X-TH INTERNATIONAL CONFERENCE OF ZOOLOGISTS

SUSTAINABLE USE AND PROTECTION OF ANIMAL WORLD IN THE CONTEXT OF CLIMATE CHANGE

dedicated to the 75th anniversary from the creation of the first research subdivisions and 60th from the foundation of the Institute of Zoology

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X-th International Conference of Zoologists

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The proceedings of the X-th International Conference of Zoologists ”Sustainable use and protection of animal world in the context of climate change”, dedicated to the 75th anniversary from the creation of the first research subdivisions and 60th from the foundation of the Institute of Zoology, organized by the Institute of Zoology, are a generalization of the latest scientific researches in the country and abroad concerning the diversity of aquatic and terrestrial animal communities, taxonomy, systematics and evolution of animals, structure and dynamics of animal populations in natural and anthropized ecosystems, population functioning and role of animals in maintaining the ecological equilibrium in the context of climate change, biological control of pests, invasive species, their ecological and social-economic impact, molecular and genetic methods in systematics, phylogeny, phylogeography and animal ecology, protection of rare, endangered and vulnerable animal species under the conditions of intense anthropogenic pressure, in the context of climate change.

The papers include useful information for zoologists, ecologists, ethologists and for professionals in the field of protection and sustainable use of natural patrimony.

The reviewed materials are approved and recommended for publishing by the Scientific Council of the Institute of Zoology.

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MONITOX INTERNATIONAL NETWORK FOR MONITORING OF ENVIRONMENTAL TOXICANTS AND RISK ASSESSMENT IN THE BLACK SEA BASIN: RESEARCH AND INTERDISCIPLINARY COOPERATION DIMENSIONS

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Abstract. The paper presents the main tackled issues and results concerning the monitoring of the toxic substances (TOXs) in the network established in the frame of BSB27 MONITOX project, implemented by «Dunarea de Jos» University of Galati, Romania (Leader Partner) in partnership with Institute of Zoology, Republic of Moldova, International Hellenic University (IHU), Greece, Institute of Geology and Seismology, Republic of Moldova (IGS), and "Danube Delta" National Institute for Research and Development, Tulcea, Romania.

Maps of pollution of water, sediments, soils and biota with TOXs built using ArcGIS and Q-GIS highlight their levels and spatial-temporal distribution in the target zones in the three countries: Danube River (Lower sector), Prut and Dniester Rivers, Danube Delta, Black Sea coast, Nestos River and delta and Northern Aegean Sea coast.

The strategy designed and results of the interdisciplinary studies performed in the period 2018-2021 on a large range of toxic pollutants in the Black Sea Basin (including NE part of Greece) are emphasized, as well as the assessment of toxicants' impact upon human health using a health risk calculator developed as ICT tool.

Introduction

The migration of toxic chemicals (TOXs) with great impact on human health in the main aquatic ecosystems and protected areas from SE Romania, Republic of Moldova and Eastern Macedonia and Thrace, Greece, affects a large number of population of the sea basin and a very important issue for inhabitants of the large basin will be the identification of the sources of TOXs in their neighboring aquatic ecosystems and groundwater - agricultural terrains (fertilizers, pesticides), insufficient industrial and domestic wastewater treatment, erosion of polluted riparian soils, accumulations of municipal garbage and hazardous wastes, oil spills, vessel discharges, atmospheric depositions, release of toxics both from natural and anthropogenic sources (industry, traffic, animal farming, burning products, port and coastal activities, hospitals, nuclear activities) [1].

There is a need for high-quality monitoring data for the Black Sea Basin (BSB) and the European Union as a whole, along with ecotoxicological data and a thorough study of risks of environmental effects of TOXs. At basin level it was identified the necessity for establishing a new set of indicators to be used in various regions in monitoring of emerging toxic pollutants in water and related environmental matrices (sediment, biota), as well as the revising of the legislation regarding the thresholds of existing contaminant substances in these compartments, as a result of new scientific knowledge [2] and published literature data in international scientific journals.

There are some aspects which must be elucidated, related to: the real influence of the geological background on migration of some trace elements, metals and other compounds from the bedrock to sediments and surface water or ground water, the influence of river or sea on the quality of the groundwater, concentration of toxics preponderant in a matrix, the pollution of lands in the vicinity of rivers and seas with a large spectrum of TOXs which could spread on large distances in other compartments and could affect human health [1].

The main threat in the region is the immense deterioration perpetrated by human activities against the natural environment, which raises imperative concern for our collective survival. Pollution has no borders/does not stop at national borders. The in-depth study of processes occurring in large, interconnected river basins and evaluation of people exposure to TOXs can be accomplished only in partnership, through cooperation based on knowledge, exchange of good practices and interdisciplinary research, conducted only in transnational networks.

The project BSB27-MONITOX is aiming at enhancing regional cross-border cooperation in the Black Sea Basin (BSB) to improve joint monitoring of environmental toxic pollution and better share and exchange of new analysis methodology, data and information on ecological state and human health impact of harmful substances. This implies:

1) to build a strong BSB network of analytical laboratories and experts (MONITOX) to elaborate a common ecotoxicological monitoring system supporting regional programmes for environmental protection and sustainable management;

2) to produce a scientific platform with harmonized information on toxics in soil, water, sediments and biota in shared riverine, deltaic and sea areas, and their potential impact on ecosystems and people.

Materials and methods

The starting point of the project were the pressing issues signalled at European level, and the common challenges in the region jointly identified through analysing European legislation and EU Directives for environmental quality assessment; international conventions regarding the toxic chemicals and wastes (Minamata Convention on Mercury, Stockholm Convention on Persistent Organic Pollutants, Basel Convention on the Control of Transboundary Movements of Hazardous Wastes and their Disposal, Rotterdam Convention on the Prior Informed Consent Procedure for Certain Hazardous Chemicals and Pesticides in International Trade); Danube and Black Sea Conventions; Convention on the Protection of the Black Sea Against Pollution; Convention Access to Information, Public Participation in Decision-making and Access to Justice in Environmental Matters (Aarhus); Convention on Environmental Impact Assessment in a transboundary context (Espoo); Convention on Wetlands of international importance (Ramsar); Convention on protection and use of transboundary water courses and international lakes (Helsinki); WHO and UNSCEAR regulations and recent scientific literature. Own experience gained in the frame of the previous implemented projects and from published research contributions of the five partners was valorized in the proposed activities and outputs [1,3].

The project is implemented by "Dunarea de Jos" University of Galati, Romania (Leader Partner) in partnership with Institute of Zoology, Republic of Moldova, International Hellenic University (IHU), Greece, Institute of Geology and Seismology, Republic of Moldova (IGS), and "Danube Delta" National Institute for Research and Development, Tulcea, Romania.

The target zones in the three countries in BSB are: Danube River (Lower sector), Prut and Dniester Rivers, Danube Delta, Black Sea coast, Nestos River and its delta and Northern Aegean Sea coast.

The **MONITOX international network strategy** of monitoring of six classes of toxic substances in seven interconnected environmental compartments (surface water, groundwater, sediments, soils, bedrock and biota) in the Black Sea Basin and border regions, along with eight types of connected investigations (geomorphological, geological/mineralogical, hydrogeological, physical, chemical, biological, microbiological, ecotoxicological), along with mapping of BSB pollution, modelling of environmental processes and risk assessment is presented in Figure 1. Our pledge is: "Through collective action, environmental protection can be achieved" [3].

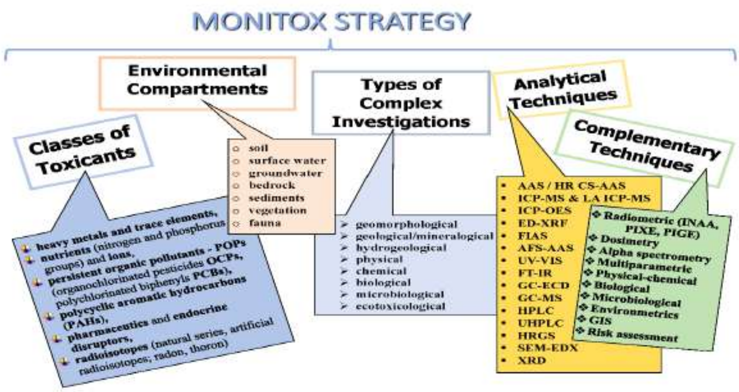

Figure 1. MONITOX international network investigation strategy

One of the project goals are the integration of the chemical, radioactivity, biological and microbiological measurements, in order to establish the surface water quality classes for the first time based on an integrated ecotoxicological quality index, with important impact on human health [3,4].

The analytical methods employed in partner institutions from Romania, Moldova, Greece and collaborating ones and used in complementarity for elemental and isotopic determination in sampled environmental materials (about 50 chemical elements), are the following: atomic absorption spectrometry (AAS), including High Resolution Continuum Source (HR CS-AAS) technique; inductively-coupled plasma mass spectrometry (ICP-MS) and laser ablation (LA) ICP-MS; inductively-coupled plasma optical emission spectrometry (ICP-OES); X-ray fluorescence analysis with energy dispersion (ED-XRF), including portable technique (p-XRF); high resolution low background gamma-ray Spectrometry (HRGS); instrumental neutron activation analysis (INAA) and ion beam techniques Particle-Induced X-ray (PIXE), Gamma-ray (PIGE) Emission using accelerated proton beams. Mercury is analyzed by flow injection for atomic spectrometry (FIAS) and a combination of fluorescence and absorbtion spectrometry (AFS-AAS). The accuracy and precision of the results are evaluated by measuring certified reference samples of similar matrix and organizing several intercomparison exercises between research laboratories [3,8]. More information is gathered for soil mineralogical constituents and microstructure using FT-IR, SEM, SEM-EDX and XRD complementary techniques.

Persistent organic pollutants (OCPs, PCBs) and pocyclic aromatic hydrocarbons (PAHs) are quantified by using gas-chromatography (GC-ECD, GC-MS) and liquid chromatography (HPLC) techniques. Moreover, oil pollutants (total petroleum hydrocarbons) in marine environment are investigated by FT-IR. UV-VIS is employed for nutrients and ions analysis in water samples, in connection with multiparametric and physical-chemical investigations.

The set of ecological indicators which are routinely monitored in the frame of national monitoring systems does not include yet the emerging toxicants, such as pharmaceutical residues and metabolites (antibiotics, anti-inflammatory, contraceptives), which are actually found in wastewater, surface water and groundwater. These are specified in EU-wide water monitoring Directive 2013/39/EU, which amended Water Framework Directive 2000/60/EC, and required a strategic approach to the pollution of water by pharmaceutical substances [1]. In the future the national monitoring systems at EU level will have to implement such indicators for emerging contaminants which might be appropriate for prioritization. Moreover, at Union level, it is foreseen to set environment quality standards (EQS) for newly identified substances, revising EQS for some existing substances in line with the scientific progress, and setting biota EQS for some pollutants [4]. An absolute novelty of investigations at maritime basin level is the adaptation and applying of Orbitrap UHPLC-MS for pharmaceuticals analysis in (Danube, Black Sea) surface water [9].

In May 2019 governments have amended the Basel Convention to include plastic waste in a legally-binding framework which will make global trade in plastic waste more transparent & better regulated. Although microplastics were not included in the proposed list of monitored contaminants, research was carried out in the project on FT-IR identification of these polymeric materials in cosmetics, personal care and everyday use products.

Radiological maps and pollution maps of water, sediments and soils will be built using ArcGIS and Q-GIS [4].

Results and discussions

The planned objectives foresee complex investigations of the processes that take place in environmental compartments of aquatic ecosystems by assessing changes in chemical composition and water quality (physico-chemical, chemical, biological, microbiological parameters) in hydrographic basins of Danube, Prut, Dniester, Nestos and Black and Aegean Seas coastlines and assessment of the impact of TOXs on ecosystems state and human health.

To achieve these plans, joint complex expeditions in target aquatic ecosystems were organized, sampling and laboratory analytical techniques were adapted, optimized and harmonized between partners and modeling of processes and evaluation of ecological and health risks was carried out. Collection of environmetal data in the periods before and after COVID-19 pandemic allowed the comparison of ecological state and evaluation of anthropogenic impacts [6,11,12].

Besides the novel implementation strategy, another specific objective of the BSB27 project is boosting awareness of the population in the Black Sea region on the environmental issues related to spread of toxicants by education and sharing scientific information and knowledge on health effects of inorganic, organic and radioactive pollutants. Dissemination of reliable, scientific information to various target groups is an important task of our project and is accomplished through ICT tools, including the assessment of toxicants impact upon human health, thus raising the level of awareness of the population in the Black Sea region regarding the environmental issues with cross-border impact: preservation of common natural resources and protected areas, prevention of spread of toxicants, hazardous waste disposal, changing behaviour on release of pollutants and reduce litter generation [5].

Digital maps of pollution, built using Q-GIS software, will be distributed through an IT platform created on the project website, together with scientific information regarding the impact of toxicants on population and aquatic ecosystems, possible sources in environment, modality of cross-border migration and accumulation in trophic chains in target areas of Black Sea region (rivers basin, deltas, sea littoral, marine environment), maximum allowed concentrations, and related legislation issues. The observed key patterns of elemental accumulation and migration in environmental compartments may be used in various environmental management programs and specific ecological studies in the Black Sea Basin.Through the created project social page on Facebook (fb.me/Monitox.project.BSB27), information on pollutants' spread in environment, their levels and danger to population (through ingestion, inhalation, dermal contact), carcinogenic effects, presence of microplastics in rivers, biota and personal care products, and good practices to reduce the impacts of TOXs upon health and ecological state are permanently distributed [5]. Innovative tools for the assessment of toxicants' impact upon human health and ecological state of aquatic ecosystems using a health risk calculator were developed. The health risk calculator (HRC) is designed as a webpage connected by a link to the project website. The HRC will also include a food toxicant calculator in order to evaluate the potential risk to human health of consumed fish or seafood, based on the estimate daily intake (EDI), the target hazard quotients (THQs), and carcinogenic risk ratio (R) as risk assessment elements [5]. A collection of digital materials in the form of a "Citizen's guide for evaluating exposure to toxicants" will be prepared for different categories of public. A great attention has to be payed to the analysis by dosimetry and alpha spectrometry of the gaseous radioactive isotopes, e.g. radon (222Rn and 220Rn) which represents the second factor of risk for population for pulmonary cancer, after smoking.

