophic competition with the zoobenthonophagous indigen carp). Populating the Stânca-Costești reservoir with silver carp and grass carp in scientifically substantiated quantities is welcome in this regard, aiming at obtaining multiple ecological and economic benefits.

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APPENDIX 1. Action of risks upon fish populations



APPENDIX 2. Scheme to reduce threats/risk factors









ALEXANDRU IOAN CUZA UNIVERSITY of IAŞI

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