The project results were distributed by the organization of informational events (international conferences [7,10] and symposium [13], regional workshops, methodological seminars, webinars, expert trainings and practical activities in laboratories), publications in international journals and methodological guides. Their sustainability will be achieved through the elaboration and initiation of new projects at various national and international funds, preparation of scientific papers in open access journals, elaboration of laboratory guides and presentations for students courses on various topics tackled by the project.

The developed strategy, knowledge and common solutions for improved joint environmental monitoring in BSB will lead to:

- a better informing of various stakeholders on the existent levels of TOXs in the region,
- understanding of complex processes which take place during TOXs migration and accumulation in food chains,
- understanding the influence of toxicants and hazardous wastes on ecological state and human health.

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ASSESSING THE IMPACT OF HYDROPOWER CONSTRUCTIONS ON FUNCTIONING OF DNIESTER AND PRUT RIVERS ECOSYSTEMS WITHIN THE HYDROECONEX PROJECT

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Abstract. The article reflects on the main issues, research methodologies and achievements of the project HydroEcoNex, a transboundary project carried out by a consortium of research institutes, NGO and a university – Institute of Zoology, International association of river keepers "Eco-Tiras" (Republic of Moldova), "Dunărea de Jos" University of Galati (Romania), as well as Ukrainian Scientific Center of Ecology of the Sea and Hydrometeorological Center for Black and Azov Seas. Among the main obtained results are the development of a common methodology with various set of indicators for assessing hydropower impact and climate change, assessment of lost ecosystem services, sharing of generated knowledge to students and researchers, endowment of the research laboratories with advanced research equipment.

Introduction

Sustainable joint usage of transboundary water resources under the impact of hydropower, climate change, are common challenges for a number of rivers of the Black Sea Basin (BSB), including the Dniester River, which is shared by Ukraine and Republic of Moldova as well as Prut river shared by Ukraine, Romania and the Republic of Moldova. At the European level, hydropower, particularly small hydropower constructions are promoted at a wide level, as these do not contribute to CO2 emissions thus being a climate friendly way of producing energy and the multiple benefits from the hydropower reservoirs, for example the water storage for drinking and irrigation, drought-preparedness, flood control, fish farming and recreational opportunities, having at the same time the negative impacts on water biodiversity and river hydromorphology. At the same time, large hydroelectric dams have an enormous adverse impact on the environment, due to significant modifications, with serious consequences on aquatic communities as well as at ecosystem level. Monitoring of the status of Dniester and Prut rivers under the impact of hydropower are very relevant, considering the role of these rivers as a major source of water for agriculture, industry and drinking water supply for the Republic of Moldova, Ukraine and Romania.

HydroEcoNex is an international project, implemented by a consortium of five partners: Institute of Zoology, International Association of River Keepers "Eco-Tiras" (Republic of Moldova), "Dunarea de Jos" University of Galați (Romania), Ukrainian Centre for the Ecology of Sea and Hydrometeorological Centre for Black and Azov Seas (Ukraine). The project HydroEcoNex aims at developing a unified system of innovative environmental monitoring for the provision with data and information essential in the transboundary and sustainable long-term monitoring of observed transformations in Black Sea Basin's river ecosystems, caused by hydropower operation under climate change. On the Dniester River, the project focuses on areas located downstream and upstream of the Dniester Hydro-energetic Complex, including the station no. 1 (HPP-1), and Hydro Power Plant No. 2 (HPP2), built directly on the Dniester River and the Hydroenergetic Station of Accumulation via Pumping (CHAP), constructed on the right bank of the Dniester River and feeding from the buffer reservoir (located between HPP- 1 and HPP-2) and the built-up reservoir on the river bank and on the Prut River in Stânca - Costești, on the both banks (Romania and the Republic of Moldova).

The current article makes an overview of the main objectives, methodology of work and achievements of the consortium partners of HydroEcoNex during 2018-2021.

Materials and methods

In order to assess the hydropower and climate change impact on transboundary rivers Dniester and Prut, the HydroEcoNex consortium used the following methods of investigation:

Research expeditions. These were carried out downstream and upstream of Dnestrovsk and Stânca-Costești Hydro-energetic complexes, including those with joint participation of several partners (Institute of Zoology - Ukrainian Scientific Center of Ecology of the Sea – Hydrometeorological Centre for Black and Azov Seas; Institute of Zoology - International Association of River Keepers "Eco-Tiras" – Hydrometeorological Centre for Black and Azov Seas; International Association of River Keepers "Eco-Tiras" – Hydrometeorological Centre for Black and Azov Seas) or individual expeditions of each partner team. The set of principal parameters were analyzed: a) physico-chemical parameters, including temperature and oxygen regimes, sediments, salinity and turbidity, water quality (main ions, nutrients, COD, BOD); b) Biological parameters (bacterio, phyto- and zooplankton, zoobenthos, fish and fish parasites; c) hydrological parameters (annual and seasonal water stocks in the investigated rivers, water level); c) climatic parameters (variability of the air temperature and precipitation). A comparison was made of the actual data with the historic data (pre-disturbance phase - before the beginning of HPP construction and operation).Laboratory analysis. The partners used the existing technical base and acquired new equipment within the project for complex assessment of water quality. The Institute of Zoology purchased modern equipment, those related to chromatography analysis as well as field equipment such as a multi-sensor measuring instrument, for measuring dissolved H2S/total sulphide in water sample under pH and temperature control and free dissolved carbon dioxide concentration directly in the water, also for the determination of the biochemical oxygen demand (BOD) under laboratory conditions, a VELP Scientifica Cooled Incubator FOC 120E with sets of VELP Scientifica Wireless BOD EVO Sensor System were purchased. The "Dunarea de Jos" University of Galati has procured in 2019 the equipment for direct analysis of solid samples through high-resolution continuum source atomic absorption spectrometry (HR CS AAS), graphite furnace technique (GF-AAS), allowing a fast analysis of trace elements in different environmental samples (soils, silts, hydrobionts, etc.) at low sensitivities and an accessory used for direct solid sample analysis by using the ContrAA 700 spectrometer, avoiding in this way the need for time costly preliminary sample preparation; in 2020 the infrastructure for environmental investigations was improved by acquiring a mercury analyzer at trace level.Modeling. Model calculations were carried out, using the numerical model RegCM4.7 for the Dniester catchment area. Such model calculations allow assessing the long-term impact of climate change on runoff parameters and the state of surface fields of temperature, humidity and precipitation and forecasting river runoff for a period of up to 10 years in according to the climatic factors. Three-dimensional spatial structure maps were developed on the distribution of the dissolved oxygen, turbidity of the fields, TDS, salinity and temperature of the water area of the Dniester estuary. A thorough statistical analysis was carried out based on a comparison of the Dniester runoff at its several representative sections in two time intervals comparable in duration, reflecting the runoff volume before the start of construction of this complex and after its commissioning.Economic valuation. The proposed methodology developed by International Association of River Keepers "Eco-Tiras" can be used at different scales – the catchment (basin) scale allow quantifying processes related to water cycle and for the implementation of monitoring plans, at the water body scale – specific functions of ecosystems, supporting certain services are analyzed. The methodological guide developed provide a theoretical description of the approach complemented by examples of evaluating the impact of hydropower and climate change in the context of Dniester River environment [1]. These estimations were based on the results of physical and biochemical parameters, identified in the framework of HydroEcoNex project. Based on these approaches, the authors of these articles tried to make an overview of the published results of the experts involved in HydroEcoNex project as well as other relevant studies tried to reveal the current changes occurring at ecosystem level which will help to identify common methods for Moldova and Ukraine for diminishing the impact.

Results and discussions

On the Prut river, the comparative analysis of the flow on the Prut's riverbed in 1961–1990 and 1991–2018 – before and after global warming, and in 1950–1975 and 1980–2017, before and after the construction of the Costesti -Stanca hydropower plant (HPP) demonstrated no significant impact on the total water discharge downstream, but only the seasonal distribution of the river's streamflow [1]. Hydropower constructions on Dniester river have caused dramatic deteriorations at ecosystem level, Such changes are for example fragmentation of river continuity, deterioration of the self-purification capacity of the river [2-3], loss of ecosystem services [4], big fluctuation of the water flows and levels [5], alteration of temperature and oxygen regimes [6], bedrock structural changes and annual stock of suspended particles [7], the rate of mineralization and dynamics of the main ions [8], penetration and distribution of alien and parasitic species [9-10]. Monitoring of the status of Dniester river under the impact of hydropower is very relevant, considering that the river is a major source of water for agriculture, industry and drinking water supply for both Moldova and Ukraine. These changes have had negative consequences on hydrobionts. In order to develop common methods of transboundary monitoring, HydroEcoNex project was developed, which united together experts from the Republic of Moldova, Ukraine and Romania for identifying the indicators of impact and propose methods of mitigation. Various tools developed during the project were systematized and presented on the project website and could be downloaded freely by different stakeholders involved in water resource management, but also the general public who care about the future of Dniester river. The project partners have made an overview of the specialized literature and own data on the impact of hydropower on freshwater ecosystems and have elaborated the set of impact indicators. The overview of historical data in comparison to the current scientific results collected by the project partners indicated that the construction of hydropower plants and the reservoirs on Dniester river led to considerable changes at ecosystem level. The research results were reflected in individual and collective research papers published by the experts of the consortium members of HydroEcoNex projects at international conferences and in various journals, including peer-reviewed high impact journals.

The project activities have led to the following main results:

- Development of a common methodology for monitoring of impacts of hydropower on transboundary river ecosystems were developed based on agreed physico-chemical, biological and climatic indicators and available best international practices, also a comprehensive methodology on economic valuation of lost ecosystem services was also elaborated;
- Capacities and awareness among key stakeholders on integrated water resource management in light of impacts caused by hydropower (HP) and climate change were increased. Several knowledge transfer workshops and public seminars were organized for various stakeholders in Odessa, Chisinau and Galati for didactic staff and students of universities, research staff, and representatives of ministries. The information and knowledge on the project results were disseminated not only to the scientific public, but also to the general public, national and local public authorities, students, interest groups and NGOs during different project activities: launching, Steering Committee Meetings, press conferences, public events, radio and TV interviews, a youth summer school, summer kayaks higher students expedition on Dniester, knowledge transfer workshops, public seminars and by means of developed communication tools - the brochure, calendars, leaflets, banners, the website (http://hydroeconex.com).
- A policy instrument (a strategy for bilateral water cooperation on joint monitoring of transboundary rivers affected by hydropower) on integrated monitoring for rivers affected by HP was developed;
- Access to technical and scientific reports, data and methodologies on transboundary monitoring of river ecosystems affected by HP was increased. The project HydroEcoNex developed various tools for mitigation the hydropower and climate change impact including a methodology for economic evaluation of lost ecosystem services, a strategy for transboundary water management, identified set of indicators for assessing the impact,

a project website, which updates the main project results including most recent publications on the main impacts assessed (www.hydroeconex.com), which serve as a platform for knowledge management and dissemination.

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MAINTAINING OF GOOD WATER QUALITY – A PREREQUISITE FOR HEALTHY FARMED FISH

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Abstract. Water quality is one of the key factors in maintenance of fish health. The current article revises the effects of various water quality parameters on fish health, methods of quality analysis and diagnosis of fish health, as well as methods for improving the water quality in fish ponds, aiming to reduce the fish health risks. The synthesis is a joint work of an international consortium formed from scientists of the Institute of Zoology and Ion Ionescu de la Brad Iasi University of Life Sciences, which allows a broader view on the fish health issue for identification of sustainable solutions for fish farmers from the Republic of Moldova and Romania.

Introduction

Maintaining of a good water quality is an important prerequisite for successful aquaculture. A range of water parameters such as water temperature, oxygen saturation, pH, carbon dioxide, ammonium nitrogen, specific pollutants (e.g. pesticides, herbicides, heavy metals, chlorine derivatives, petroleum products etc.) can harm fish in various ways – from physical damage of skin (erosion), gills and internal organs to functional alterations, such as the lower metabolism and ventilation rates, change in circulatory parameters, influence on immune system, which increase their susceptibility to diseases. In addition, some farm management practices, such as improper feed quality and quantities, excessive fertilization or stock density and inappropriate stocking time can also contribute to worsening of the water quality and fish health. For example, feeding fish on high lipid content commercial feed may lead to accumulation of fat in their bodies and, consequently, to bioaccumulation of many toxic pollutants, e.g. PCBs [4, 5].

Thus, periodic investigation of water quality parameters and also of the health status of fish may prevent significant production losses in fish ponds. Measurements of the hydrobiological and hydrochemical conditions along with the fish health status at different production stages or when major fish health issues are encountered are extremely important. The current article made a synthesis of methods for water quality analysis and diagnosis of fish health, various quality parameters affecting fish health, as well as fish pond management measures for ensuring a good water quality for an adequate fish health.

Materials and methods

The diagnosis of fish health status is a rather difficult task, since the fish and water are not sampled during the same period of time when the pollution occurred. Hence, it is necessary to use all the available farm information and relevant field and laboratory analysis to detect the cause of the harm to fish and, where appropriate, to aquatic invertebrates, which provide fish food resources but, also, contribute to the maintenance of the fish ponds. The field investigations shall involve firstly an assessment of previous records of factors, which might have produced the changes at ecosystem level, and recent main factors, responsible for impoverishment of water quality and fish health status. The next steps are to perform the necessary physico-chemical and hydrobiological analyses of the water. If necessary, the bottom sediments, periphyton and then the fish themselves should be examined. Compared to other animal species examined when necessary, fish require little visible contact with the examiner. Thus, specific fishing techniques are required for direct examination of catches, from which fish with an apparent phenotypic pathology or lethargic fish are selected. The examination is based on a detailed history, direct clinical examination, necropsy examination and laboratory examination.

For assessment of the water quality, samples shall be collected for hydrochemical analysis (some measurements are carried out *in situ*, e.g. O2, temperature, pH, transparency, conductivity etc). To have a complete picture, the status of the zooplankton, phytoplankton and benthic communities shall also be assessed.

Besides chemical and hydrobiological analysis, it is important to obtain information from the fish farmers. In order to establish a dialogue, an appropriate simple language shall be used, without the use of scientific or technical terms. The clinical interrogation does not have to be an indictment aimed at discovering the guilt of fish farmer or pond owner. Collateral questions can be used to discover errors in technological exploitation, because there is a tendency to overlook these mistakes. It is important to get information on the used feed supplements (such as vitamins, antiseptics), other substances added to water (lime-based preparations, drugs etc.).

Field examination includes direct observation (inspection) of fish in the water body, in the net or on the shore. Biometric and weight measurements data shall be collected. Fish movements need to be observed as well, in order to register if there are any exaggerated or apathetic swimming movements. Under some stressful conditions, fish may even jump out of the water or swim in a circle or in a spiral. The intense decrease of dissolved oxygen or the diseases accompanied by respiratory disorders, most often associated with gill injuries, will cause prolonged swimming at the water surface or swimming with open mouth. Some disorders may cause swimming in a lateral direction. The examination of the body shape and dimensions, tone and vigour of the movements, appearance of the body surface, fins, scales, gills and eyes can be examined in the field, but also examined more thoroughly in the laboratory, where the presence of wounds, macroscopic parasites, pigmented spots, tumours, edema need can be evaluated. Hand magnifiers may also be used for inspection.

Within the framework of the on-going project 2 SOFT/1.2/47 Team up for healthy fish in aquaculture systems in the Prut River basin - HealthyFish (Joint Operational Program Romania-Republic of Moldova 2014-2020, funded by the European Union), a joint expedition was carried out with the participation of researchers from the Institute of Zoology and the Ion Ionescu de la Brad Iasi University of Life Sciences. The expedition was focused on fish farm Rompescaris SRL (Podu-Iloaiei, Iași county) and S.C. Piscicola S.A. - Drăcșani fish farm (Botoșani county) (Figure1).

Figure 1. a) Joint field trip carried out by the team members of the Institute of Zoology and- "Ion Ionescu de la Brad" Iasi University of Life Sciences at fish farms in Romania, on 25 of May, 2021; b) knowledge sharing between researchers and farmers regarding fish infestation

Researchers and farmers discussed in detail the problems faced by fish farmers and ways to solve them. Hydrochemical, hydrobiological, ichthyological and ichthyoparasitological samples were collected. Some investigations were performed *in situ*, for example, pH, Eh, O2, N-NH4+, N-NO2-, N-NO3-, mineral phosphorus, conductivity, etc. Fish health was also analysed primarily under field conditions.

Results and discussions

Water temperature and its oxygen saturation are among the monitored parameters of water quality in fish growth. These factors, along with the synergistic effect of other coexisting stressful conditions lead to various problems such as discomfort to the fish, disease prevalence and in the worst case – to mass deaths. For example, the change of water temperature can lead to intensification of the metabolic rate of fish, as fish requires more energy to cope with the increased water temperature. Temperature increase also intensifies organic pollution of water, which, in turn, leads to a decrease in the content of dissolved oxygen and, as a consequence, creates a favourable environment for the growth of pathogenic bacteria and parasite invasions [2, 3]. Favourable thermal water conditions for carp vary from 0-1° C to 25° C. Above this value fish usually dies, this being also determined by the lack of oxygen, which correlates with the water temperature. Sharp changes with 10-15° C cause shock to large fish, loss of balance, inactivity, stopping of movement of the gills and finally – suffocation. Young fish are more tolerant, but larvae or juveniles, on the contrary, are more sensitive, as only a small difference of 1-2° C can lead to significant physiological changes or even perishment of larvae.

Oxygen saturation of water is a major factor in fish health. Carp can tolerate low concentrations of oxygen in the water, but such species as perch as pike, vice versa, are sensitive to oxygen deficiency. The lowest critical oxygen level in carp ponds is approximately of 3-4 mg/L. Some signs of severe oxygen deficiency are the death of fish with open mouths and gills, enlargement and redness of the lower lip. In addition, if the pH is too low or too high, damages may also occur, with haemorrhages of the gills and on the lower part of the body. Also research showed that low pH levels can facilitate the metal release from rocks and sediments, which can affect the metabolism of fish and its ability to take up water through the gills [2, 5, 7].

A consequence of the decrease in the amount of dissolved oxygen is the excessive development of cyanophyta algae caused by excess of nutrients. Algal bloom is one of the main causes of the mass perishing of such species as carp, silver carp, bighead carp, grass carp, pikeperch, catfish, crucian carp. Some fish species are even able to accumulate their toxins throughout life through bioaccumulation, thus posing a risk to consumers [2, 7]. Fish death is also often caused by the rapid decay of aquatic vegetation, which contributes to oxygen deficiency and the formation of hydrogen sulphide (H2S), which becomes dangerous at a concentration of 1-10 mg/L. Presence of toxic substances in water may cause poisoning and considerable losses to farmed fish. In spite of the protection ensured by the tough epithelium, scales and secreted mucus, some toxic substances can cause lesions to the tegument, the fins and also damage the ability to secrete mucus and control internal osmotic pressure [2, 5]. Such toxic compounds as heavy metals, chlorine derivatives, pesticides, petroleum products may damage not only the skin, but also internal organs, e.g. liver, thus increasing the susceptibility of fish to disease. Contamination with heavy metals with bioaccumulation properties (accumulation in food chains), such as mercury, even at low concentrations can often cause various diseases not only to fish, but also to consumers of fish products, including humans. Intensive feeding on lipid rich feed will accelerate this process, as fish body fat will accumulate toxins [5]. Also phenol and pesticides, along with chlorine derivatives, even at small concentrations can cause disorders of the nervous system of fish and liver degeneration. Among the most harmful compounds, which are used as insecticides in agriculture, are those containing mercury or gamma-hexachlorocyclohexane (gamma-HCH), as they may accumulate in the muscles of fish, becoming in this way highly toxic to humans.Various components of agricultural waste, as well as organic waste resulting from animal husbandry can cause toxicity in fish and increase the incidence of various diseases. Excessive organic fertilizers can lead to direct damage to fish epithelium. Lime used in water disinfection increases the pH of the water and can influence /damage to the respiratory epithelium of fish.Free ammonia (NH3) and ammonium ions (NH4+) are among the most common toxic substances, the source of which is the breakdown of protein substances and urine or their penetration into sewage from fertilized agricultural lands. Ammonia usually causes problems for intensive farming systems, especially recirculatory ones [1, 5]. Fish may tolerate concentrations of up to 5-10 mg/L of total ammonia in neutral water (pH 7), but in alkaline waters (pH>7) already an amount of 0.2-0.5 mg/L causes convulsions in fish and bleeding of gills. The first signs of ammonia toxicity in fish include a slight restlessness and increased rate of respiration. Fish usually congregate close to the water surface. In later stages, with intensification of ammonia toxicity, fish gasp for air, their restlessness increases with rapid movements and respiration becomes irregular, then a stage of intense activity follows. In the worst cases, the fish can lose their balance, leap out of the water, affected fish lie on their side and spasmodically open widely their mouths and gill opercula [2, 5, 7]. Intensive feeding with a high nitrogen diet may lead to ammonia poisoning, which in combination with other additional stresses, e.g. an abrupt oxygen deficit, or sudden changes in water temperature, may also worsen the fish health status.Along with pollution, poor maintenance of fish pond such as periodic disinfection for algal control, low water level, too high stocking density of reared fish, poor quality or excessive feeding are common causes, which lead to a decrease in water quality, but also in the health status of fish. Some feed supplements, e.g. excess quantity of molasses, feed covered with mold and remnants of fermented agricultural waste may have a negative impact on fish health due to formation of toxic compounds (amines and peroxides), which alter the intestinal bacterial flora. Overcrowding of fry and fingerlings coupled with low oxygen level can lead to quick spread of parasitic diseases, while improper temperatures, nutritional imbalances, chronic exposure to toxic substances, including PCBs, or to high content of suspended solids (200-300 mg/L) can lead to development of the spring viremia and tail rot infections [1-2].This, an optimization of water quality conditions along proper control of feeding of fish are important prerequisites for ensuring a healthy state of fish stock. Also stocking the ponds with the right number of fish and at the right time in the spring are other important preventive measures.Combating the algal blooms is sometimes difficult, because some resistant forms of cyanobacteria, once propagated in the fish ponds, are able to survive for many years in the bottom sediments. Treatment with slaked lime or chlorination of sediments of the fish pond in question (after removing the fish from it), in which a significant amount of cyanobacteria has developed, is very important, even if it is known that many useful organisms will also be destroyed as well. The extent of the phenomenon depends directly on the amount of nutrients in the water and the thermal regime of the fish pond, therefore, in order to prevent or reduce the intensity of algal bloom, it is important to reduce the amount of nutrients that fall into it. The natural food, made up of detritus, bacterial colonies, aquatic plants, plankton, benthos, insects and their larvae, is extremely important, as a source of protein in the diet of fish, which would otherwise be provided exclusively by fishmeal, more expensive or by a number of components of animal or vegetable origin, often incomplete in relation to the fish requirements.In the larval or brood stage, micro-algae are used as food, especially the green ones, and often the aquatic crustacean *Artemia salina*. This species lives in saline watersheds and feeds on diatoms and green algae. The production of natural nutrients in ponds can be maintained by applying various mineral and organic fertilizers (including organic and agricultural animal waste). These products increase the primary phytoplankton production and the production of bacteria, which represent an important food source for invertebrates. Some species of bacteria and most invertebrates are the main source of protein in the nutrition of high-energy pond fish to maintain optimal growth. Thus, the natural basis of fish feed is an important factor in the efficient feeding of fish. During the summer growing season, the full value of the ration is obtained by increasing the amount of natural food in the pond. Formulas

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for calculation of quantitative indicators of the natural feed base for different fish farming areas are available [8-11]. Based on the available quantitative indicators of the natural food base in reservoirs, the trophic potential or food base of the water body is calculated. For example, it has been shown that for breeders and remontants of carp, natural feed should form 75-80% of the total feed, for two-year-old fish - 40-50%, and for one-year-old fingerlings - 25-30% [4-7].

For increasing in the resistance and tolerance of the nutritive basis of fish, the trace elements can be used [6]. The permanent monitoring of the hydrochemical regime, particularly of the water temperature, oxygen and nutrient level, as well as of phytoplankton, zooplankton and zoobenthos communities will allow taking appropriate measures in the right time to avoid fish production losses.

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ENVIRONMENTAL TOXICANTS EVALUATION IN A MODERN MONITORING SYSTEM - ROMANIAN MONITOX NETWORK AREA

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Abstract. In this paper a class consisting of 8 environmental toxicants were selected to assess Danube River water quality between Calarasi-Silistra sector until the river flow into the Black Sea, the Romanian Monitox Network Area. A comprehnsive comparison between 2 years of investigations in terms of nutrients contribution, such as different forms of nitrogen (ammonium nitrogen (N-NH4+), nitrite nitrogen (N-NO2-), nitrate nitrogen (N-NO3-), organic nitrogen, total nitrogen (TN)) and phosphorus (orthophosphate phosphorus (P-PO4 -3), total phosphorus (TP)), was made in order to provide information on the spatial and temporal variations of this river water quality. Sampling was performed according to European standards and quantitatively analysed using molecular spectrophotometry using UV VIS spectrophotometer Perkin Elmer Lambda 650S. The obtained results showed differences in nitrite nitrogen loads in surface water samples with higher values in the samples collected from the sampling points situated at the mouths of the Danube River into the Black Sea in 2020. Regarding quality classes, the surface waters were generally framed, in good quality class. The nutrients concentrations, combined in CCME WQI index, give an overview on surface water quality assessement in Romanian Monitox Network Area, with a good quality of Lower Danube (Constanta in 2019 and 2020) and fair quality of Danube Delta in 2020, but very closed to good quality.

Introduction

At the end of a 2860 km journey from its spring in the Black Forest Mountains, more precisely from Donaueschingen in Germany, to Pontus Euxinus (Black Sea), the Danube has been building, for over 12000 years by now, one of the most characteristic and beautiful deltas in Europe and even across the world [2].

For all its ranking second in size in Europe (after the Volga delta) and 20nd in the world, the Danube Delta's amazing landscape and fauna, birds in particular, represent a great scientific attraction, actually a study laboratory on the formation of deltaic, touristic and economic (natural renewable resources) ecosystems [2].

The Delta area is estimated at 4180 km2, 84% of which (3510 km2) lies on Romanian soil teritorry, between the three main Danube arms: Sfantu Gheorghe, Sulina and Chilia, listed in decreasing age [2]. Referring to the Black Sea "0" level, 20,5% of the Delta area lies below this point and 79,5% above it. The greatest extension (54,5%) has the territory comprised between

0 and 1 meter high [2].

At European legislative level, there are concerns for improving the quality of surface waters in European Directives. Romania implements these directives (Annex P5.1.)

According to EU Water Framework Directive ecological status is an expression of the quality of the structure and functioning of aquatic ecosystems associated with surface waters. Furthermore, the WFD provides a selection of the most relevant quality elements for the classification of ecological status. Water Framework Directive established 5 quality classes color-coded, for surface waters (very good - blue, good – green, moderate – yellow, poor – orange, bad – red). For chemical indicators, all the concentrations values were reported to the Romanian Order 161/ 2006 which is the transposed of Water Framework Directive into Romanian Legislation.

The classification into the quality classes was made in accordance with Romanian Order 161/2006, regarding the classification of surface water quality to determine the ecological status of water bodies, Table no. 6, Elements and biological quality standards, chemical and physical-chemical for setting ecological status of surface waters, Annex C, Elements and chemical, physical-chemical quality standards in water.

In the last years, many quality indexes were developed, integrating different physical-chemical indicators. Such an index is Canadian Council of Ministers of Environment Water Quality Index. This index takes into account nutrients concentrations and it is a useful tool for water quality management, because a single value describes the water quality.

Materials and methods

During the national trip, there were collected in 2019 and 2020, 50 surface waters samples for nutrients analysis.

For the dissolved forms (nitrite, nitrate, ammonia, orthophosphate) the surface water samples were stored at 2-5oC, for maximum 24 hours before analysis. For the analysis of total nitrogen, the samples were preserved on the field with 1 mL H2SO4 concentrate for 100 mL sample, store at 40 C and analyzed in the laboratory. Determination of dissolved nutrients was made on filtered samples and total forms on unfiltered samples using UVVIS Lambda 10 PerkinElmer spectrophotometer, using ISO standards. The final results were expressed in mg/L. All the reagents have very good quality analytical grade. For quality assurance were made flow charts with the specific certified reference materials.

The ammonium concentration, expressed as N-NH4+, was determined at 655 nm by measuring the absorption of the blue compound formed by the reaction of ammonium ion with salicylate and hypochlorite ions in the presence of sodium nitroprusside, according with SR ISO 7150-1, Water quality, Determination of ammonium, Part 1.

Manual spectrometric method at UV-VIS Lambda 10 Perkin Elmer Spectrometer (SR ISO 7150-1 2001).

Nitrite (N-NO2-) was determined through formation of reddish purple azo dye color, produced at pH 2.0 to 2.5 by coupling diazotized sulfanilamide with N-(1-naphthyl)- ethylenediamine dihydrochloride, according with SR EN 26667/ISO 6777/2002, Determination of nitrite, Molecular absorption spectrometric method, using the UVVIS Lambda 10 Perkin Elmer Spectrometer at 540 nm. The analysis was made on filtered water (SR EN 26667/ISO 6777/2002).

The nitrate (N-NO3-) was determined according with SR ISO 7890-3:2000 – Water quality, Determination of nitrate, Part 3. Spectrometric method using sulfosalicylic acid, by spectrometric measurement of yellow compound absorbance formed by reaction of sulfosalicylic acid (formed by addition of sodium salicylate in the sample and sulfuric acid) with nitrate followed by treatment with alkaline solution, at UVVIS Lambda 10 Perkin Elmer Spectrometer (415 nm) (SR ISO 7890-3 2000). Determination of phosphorus content in surface waters was made according with SR EN 6878/2005, Water quality, Determination of phosphorus, Ammonium molybdate spectrometric method using UVVIS Lambda 10 Perkin Elmer Spectrometer at 880 nm. For total phosphorus, the samples were treated on unfiltered water. Ammonium molybdate and potassium antimonyl tartrate react in acid medium with orthophosphate to form a heteropoly acid – phosphomolybdic acid – that is reduced to intensely colored molybdenum blue by ascorbic acid (SR EN 6878 2005). In order to evaluate the surface waters quality in an integrated index, it was computed CCME WQI – Canadian Council of Ministers of Environment Water Quality Index. The elements that underlie the calculation of this index are: scope (F1), frequency (F2), amplitude (F3), excursion [1,4]. With the computed values for this index, the surface water quality is classified in 5 classes, 0 – 44 (poor quality), 45-64 marginal quality, 65- 79 fair quality, 80-94 good quality, 95-100 (excellent quality) [3,6].

Results and discussions

Since the end of 1970 until 1990, the nutrient emission from the anthropogenic sources along the Danube River had strongly influenced the amount of the nutrients accumulated into the Danube Delta's ecosystems [2,5].In surface waters, nutrients are represented by nitrogen and phosphorus in dissolved and total forms.In water bodies, ammonium is on the one hand a measure of the reduced inorganic form of nitrogen and in the other hand it consists of dissolved ammonia (NH3) and the ammonium ion (NH4+). In general, nitrogen is an essential plant nutrient and although ammonia is only a small component of the nitrogen cycle, it contributes to the trophic state of a water body. Water bodies eutrophication process consists in prolific algal growths, that have deleterious impacts on other aquatic life, drinking water supplies, and recreation and is determined by excess of ammonia concentrations. In surface waters, nitrite is an intermediate form of nitrogen compounds. It is an unstable form that is either rapidly oxidized to nitrate by nitrification process, or reduced to nitrogen gas by denitrification process. For plants, nitrite is a nutrient source. Nitrite boosts plants proliferation. For aquatic life, nitrite is toxic at relatively low concentrations.The most stable and oxidized form of nitrogen is nitrate. It results from the complete oxidation of all nitrogen compounds. Plants use nitrate as primary form of nutrients to stimulate plant growth. Excessive amounts of nitrogen may result in phytoplankton or macrophyte proliferations.

Total nitrogen is a sum of all forms of nitrogen (organic and inorganic). Nitrogen is an essential plant element and is often the limiting nutrient in marine waters. The importance of nitrogen in the aquatic environment varies according to the relative amounts of nitrogen forms presence, as ammonia, nitrite, nitrate or as organic nitrogen.

Phosphorus in surface waters is represented by inorganic and organic forms. The inorganic oxidized form of soluble phosphorus, is phosphate. This form of phosphorus is the most readily available for uptake during photosynthesis. High concentrations of orthophosphate generally occur in conjunction with algal blooms.

Total phosphorus is a measure of the inorganic oxidized form of soluble phosphorus. During photosynthesis, total phosphorus is available for uptake.

In this study, we evaluated from nutrients point of view, first, the quality classes for surface waters collected from the target areas of Monitox Network in Romanian sector and second to quantify the surface waters quality in terms of integrated indices.

According with Romanian legislation, it was established 5 quality classes, in accordance with EU Water Framework Directive, for each chemical indicator, with specific limits.

For ammonia nitrogen, in 2019, 93.75 % of surface waters were framed into the first quality class (very good quality class) and 6.25 % in second quality class (good quality class) and in 2020 all the surface waters had a very good quality class.

For nitrite nitrogen, the surface waters are framed in very good quality class (15.62% - 2019) good quality class (81.25 % - 2019, 60% - 2020), and moderate quality class (3.13 % - 2019, 40% -2020).

Table 1. CCME WQI values for surface waters of Romanian Monitox Network Area

Sampling points 2019		CCME – WQI	
		2020	
Lower Danube (Constanta)	Fetesti	89.785	83.859
	Ostrov (bac pass)	89.788	89.717
	Dunare veche Ostrov	89.785	89.789
	Cernavoda bridge	79.583	89.712
	Cernavoda Seimeni	89.790	89.722
Lower Danube (Tulcea)	Braila upstream (mineral port)	89.789	89.728
	Braila downstream (mineral port)	89.789	89.719
	Galati downstream (mineral port)	89.791	89.718
	Siret upstream	89.757	78.957
	Siret upstream	79.581	79.057
	Galati town downstream	89.789	89.741
	Prut Giurgiulesti	100.000	89.735
	Prut downstream	79.580	89.722
	Reni downstream	89.790	89.733
	Isaccea downstream	89.790	89.751

Danube Delta	Ceatal Chilia	89.771	89.738
	ChiliaVeche upstream	89.793	78.937
	ChiliaVeche downstream	89.791	78.947
	Musura bay (river mouth)	89.787	79.310
	Sulina branch (river mouth)	89.793	79.039
	Sfantu Gheorghe branch (river mouth)	89.793	79.077
	Sf Gheorghe upstream	89.792	79.064
	Sacalin	89.789	79.313
	Ceatal Sf Gheorghe	89.790	78.423
	Aval Izmail	89.791	79.493

For nitrate nitrogen, 50% of surface waters were framed in very good quality class in 2019 and 40% in 2020, and 50 % in good quality class in 2019 and 60% in 2020.

Total nitrogen had values corresponding to the second quality class in 93.75% of surface waters in 2019, and 100% in 2020, in Monitox network area and only 6.25 % for third quality class in 2019 (Cernavodă bridge and Prut downstream).

For dissolved phosphorus, 84.37% of surface waters selected are framed in very good quality class in 2019 and 100% in 2020, and 15.25% in second quality class in 2019. For total phosphorus, 68.75 % of samples are framed in very good quality class in 2019 and 80% in 2020, and 31.25% in second quality class in 2019 and 20% in 2020.

The obtained results showed differences in nitrite nitrogen loads in surface water samples with higher values in the samples collected from the sampling points situated at the mouths of the Danube River into the Black Sea in 2020. Concerning the total nitrogen levels in Danube waters, significant higher values were obtained in 2019, when the organic nitrogen fraction of the total nitrogen was much higher than the others nitrogen fractions, different from the data obtained in 2020, when the organic nitrogen and the sum of its inorganic forms from the total nitrogen had similar values. Regarding the phosphorus compounds, the concentrations determined in the two studied periods are not significantly different.

The computed values of CCME WQI index for surface waters sampled in Romanian Monitox Network Area, in 2019 and 2020, showed that in 2020, the surface waters quality decreased, comparing to 2019, due to nitrite concentrations. At 2019 level, 4% of surface waters had an exccelent quality, 82 % good quality and 12 % fair quality. In 2020, 56% of surface waters have good quality and 44% fair quality. At integrative level, Lower Danube (Constanta) had a good quality in 2019 and 2020 and Danube Delta in 2020 had a fair quality (table 1).

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ECONOMIC ASSESSMENT OF THE HABITAT SERVICES AND BIODIVERSITY LOSSES UNDER THE DNIESTER HYDROPOWER COMPLEX IMPACTS

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Abstract. There are presented results of the economic valuation of the habitat service and biodiversity losses caused by the Dniester hydropower complex functioning. Habitat services were evaluated for two bird species (glossy ibis, Plegadis falcinellus, and yellow heron, Ardeola ralloides) and fish spawning grounds. The evaluation of biodiversity services was carried out for the Ramsar sites "Lower Dniester" and "Unguri - Holosnita". The current cost of discussed losses in the Moldavian part of the Dniester basin is about USD 6.7-7.1 million.

Introduction

The habitats provide everything that an individual plant or animal needs to survive: food, water, shelter. In this structure, each ecosystem provides different habitats that are of great importance to the life cycle of species. At the same time, habitat services highlight the importance of the ecosystems themselves in providing habitats for both sedentary native and migratory species. Along with this, habitats contribute to the maintenance of biological and genetic diversity of various populations. Habitats with an exceptionally high number of species, making them the most biologically diverse, are known as "biodiversity hotspots".

A significant part of the world's biological resources is characteristic for developing and transitional countries where they are under the greatest threat of anthropogenic pressure, both inside and outside the developing world. These threats, along with population and economic growth, include new challenges, and the hydropower development is among them [12].

In particular, the Dniester River flow regulation, carried out in the interests of the Dniester Hydropower Complex (DHPC) functioning, significantly changes its natural seasonal flow [6]; this often leads to the drainage of Dniester delta in the spring-summer period. The flow regulation affects negatively not only the local population's well-being, but also all living organisms: fish, amphibians, birds. Therefore, understanding the role of habitats and their economic value is extremely important as a tool for highlighting and quantifying the range of benefits through them obtained, thus allowing their direct comparison with other trajectories of sustainable development [3].

In recent decades, significant researches have been undertaken in this direction. Most of them have been done in developed countries [4, 5 8], but with limited use in developing world [3, 9]. This situation is due to the lack or weakness of local research capacity, which is insufficient to raise awareness of the habitats importance in the ecosystems and biodiversity conservation. Alternatively, the use of non-economic methods, such as questionnaires, focus groups, participatory participation and other tools [10, 11], allows only the limited solutions of problems under consideration. Moreover, the results of such assessments vary significantly for different ecosystem services, sometimes by several orders of magnitude, due to the fact that their values were estimated without participation of market approaches [7]. Such methods do not provide meaningful results that can be used for policy development at the national and international levels.

Materials and methods

As an object of this study, the economic valuation of a number of habitats services and biodiversity in the Dniester basin, most affected by the DHPC functioning, has been carried out. Its destructive influence on main representatives of the Dniester delta's natural ecosystems has manifested in a catastrophic reduction (almost by 80%) of its fauna. In particular, in the last decades about 160 species of birds have disappeared here, and currently a number of their species that form a background for the Dniester delta's biodiversity are on the verge of extinction [16]. The economic valuation was carried out for two bird species: glossy ibis (*Plegadis falcinellus*) and yellow heron (*Ardeola ralloides*). They both are listed in the red books of Moldova and Ukraine.

Also, after the DHPC construction, due to a decrease in the Dniester runoff, most of the spawning wetlands ceased to be periodically flooded which has a significant impact on phytophilic fish, laying spawn on underwater vegetation. This led to a need to evaluate the loss of ecosystem services supporting the fish habitats.

The economic valuation of biodiversity, considered as an ecological resource, was carried out for the Ramsar site "Low Dniester".

As to the methodic used, the economic valuation was primary carried out, using the method of replacement cost of an animal world representative. According to this method, the total cost of a biological species restoration Ci is determined as follows [14]:

$$C_i = V_i * N_i \quad (1),$$

where: V_i replacement cost of one individual of *i*-type species;

N_i total number of individuals of i-th species living within the study area.

To calculate the replacement cost of one individual (V*i*), there are used a resource value (*kr*) and increasing (*km*) coefficients indicating that species belongs to those included in the Red Book or subjected to international treaties, including Convention on the International Trade in Endangered Species of Wild Fauna and Flora (CITES) :

$$V_i = k_r * k_m \quad (2),$$

where: k_r - resource value coefficient taking into account the resource value of wildlife objects; it is taken as a fine size for the destruction of one specimen species in accordance with the law;

k_m - increasing coefficients.

In particular, according to [14], k_m equal:

2 - for species, covered by international treaties;

3 - for wild animals belonging to species included in the Red Data Book.

The method of replacement cost was used to evaluate habitat services for bird species and fish spawning grounds. The estimation of costs of the Dniester River wetlands' biodiversity conservation was based on the approach using a so called "reference value" [2].

Results and discussions

Maintaining the bird habitats

Before the DHPC construction, one of the most widespread birds in the Dniester delta was glossy ibis, Plegadis falcinellus; in 1970-1982, 2500-3000 its adult individuals nested stably here. In 1988-2002 the number of ibis nesting in the delta decreased in 8-11 - 14-25 times, fluctuating only within 100-350 adults. In 2003-2009, the decline continued (up to 40-150 adults), and in subsequent years (2010-2015) this bird almost completely disappeared from the delta as a nesting species [16].

According to the Ukrainian legislation, a fine for the destruction of one glossy ibis specimen is 12,063 hryvnas (~ 434 US dollars) that, taking into account the increasing coefficient km, equal to ~1,302 USD. Considering the amount of the fine as a kind of compensation for the lost of this environmental service, the total losses from reducing the glossy ibises as a result of hydropower negative impacts on the environmental conditions in the Dniester delta is about 3.9 million USD, even without taking into account the coefficient of reproducibility. In reality, this figure could be much larger.

Another vulnerable bird species under this economic evaluation - yellow heron, *Ardeola ralloides* - plays an essential role in wetland ecosystems, being the most important link in food chains. Since the yellow heron is very sensitive to even minor changes in the habitat where it is developing, it can be used as an indicator of such changes. So, before the start of DHPC construction and operation (1970-1982), 600-900 adult specimens of yellow heron nested in the Dniester delta. In 1985-1999 its number decreased to 200-300 individuals, and in 2000-2012 only 30-120 adults nested here (*ibid*). According to the Ukrainian legislation, a fine for the destruction of one yellow heron is, as in the case of the glossy ibis, 12063 hryvnia, or taking into account the multiplying coefficient (km = 3) is also equal ~ 1300 USD. If to assume that during the period of this review the number of yellow heron decreased by 675 specimens, then the total amount of losses from such reduction is about 900 thousand USD, excluding the species reproduction rate.

These estimates are especially important, considering that according to the prognoses [16], in the next 20 years, with the continuing violation of the scientifically grounded rules of DHPC functioning, in the Dniester mouth the final degradation of freshwater ecosystems and the disappearance of water bird main species will occur.

Maintaining the fish spawning grounds

Currently, in the Dniester floodplain from Naslavcea to Dubasari about 4,098 hectares of flooded spawning grounds are practically lost, which is about 14% of their total area that existed here earlier, before the DHPC construction [13]. The most extensive lacustrine-over flow system was in the Lower Dniester part that began below Bendery. Here, the floodplain reaches 2-3 km, expanding in some places to 17-18 km. Before the Dniester flow regulation, during the spring-summer high water, when it rose to 1.5 m above the low-water level, the entire floodplain was flooded. When the water level rose to two meters, the floodplain from Bendery to the Dniester mouth turned into a continuous reservoir with a total area of about 35 thousand hectares, serving as a spawning ground for phytophilous fish species and feeding their juveniles [17]. However at present, more than 20 thousand ha of valuable spawning grounds have been lost in the Dniester lower reaches, on the territory of Moldova only. The total loss of spawning grounds in the Dniester basin within the country is about 24.1 thousand ha.

At the same time, the specific feature of fish spawning migrations in the Dniester is their direct dependence on floods water level, their number, duration, and water temperature. The condition of the Dniester lacustrine-flooded system directly determines the fishery catches both in the river and Dniester Liman [17]. The required depth of spawning grounds is 0.5-1.0 m.

The economic valuation of fish spawning habitat losses was carried out, using the replacement cost of regulated spawning grounds. The importance of such facilities is confirmed by the experience of their use near the village of Hlinaia (area 2.98 ha, water volume 54.2 thousand m3). This experiment was organized to compensate for the lost areas of natural meadow spawning grounds due to the Dniester swallowing and its hydrological regime deterioration [15].

So, in 2019, about 4,742 specimens of larvae of different fish species were released from this spawning ground (by catching with a net). By catching with a Kori net this figure was 493,700 specimens of common carp larvae. Consequently, 165,671 fries can be obtained per ha. Since 3,456 ha of spawning grounds were lost, the possibility of reproduction of 573 million larvae was lost. Taking into account the current cost of 1 million larvae is 9,000 lei, the cost of spawning grounds loss is 5.2 million lei, or about 287 thousand USD per year.

However, this loss is underestimated, because it does not fully reflect the value of spawning grounds as reproductive ecosystems and the costs of their creation. At the costs of one hectare of regulated spawning grounds creation of 10-12 thousand lei, the compensation cost for lost spawning grounds will amount to 35-41 million lei (~ 1.9-2.3 million USD at the exchange rate of 18 lei per USD).

Economic evaluation of biodiversity

Economic valuation of the costs of the Ramsar site “Lower Dniester” biodiversity was based on the approach using a so called “reference value”. Such reference value was obtained based on available information on the cost of maintenance/conservation of biodiversity services, by calculating the average cost per unit area. This approach is close to the “*benefit transfer*” method that is used in situations where significant local expert knowledge and resources cannot be provided (GEF, 2018).

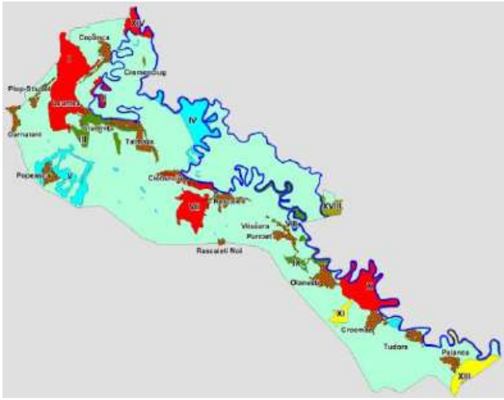

Figure 1. Key territories of the Ramsar site "Lower Dniester" by their importance: red - international; blue - national; yellow - local

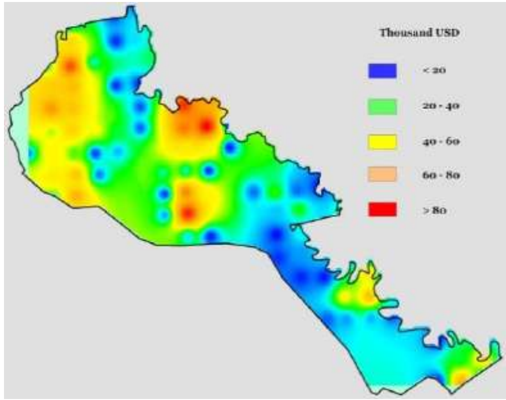

Figure 2. Economic value of biodiversity in the Ramsar site "Lower Dniester"

In the presented study, the value of biodiversity, accepted as a "reference value", was found by averaging the foreign information on the values of particularly rich in relation to their biodiversity territories. As a result, two indicators were identified as the reference values for our economic valuation: the average minimum (3,520 USD) and the average maximum ($6,705 USD), both per hectare. The estimation was carried out for the "key areas" of the Lower Dniester, as the most biodiversity-rich section of the Dniester basin (fig. 1). Additionally, to take into account the quality and productivity of ecosystems, an additional, so-named *estimating coefficient*, depending on ecosystems fragmentation, was introduced into the reference values.

Spatial interpolation of the evaluation results (fig. 2) demonstrates clear territorial differences in the biodiversity values. Based on the total area of especially valuable territories of the Lower Dniester (11,160 hectares), the assessment of its biodiversity is estimated at 11.3 thousand USD according to the average minimum value and 21.5 thousand USD - according to the average maximum value. Hence, the loss of biodiversity of this unique natural system will lead to colossal economic losses of its ecosystem services, ranging from 126 to 240 million USD.

The obtained estimates of the economic value of the Lower Dniester's biodiversity, with a certain degree of conditionality, were used for the analogous estimation of the Ramsar site "Unguri - Holosnita". The territory of this site includes six natural complexes with a total area of ~ 3720 hectares. The economic value of this site's biodiversity services ranges from US $ 42 million to US $ 80 million, depending on the selected "reference value".

Conclusions

The study showed the current cost of maintaining the habitat and biodiversity services in the Moldavian part of the Dniester River basin, lost as a result of the impacts, is about 6.7-7.1 million USD. The cost of these ecosystems service potential loss that, with varying degrees of risk, could result due to the DHPC continued operation, is estimated at USD 168-320 million.

Certain expenses of the performed economic valuation are objectively caused by the absence in Moldova of an appropriate regulatory framework for this type of assessment, which leads to the use of available analogues. Moldova desperately needs the development of official, scientifically grounded methodological documents on the economic valuation of ecosystem services, which should be based on advanced world and European approaches. A high-quality and reliable evaluation of the ecosystem service losses caused by the intensification of hydro-power development is unthinkable without the creation and permanent functioning of an environmental monitoring system, including a full set of indicators of impact on ecosystems and their state, with free access to its data.

Further improving the approaches and methods for the economic valuation of ecosystem services is an important area for new research in this field. From this viewpoint, the study makes a certain contribution to the search for these problems solution. It demonstrates the country's national experience in such solution scientific substantiation based on quantitative analysis and study of the composition and state of important ecosystems and natural complexes on the whole.

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SPECIFIC FEATURES OF THE SPATIAL STRUCTURE OF HYDROPHYSICAL FIELDS IN THE DNIESTER ESTUARY IN THE AUTUMN 2020

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Abstract. The analysis of the features of the structure of the fields of salinity, dissolved oxygen and turbidity of water masses of the estuary was carried out according to the data of expeditionary studies using the hydrological multiparameter sonde. The negative role of the approach shipping channel for the ecosystem of the Dniester estuary is determined. It is shown that the modern regulation of the Dniester runoff leads to a deterioration in the quality of the estuary waters.

Introduction

River flow regulation by dams and channel reservoirs creation has both positive and negative consequences for river ecosystems and water economy of the region. The total river flow is redistributed and transformed over time; the water balance changes; stream velocity in the reservoir part decreases; below the dam, the runoff of suspended particles decreases ("solid runoff"); the hydrochemical regime changes. This article presents an analysis of the modern spatial distribution features of water parameters in the water area of the Dniester estuary, as well as the influence of the approach channel from the Black Sea, through the Tsaregradskoe mouth, to the seaport Belgorod-Dnestrovsky (hereinafter referred to as "SP") on the formation of specific conditions that affect the functioning ecosystems in the estuary.The Dniester navigable approach channel (hereinafter referred to as "NAC") was dug in 1971 and has a length of 19.7 km, a width of 60 m, and a depth of 4.5 m [11]. Salt sea water, together with marine hydrobionts, moving in the bottom layer along the NAC, got the opportunity to penetrate far into the desalinated water area of the Dniester estuary. Traffic rules in navigable channels provide that the draft of vessels must be less than the depth in the canals by at least 20 cm [12]. When ships move along the NAC, a complex circulation of water arises in it, which promotes mixing, enrichment of water masses with oxygen and weakening their stratification. The intensity of navigation along the NAC to the SP has sharply decreased in recent years; therefore, it can be assumed that specific hydrological and hydrochemical conditions have arisen in the estuary, which in a certain way affect the vital activity of ecosystems.

Materials and methods

The initial data for the analysis of the situation were obtained during complex hydrological surveys of the Dniester estuary in September - October 2020 as part of expeditionary studies under the HydroEcoNex project. For measurements, we used a multi-parameter hydroprobe "EXO1" from XYLEM (USA) with specialized calibrated sensors from the same company.

Brief technical characteristics of EXO1 hydroprobe sensors [13]. Soundings were carried out from a motor boat. A GPS navigator was used for positioning.

The following water parameters were obtained, practically for the entire water area of the Dniester estuary, from the surface to the bottom: temperature, salinity, dissolved oxygen, mineralization and turbidity. Sampling discreteness, in time, was 500 milliseconds. On the survey verticals, where complex hydrological conditions were noted, the sampling discreteness by layers did not exceed one centimeter (the resolution of the pressure sensor was 4 mm). The declared density of data collection, up to this point, has not yet been involved in the Dniester estuary, thus the obtained data made it possible to describe in more detail the features of the structure and reveal new features of hydrophysical fields in comparison with previous studies [2,3,6,9,11].

Results and discussions

It should be noted that the survey of the Dniester estuary water area was carried out for two days (October 23 and 24, 2020), so we observed and describe the three-dimensional structure of the estuary waters. Wind speeds more than 8 m/s were not recorded during October 2020. Southwestern wind from 1 to 5 m/s was constantly acting for more than three days from October 21. Thus, the survey was carried out practically at a stationary (in modulus) wind field and a steady spatial structure of the estuary waters, typical for the autumn season.

The spatial distribution of the water salinity of the Dniester estuary in the surface and bottom layers is shown in Fig. 1. The surface structure of the salinity field is determined by: the interaction of the waters of the estuary itself with the freshwater runoff of the Dniester; water exchange with sea waters (through the Tsaregradskoe mouth); and wind action. The salinity of the northern part of the estuary (less than 0.6 PSU, Fig. 1a) is almost completely determined by the Dniester runoff. The salinity of the river waters of the lower Dniester fluctuates in the range of 0.19 - 0.37 PSU). In the northern part of the estuary, there is a water mass that is practically homogeneous in terms of thermohaline parameters, filling this entire part of the water area and formed by the hydrometeorological conditions described above. The southern part of the estuary is occupied by estuary waters with a salinity of 1 - 5 PSU, which have already been transformed when interacting with sea water. The intermediate-frontal gradient zone, peculiar in salinity, is located in the middle and narrow part of the estuary and separates the fresh waters of the northern water area and the actual estuary water mass in the southern part. A feature of this zone is the presence of areas of high salinity gradients in the SP area, which gradually erode in the northeastern direction. This is due to the configuration of the banks and hydrodynamic processes of interaction between river and estuary waters.

The structure of the bottom salinity field, Fig. 1b, has specific features that were first identified for the Dniester estuary water area. In the southern part of the estuary, the salinity of bottom waters (1-5 PSU) is due to the interaction of sea and estuary waters. The main feature of the salinity field is the presence of a seawater wedge (with salinity up to 14.4 PSU), which penetrates through the 4.5 m deep NAC (with an average estuary depth in this part of less than 2 m [11]). As a general feature of the salinity field structure, we note that a weak wind from the south and southwest contributed to the “shift” of the estuary water upper layer throughout the entire water area in the northeast direction, and its place was filled with less saline water due to currents of the surface layers directed towards the southern, lower part of the estuary; therefore, the salinity near the western coast is less than that of the eastern one, Fig. 1a. Thus, four water masses of the Dniester estuary should be distinguished: fresh waters of the northern water area (salinity less than 0.6 PSU); intermediate frontal waters in the central and narrow area (0.6 - 2.0 PSU); directly estuary waters in the southern part (2.0 - 5.0 PSU); and sea waters in the NAC (more than 10 PSU).The water temperature field during the expedition was practically uniform (14.5-15.5 °C), except for the temperature of the water mass in the NAC zone, the vertical structure of which will be described below. Thus, it can be noted that all water masses of the estuary have practically the same water temperature in the autumn period, which is generally predetermined by the weather conditions of the season.The spatial structure of the surface and bottom waters mineralization generally agrees with the hydrological and hydrobiological processes taking place in the estuary. As already noted, the northern part of the estuary is under the influence of the Dniester runoff, therefore, here salinity has values of 400-600 mg/l typical for fresh waters. The commissioning of the Dniester HPP led to a noticeable smoothing of the seasonal hydrograph of the Dniester runoff and reduced the intensity of runoff through the estuary. As a result, the accumulation of mineral and organic compounds of nitrogen and phosphorus has increased in the estuary, water bloom has become a common phenomenon, as well as the appearance of hypoxia zones. On the other hand, the NAC contributed to an increase in the inflow of seawater into the estuary water area and, accordingly, to a mineralization increase. The mineralization of water masses in the southern part of the estuary increases towards the sea from 1000 to 5000 mg/l. The zone of the NAC stands out especially, the mineralization here is more than 15 g/l, which significantly exceeds the background value.

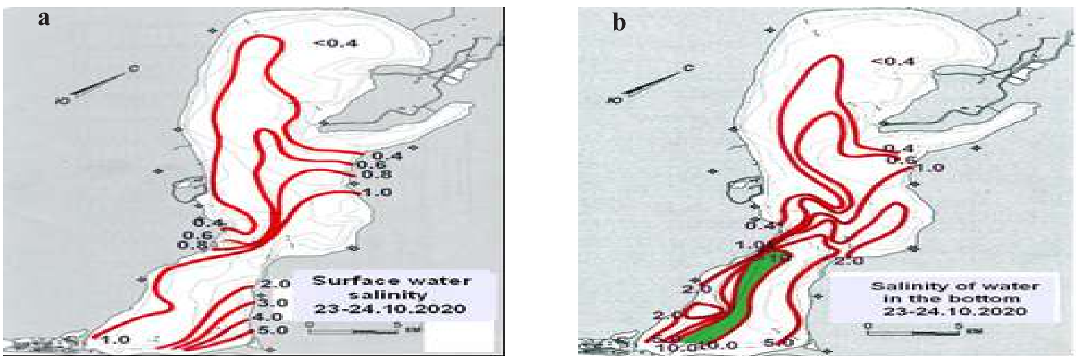

Figure 1. Spatial distribution of the Dniester estuary water salinity according to the results of a complex hydrophysical survey on October 23-24, 2020: a) the surface layer, b) the bottom layer. (The geographic diagram of the boundaries and bottom bathymetry of the Dniester estuary is taken from [11])

The estuary waters in the central and southern parts, with the exception of a thin bottom layer of 5 - 20 cm, contain at least 95% of dissolved oxygen, and in some zones, values of more than 140% were recorded, what is typical for polytrophic and hypertrophic waters and indicates their significant pollution [5,8]. The zones in which the oxygen content at the bottom is less than 100% (and in the rest of the water area in the entire volume of water the saturation is more than 100%) are shown in Fig. 2. Two isolated zones with oxygen saturation less than 50% were clearly determined in the bottom layer of the northern and central regions of the Dniester estuary. These zones were also noted during the expeditionary work on **September 14, 2020, Fig. 2b.** The expedition in September 2020 carried out observations in the middle sector of the estuary, from the mouth of the Glubokiy Turunchuk river, down to the narrow part of the estuary, between Belgorod-Dnestrovsky city and Roksolany village, Fig. 2b. The higher water temperature (about 22°C) during the survey on September 14, 2020 led to an expansion of the hypoxic zone compared to that recorded during the survey at the end of October. The metabolic activity of aquatic organisms, which is caused by an increase in the amount of dissolved and suspended forms of nitrogen in river and estuary waters in warm seasons, became especially active after the regulation of the river flow [1]. Destructive processes intensified in autumn, during the low-water period, and caused a deficiency of dissolved oxygen in the bottom layers, in places with low water exchange, especially in the northwestern part of the estuary and at the Sukholuzhie area [10].

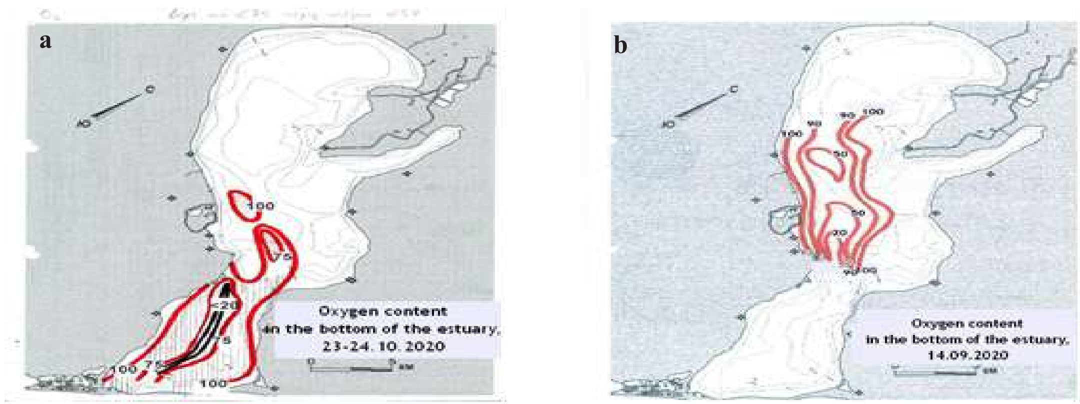

Figure 2. Spatial distribution of the content of dissolved oxygen (%) in the bottom layer of the Dniester estuary waters based on the results of complex hydrophysical surveys in September-October 2020

A certain deficit in the oxygen content in the bottom layer is observed practically throughout the entire water area of the southern part of the estuary, with the exception of the coastal zones. The NAK zone forms a water mass, which has a separate hydrological regime and characteristics that differ from the rest of the estuary. The NAK is one of the main ways of penetration of seawater in the bottom layer into the overlying sections of the estuary. The invasion of seawater into the estuary in conditions of low water, with a decrease in the river flow for natural reasons or due to the interception of the flow by reservoirs, during strong surge phenomena, can oppress not only the freshwater delta ecosystem, but also create threats in the river bed itself, in places of fresh water intake for water supply [7].Let us consider the graphs of the vertical distribution of the hydrological and hydrochemical parameters of the waters at the station located directly in the NAC in the area of the SP, Fig. 3.Figures 3a, 3b clearly show sharp jumps in the values of salinity and water temperature, which form a halocline and a thermocline, respectively, a pycnocline is created in the density field. The uniqueness of the hydrological situation lies in the fact that, in contrast to the negative density gradient created by an increase in temperature to the bottom, an increase in salinity is a more significant factor for creating a sharp pycnocline. The pycnocline prevents the penetration of oxygen into the underlying layers and promotes the accumulation of hydrogen sulfide at the bottom in the absence of mechanical mixing. Detritus accumulates on isopycnic surfaces in the water column in the NAC and in the port water area. Low current velocities, natural and artificial isolation of the NAC and SP waters accelerate the formation of a pycnocline in comparison with neighboring estuary sections and create noticeable differences in the hydrophysical and hydrochemical characteristics of water.In fact, a two-layer structure (homogeneous for each layer vertically) is observed, in the upper part of which there is estuary water mass, and in the lower part there is seawater with its own specific water masses characteristics. The upper layer of the estuary water with a thickness of 1.2 m has a water temperature characteristic as the entire estuary - about 15oC and salinity of about 1 PES. The lower layer - from 1.7 m to the bottom has a noticeably higher temperature of 17-20°C and salinity - 13.8 - 14.2 PSU. Sea water obtained such characteristics in September. That is, water exchange in the NAC did not occur for at least about a month. The upper and lower water masses are separated by a density jump layer, the thickness of which is only 0.5 m, but with very high values of the average gradients over the layer: for temperature - 4oC/m, salinity - up to 25 PSU/m. The indicated values of the gradients have never been before recorded in the water area of the Dniester estuary.

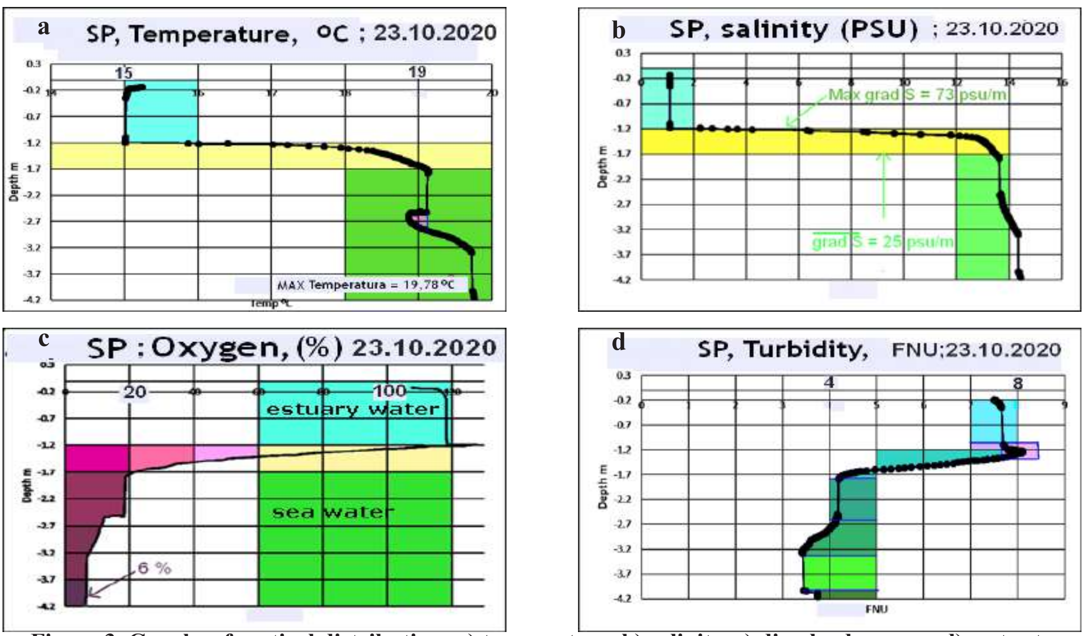

Figure 3. Graphs of vertical distribution: a) temperature, b) salinity, c) dissolved oxygen, d) water turbidity of the Dniester estuary on the spot at the entrance to the SP according to the results of a complex hydrophysical survey on October 23-24, 2020.

The formation of a sharp pycnocline in semi-enclosed water areas serves as a “hard cover” that prevents the penetration of oxygen into the interior and contributes to its almost complete consumption in the bottom layer. In the NAC and adjacent areas in the hot summer-autumn period, saprobiotic conditions are created, and the death of bottom fauna may occur. From the bottom layer, not only biogenic substances, but also hydrogen sulfide and other gases (methane, ammonia, mercaptans, etc.), formed during the decomposition of organic matter under anoxia and hypoxia, enter the water column and even into the surface layer [8]. This is evidenced by the graph of the vertical distribution of dissolved oxygen, Fig. 3c. If oxygen saturation in the upper layer reaches 120% or more, then below the pycnocline its content in the waters decreases from 20% under the jump layer to 6% at the bottom.

The vertical distribution of water turbidity can supplement information on the fine-structured features of the hydrological and hydrobiological characteristics of waters, Fig. 3d. The surface water of the Dniester estuary had a turbidity in the central part of 8-10 NFU, in the northern part - 13-19 NFU, in the southern - 11-17 NFU, and in the near-surface layer, from 0 to 5 cm, the turbidity values were about 2-5 units above.

Formally, the ratio between the unit of turbidity in terms of formazin and the solid particles amount in water of 1unit FNU corresponds to the content of 0.58 mg/l of kaolin [4]. Liman water which occupies the upper layer with a thickness of 1.2 m has a value of about 7.8 FNU (excluding the upper 5 cm). The vertical variation of oxygen and turbidity in a thin about 3 cm thick layer on the 1.20 m horizon is abnormally released and indicates that it is a hypertrophic layer with a higher oxygen content - 128% (+10%) and solid suspended particles - 8.11 NFU (+0.3 FNU units) than in the higher and lower horizons (most likely, detritus and dead organic matter are concentrated in this thin layer). This layer cannot go dipper due to large gradients of water density, i.e. it is located at the interface between two dissimilar water masses.

In the jump layer, the turbidity values decrease to 4.30 units. Below the turbidity continues to decrease to 3.41 NFU, practically correlating with the change (decrease) in the amount of oxygen with depth, Fig. 3c, 3d. Obviously, with a decrease in the amount of dissolved oxygen in the seawater of the NAC, its transparency increases, since turbid estuary waters rich in suspended solids cannot penetrate into these layers. A similar phenomenon is observed on the seashore during the upwelling of deep sea waters, when very cold, but transparent and “contaminated” with hydrogen sulfide waters come to the shore under the action of the wind of the corresponding directions.

Conclusions

Expeditionary studies of the spatial structure of the waters of the Dniester estuary made it possible for the first time to reveal sharp differences in the values of the hydrophysical characteristics of the waters of the estuary and the waters of the NAC.

The features of the spatial structure of the salinity field of the estuary waters made it possible to distinguish four water masses.

The relatively stationary zones of the estuary water area where the oxygen content in the bottom waters is less than 50% have been determined. Sharp differences in the values of temperature, salinity, mineralization, turbidity, and the content of dissolved oxygen in the vertical structure of water in the NAC were revealed for the first time. These differences are due to the lack of navigation in the Channel. Significant gradients of temperature and salinity in the PC (- 4oC/m and up to 25 PSU/m, respectively) form a powerful pycnocline under certain hydrometeorological conditions.

Under the pycnocline in the bottom and middle layers of the NAC, the content of dissolved oxygen decreases from 20% to 6%; therefore, these layers may contain hydrogen sulphide. The redox line may rise into the water column in some situations. This leads to a significant deterioration of ecological conditions in the waters of the estuary and the oppression of the biota living in it.

The flow pass through the estuary has noticeably slowed down, the accumulation of mineral and organic compounds of nitrogen and phosphorus has increased primarily because of the regulation of the Dniester runoff. Large values of the content of dissolved oxygen and mineralization in the waters of the estuary indicate unfavorable biochemical processes: in the estuary, “blooming” of waters has become a common phenomenon, as well as the appearance of hypoxia zones, which is characteristic of significantly polluted polytrophic and hypertrophic waters.

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THE SEASONAL DYNAMICS OF PHYTOPLANKTON AND WATER QUALITY IN THE PRUT RIVER LOWER SECTOR

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Abstract. The article presents the results on qualitative and quantitative indicators of phytoplankton of the Prut River lower sector within the Republic of Moldova in 2018-2020. In phytoplankton compositions 72 species were identified. Seasonal and long-term patterns of phytoplankton development have been established in Prut River lower sector. The numbers of phytoplankton changed within the limits 1,06 to 20,32 mln. cell/l, with biomass 1,23-21,64 g/m3. The values of saprobic indexes, estimated on the basis of species-indicators (52 species) from phytoplankton composition, which are in proportion of 58% typically β-mezosaprobic, confirm the following: the water quality of lower Prut River sector in the period 2018-2020 was satisfactory for the development of phytoplankton and was attributed mainly to II-III (good-moderately polluted) quality classes.

Introduction

The Prut River is the second longest and the last major tributary of the Danube, with its confluence located just upstream of the Danube Delta [7]. The study of phytoplankton development and water quality of lower Prut river sector are of particular importance in terms of eutrophication and continuous pollution. Photoautotrophic plankton is the major primary producer of organic carbon in the waters. Phytoplankton composition and biomass provides an integrated view on water-quality conditions, an indication of eutrophication. Reaction of the phytoplankton to the natural and anthropogenic factors is closely connected with changes in algae abundance, biomass and species composition and it is a reliable indicator of the aquatic ecosystems [4, 5].

Material and methods

Researches of phytoplankton were carried out seasonal during 2018-2020 from lower Prut River sector (Cahul, Caslita-Prut and Giurgiulesti sampling points) in the research of the Laboratory of Hydrobiology and Ecotoxicology of the Institute of Zoology. Algae species identification was performed using the microscope MIKMED-2 (LOMO) and identifying keys [2]. To estimate the trophic dynamics seasonal and annual values of biomass were used. Sampling and analysis of phytoplankton was carried out by conventional hydrobiological methods [1-3,6].

Results and discussion

In phytoplankton compositions of the lower Prut River sector during 2018-2020, 72 species were identified, and represented by 5 taxonomical groups: *Cyanophyta*–8, *Bacillariophyta*–38, *Chrysophyta*-2, *Euglenophyta*–8, *Chlorophyta*–16 (*Chlorococcophyceae*-14, *Volvocineae*-1, *Desmidiales*-1). It was found that the phytoplankton species of the following groups predominated: *Cyanophyta, Bacillariophyta* and *Chlorophyta.* (Tab.1.). The basis of the floristic diversity of lower Prut River sector consists of such species as: *Aphanizomenon flos-aquae (L.)*

Ralfs, *Oscillatoria lacustris (Kleb.)*, from blue-green algae, *Cyclotella kuetzingiana Thw.*, *Navicula cryptocephala Kutz.*, *Synedra acus Kutz., Synedra ulna (Nitz.)* from diatoms algae, *Monoraphidium Komarkovae (Nyg.), Monoraphidium contortum Thur.* and *Scenedesmus quadricauda Turp.* from green algae. The quantitative parameters of phytoplankton vary widely in seasonal and annual aspect.

Table 1. The number of planktonic algae found in the lower Prut river sector during 2018-2020

Algal groups	Cahul	Caslita-Prut	Giurgiulesti
Cyanophyta (Cyanobacteria)	3	4	7
Bacillariophyta	27	26	34
Chrysophyta	-	2	1
Euglenophyta	2	7	4
Chlorophyta:			
Chlorococcophyceae	7	11	9
Volvocineae	-	1	-
Desmidiales	-	1	-
Total	**39**	**52**	**55**

The number of phytoplankton species in lower Prut River sector was in limits 1,06- 20,32 mln. cell/l, and biomass 1-21,94 g/m3 in spring, 1,61-18,68 mln. cell/l, with biomass 1,9-10,36 g/ m3 in the summer, 1,36-6,88 mln. cell/l, and biomass 1,23-6,44 g/m3 during autumn (Fig.1.).

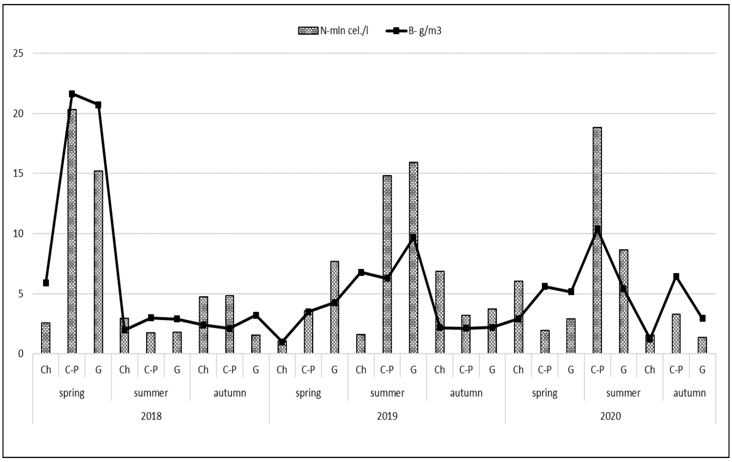

Figure 1. Dynamics of quantity (N-mln cell/l) and biomass (B-g/m3) of phytoplankton in the lower Prut river sector (C-Cahul, C-P - Caslita-Prut and G-Giurgiulesti sampling points) during 2018-2020.

The highest values of phytoplankton species number and their biomass were attested at Caslita-Prut and Giurgiulesti during spring 2018 and at Caslita -Prut sampling points during summer time 2020. In lower sector the preponderance of *Cyanophyta, Bacillariophyta* and *Chlorophyta* in the forming of the number of algae species was attested. Species of algae which are growing in large quantities were represented by: *Aphanizomenon flos-aquae (L.) Ralfs, Os-*

cillatoria planctonica (Wolocz.), *Oscillatoria lacustris (Kleb.)*, *Synechocistys aquatilis (Sanv.)*, *Asterionella formosa Hass., Nitzschia acicularis W.Sm., Monoraphidium contortum Thur., Scenedesmus falctus Chod*.

The main part in the formation of phytoplankton biomass was *Bacillariophyta* algae: *Cocconeis placentula (Ehr.), Cymatopleura solea (Breb.) W.Sm., Cyclotella Kuetzingiana Thw., Nitzschia sigmoidea (Ehr.) W.Sm., Gyrosigma acuminatum (Kutz.), Rhoicosphenia curvata (Kutz.), Synedra ulna (Nitzh.)Ehr., Surirella robusta Ehr. v.splendida and mostly in summer time presented by Euglenophyta species: Trachelomonas hispida (Perty) Stein., Euglena polymorpha Dang., Lepocinclis fusiformis (Lemm), Phacus pleuronectes (Mull.)*.

For the estimation of trophicity of lower Prut river sector, according to the criteria of classification and trophicity categories of continental aquatic ecosystems [2], were used the seasonal and multiannual values of phytoplankton biomass. Thus, according to the values of phytoplankton biomass, which in 2018-2020 varied within the limits of 1,00-21,64 g / m3, the lower Prut River sector was attributed to the category of "eutrophic" trophicity, periodically "polytrophic" (Fig.1.).

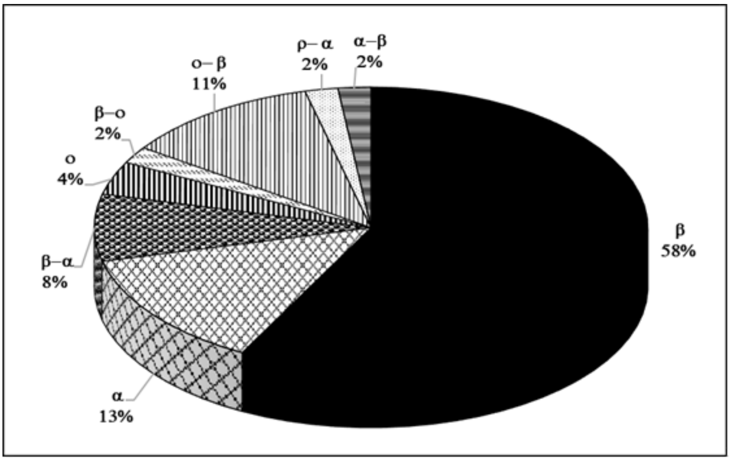

Figure 2. Distribution of phytoplankton indicator types according to the zone of saprobity in lower Prut river sector 2018-2020

From whole number of species of algae, identified in Prut river lower sector (72 species and varieties of phytoplankton) 52 were indicators of water saprobity, the most frequently met, were the following: *Aphanizomenon flos-aquae (L.) Ralfs*, *Asterionella formosa Hass., Cyclotella Kuetzingiana Thw., Gomphonema olivaceum (Lyngb.), Hantyschia amphyoxis (Grun.), Navicula gracilis (Ehr.), Navicula cryptocephala (Kutz.), Surirella robusta (Ehr.), Euglena polymorpha(Dang.)., Trachelomonas hispida (Perty) Stein., Scenedesmus quadricauda Turp*. more than 58 % of them were β-mesosaprobe species. α -mesosaprobe species made 13 %, the most frequent of which were: *Nitzschia acicularis W.Sm., Nitzschia palea (Kutz.), Euglena polymorpha Dang., Closterium acerosum (Ehr.)*; O-β mesosaprobe species were 11 %, the most frequent of which were the species: *Anabaena spiroides (Lemm.), Epithemia ze-*

bra (Ehr.), Fragilaria capucina (Desm.), Melosira italica (Ehr.); species β - α-mesosaprobic (8%) were represented by: *Merismopedia tenuissima (Lemm), Cymatopleura solea (Breb.), Navicula cincta (Pant.), Navicula hungarica v. capitata (Grun.)*; species o-oligosaprobic (4%) were presented by: *Dinobryon sertularia (Ehr.), Ophiocitium capitatum (Woll.)*; and species: β-O-mesosaprobic were accoumting for 6% *(Navicula gracilis (Ehr.)*), α-β mesosaprobic (*Cyclotella meneghiniana (Kutz)*), ρ-α- mesosaprobic *(Chlorella vulgaris (Beier.)*). (Fig.2.). Species with preference to the χ- xenosaprobe zone have not attested in Prut river lower sector, these however beingmet in the previous years [4, 5].

The saprobic index values were established within the limits 1,98-2,3 in spring time, 1,92-2,34 in summer and 1,9-2,3 at lower Prut river sector, with high values at Cahul sampling point. According to saprobic value indices, the water quality of lower Prut river sector sector was satisfactory for the development of phytoplankton and was attributed mainly to II-III (good-moderately polluted) quality classes.

Conclusions

The basis of the floristic diversity of the lower Prut river sector consists of groups: *Bacillariophyta, Cyanophyta, Chlorophyta, Euglenophyta and Chrysophyta*.

Higher values of quantitative phytoplankton parameters were recorded during the spring and summer periods. In most cases the phytoplankton biomass values referred to the trophic category "eutrophic" sometimes "polytrophic".

According to saprobic index values indices, the water quality of lower Prut river sector was satisfactory for the development of phytoplankton and was attributed mainly to II-III (good-moderately polluted) quality classes.

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THE STATE OF ZOOPLANKTON COMMUNITIES IN THE LOWER DNIESTER AREA UNDER THE CONDITIONS OF RIVER REGULATION AND ACTUAL CLIMATIC CHANGES

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Abstract. The study focuses on zooplankton communities of the Lower Dniester. The quantitative indicators of plankton are given and the annual dynamics is described. An assessment of the current state of the river according to the state of zooplankton communities is presented, together with a comparison with historical data, at different stages of river regulation. The changes that occurred in the river zooplankton since the 1950s were analyzed. The relationships between individual characteristics of planktonic communities and the physical and chemical characteristics of the river was also investigated. A comparison of actual data with those collected during 70-80s revealed no significant changes in the structure of the zooplankton community. The proportion of different groups of zooplankton organisms changed insignificantly, the saprobity indices improved slightly, and the average zooplankton biomass did not change. However, compared to the period before 1950s, prior to river regulation for hydropower purposes the role of rotifers in the community was reduced. It might be difficult to identify the main factor determining the development of zooplankton in the Lower Dniester, in order to understand the processes taking place in planktonic communities, it is necessary to analyze the complex impact of hydrological and hydrochemical factors on planktonic communities.

Introduction

The Dniester is one of the four largest rivers in Ukraine, the largest river in Moldova, and the biggest source of freshwater in Ukraine and the only major source in Moldova.

In recent years, several factors, including the hydrochemical and hydrobiological status of the river ecosystems have produced significant changes.

Dniester river has undergone significant modifications, following the construction of the Dubasari (1953) and Novodnestrovsc (1980) reservoirs, which caused the rupture of the longitudinal connectivity of the river, the disruption of the hydrological, thermal and hydrochemical regimes. This resulted in harmful effects first of all on the hydromorphological balance of the rivers, but also on the aquatic fauna and habitats. Therefore, the ecological state of the river is of particular interest for research and monitoring.

Since anthropogenic pollution is multifactorial, in order to assess the effect of its impact on aquatic ecosystems, it is necessary that along with physicochemical studies, observations of the state of communities of aquatic organisms are carried out. In appraising the state of aquatic ecosystems according to hydrobiological parameters, zooplankton is one of the main biotic components and has an extremely important role in the structure and functioning of ecosystems, including participation in self-purification processes.

Zooplankton is a multi-species community, in which, as a rule, the following groups of invertebrates are distinguished: rotifers (Rotatoria), cladocerans (Cladocera) and copepods (Copepoda). Changes in the structure of zooplankton are of great importance in the functional organization and state of the entire aquatic ecosystem [11].

The hydroecological history of Dniester River and Dniester estuary may be divided into three periods: 1. pre-regulated period; 2. partly regulated period (starts in 1954 after Dubossary Reservoir and the Dubossary Hydroelectric Power Plant were put into operation); 3. fully regulated period (from 1983 when the Dniester Reservoir and the Dniester Hydroelectric Power Plant were put into operation).

Although the research papers on the state of zooplankton in the lower Dniester were published in the first half of the last century, most of them were either purely faunistic, without describing the quantitative characteristics, focusing on peculiarities of the ranges of certain species [6,14], or describing only small sections of the river basin. Complex studies on the topic were undertaken only in the 50s of the last century - Markovsky, Yaroshenko and Grimalsky [10, 15, 23].

These studies provided detailed information on the taxonomic composition, spatial and seasonal variability of the zooplankton. Some brief data on the zooplankton of the Lower Dniester and Dniester delta were given in the works of Burnashev [7, 8], Grimalsky in 1968 [9]. Large-scale studies on zooplankton in the lower Dniester was done in the work carried out by Naberezhny, Climenco, [4, 12, 13, 16, 17].

However, the first author offered rather a brief description of the zooplankton, without referring particularly to the Lower Dniester, and the second author considered data only from the seasonal point of view.

Thus, the latest studies of zooplanktonic communities in the lower Dniester were carried out mainly in the spring-summer period, as the periods of the greatest development of zooplanktonic organisms. However, the year-around monitoring can reflect the effect of climatic changes on plankton even more clearly than only monitoring in the spring and summer, for example, changes in the quantitative and qualitative structure of communities as a result of changes in the timing of plankton development.

Therefore, now it becomes critically important to study the annual dynamics of zooplankton communities and revealing the patterns of the changes occurring at biocenosis level.

The paper was elaborated on the basis of data obtained under BSB27 ” Black Sea Basin interdisciplinary cooperation network for sustainable joint monitoring of environmental toxicants migration, improved evaluation of ecological state and human health impact of harmful substances, and public exposure prevention (MONITOX)”, BSB165 „Creating a system of innovative transboundary monitoring of the transformations of the Black Sea river ecosistems under the impact of hydropower development and climate change”, State Program 20.80009.7007.06 Determining the changes of aquatic environment, assessing the migration and impact of pollutants, establishing the patterns of the functioning of hydrobiocenoses and preventing the negative effects on ecosystems, and the part of ecological monitoring of Lower Dniester National Nature Reserve.

Materials and methods

This paper presents the results of investigations of zooplankton communities of the Dniester river, in the lower sector (Vadul lui Voda - Palanca) on the territory of the Republic of Moldova and the delta Dniester river (Palanca - Maiaki) of Ukraine. Zooplankton samples on Lower Dniester were collected during 2020, linearly including all seasons as part of the complex research of the Laboratory of Hydrobiology and Ecotoxicology of the Institute of Zoology. The complex expeditions were carried out on the Lower Dniester ecosystem (Vadul lui Voda, Varnita, Sucleia, Palanca).The zooplankton sampling from Dniester delta was collected on all seasons, on two monitoring points near the village Mayaki and near the 51-th km of road Odessa-Reni (st.Palanca) by the Ukrainian Scientific Centre of Ecology of the Sea. The collection of zooplankton material was performed together with hydrological, hydrochemical and other biological samples. Sampling and subsequent processing was carried out by standard methods [1, 3]. The samples were collected using the Apstein zooplankton net (№ 55) by filtering a quantity of 100 l of water. The collected zooplankton material was fixed immediately in the field with formalin solution (40 %). The quantitative counting of zooplankton was carried out using Bogorov counting chamber and the binocular stereo zoom Discovery V8 ZEISS, using three replications. The density (N – ind/m3) of the organisms was calculated to cubic meter. Identification of the main zooplankton groups (Rotatoria, Copepoda, Cladocera) was carried out up to the highest possible level, with the use of the microscope Axio Imager A.2 (Zeiss). The determination of the taxonomic structure of the zooplankton groups was carried out with the use of identification guides [16, 21] The estimation of the investigated aquatic ecosystems and water quality was performed by saprobiological analysis based on an existing saprobiont system [3, 5].

Results and Discussion

Research on the monitoring of zooplankton communities in the borders of the Republic of Moldova in the Dniester ecosystem, including its medial and lower sector, began in the 1947s and is reflected in numerous works [4, 16, 17, 22].

During 2020, the development of zooplankton in the Lower Dniester showed both seasonal and within station variations. The specific diversity of zooplankton during 2020 in the lower sector of the Dniester (Vadul lui Voda - Palanca) was represented by 72 taxonomic units (fig. 1), with the predominance of the group of rotifers which constituted 64% or 46 taxonomic units of the total number. Copepods were represented by 15 (21%) taxonomic units including the stages of nauplii and copepods and cladoceres 11 units or 15%. The diversity of zooplankton in the Dniester delta constituted 37 taxonomic units: Rotatoria - 23 (62%), Cladocera 5 (13%), Copepoda -4 (11%) and other groups of organisms (Varia) - 5 (14%) taxonomic units fig 1, b. As reflected in previous investigation published earlier [18, 19], the main share in the formation of specific diversity in the investigation period belongs to rotifers.

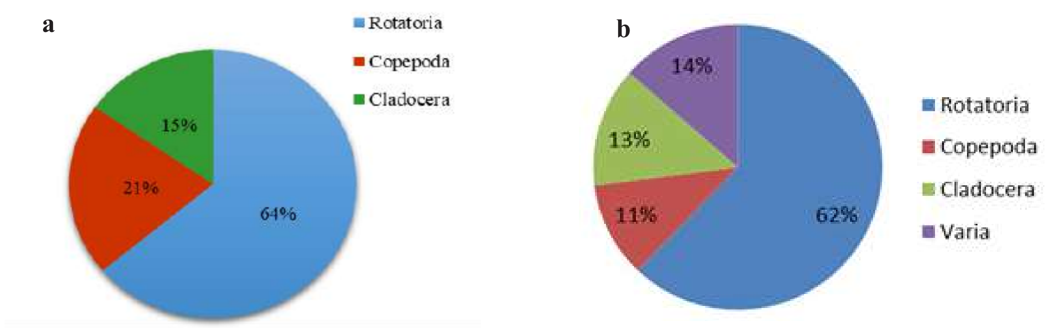


Figure 1. Taxonomic diversity and the contribution of the main groups of zooplankton in the Lower Dniester, 2020

It is known that the composition of zooplankton communities can differ significantly depending on the geographical conditions of the researched areas, but also on different hydrological, chemical and biotic factors. The specific diversity during the year along the river varied within the limits of 4-45 taxonomic units, with the specific richness increased at st. Palanca.

Following the abundant rainfall, during 2020, which conditioned the overflow of large waters and the flooding of some sectors of the ecosystem of Dniester river, in the composition of riparian zooplankton more and more limnophilous species appear, with increased preferences for organic substances such as *Platyias quadricornis* (Ehrenberg, 1838), *Brachionus calyciflorus* Pallas, 1776, *Brachionus budapestinensis* Daday, 1885, *Echlanis dilatata* Ehrenberg, 1832, *Filinia longiseta* (Ehrenberg, 1834) species that prefer vegetation thickets – e.g. *Lophocharis oxysternon* (Gosse, 1851), *Lecane (Monostyla) closterocerca* (Schmarda, 1859) and species characteristic for swampy areas - *Eudactylota eudactylota* (Gosse, 1886) (st. Palanca).

From the analysis of the data of the last three years, related to the qualitative composition of zooplankton organisms, a trend of increasing specific diversity along the Lower Dniester river was observed. Figure 2 shows the distribution of the number of taxa registered on the Lower Dniester in the dynamics, during 2020 and previous two years.

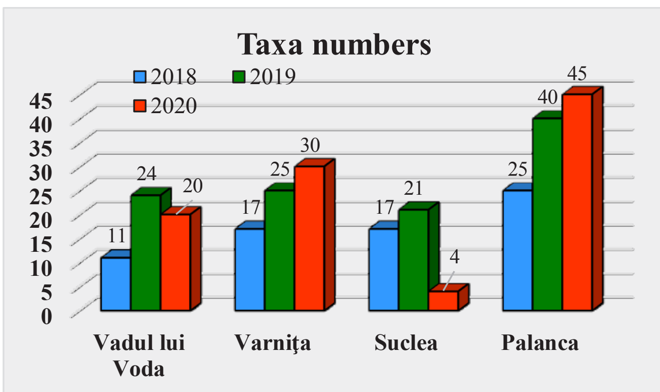


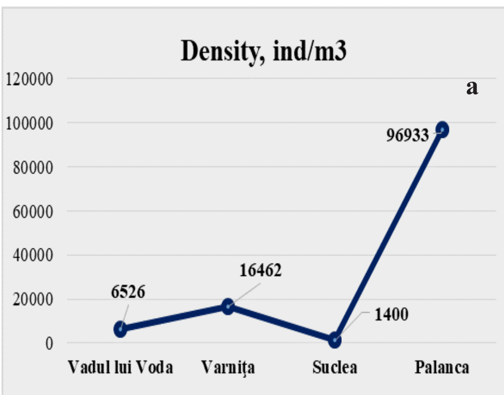
Figure 2. Distribution of the number of zooplankton taxa along the Lower Dniester during 2018-2020

From the obtained data, an increase in the composition of zooplankton diversity from 2018 to 2020 at all sampling stations could be seen.

The increase in the diversity and quantitative parameters in the Lower Dniester sector during 2020 was not only due to favorable climatic conditions and factors linked to this period but also to the more often sample collection (monthly) during 2020. Along the Lower Dniester River up to the river mouth, zooplankton diversity increased to 45 taxonomic units (st.Palanca).

The peculiarities of the qualitative structure and quantitative composition of zooplankton were mainly determined by the hydrological regime of the Lower Dniester, which is unstable and directly dependent on the hydrological regime of the Dubasari reservoir, thus favoring the proliferation of zooplankton in the Lower Dniester. The data showed that, the contribution of the Dubasari accumulation lake to the formation of zooplankton community in the lower Dniester is insignificant. At Vadul lui Voda st. the lowest values of composition and development of zooplankton (fig. 2-3) was observed, with an increase towards the river mouth. The reduction of the composition of specific diversity and quantitative parameters of zooplankton development at Sucleia station was influenced by the hydrological conditions as well as by unfavorable sanitary-biological conditions due to discharge of industrial-municipal wastewater.

Quantitative development of zooplankton in the the Dniester river during 2020 [2] was largely determined by unstable climatic conditions and water level fluctuations, which led to an increase in the development of the number, compared to previous years, when the main contribution to the formation of zooplankton biomass was made up by rotifers, and to the formation of crustacean biomass, especially copepods. The average density (figure 3 A-B) of the zooplankton of lower Dniester during 2020 varied in the limits of 1.4 - 96.9 thousand ind /m3, with a maximum development at Palanca station and minimum at Sucleia station.



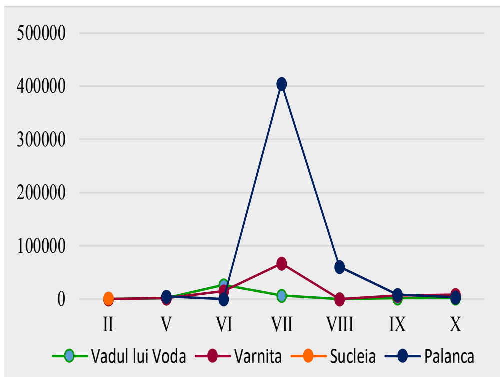


Figure 3. Abundance (A) of zooplankton and seasonal distribution the density (B) on the course of Lower Dniester during 2020

Along the river, from Vadul lui Voda station to Varnita, an increase in the abundance of zooplankton by 2.5 times was observed, however a decrease towards st. Sucleia and a significant increase in st. Palanca, where the average value of the density was 96.9 thousand ind /

m3 was encountered. The less favorable conditions were encountered at Varnita station, where the average values of the abundance was 1400 ind / 3. Figure 3B shows the parameters of the number of zooplankton communities during the investigation period. In the seasonal aspect, at all monitoring points, both the specific diversity and the quantitative parameters of the lower Dniester zooplankton reached the maximum values in July-August.

Quantitative indicators of the seasonal development of different zooplankton groups from Dniester Delta are shown in Figure 4. It is noteworthy that although the first maximum of development was the largest by abundance, the largest by biomass was the second maximum, which occurred in September.

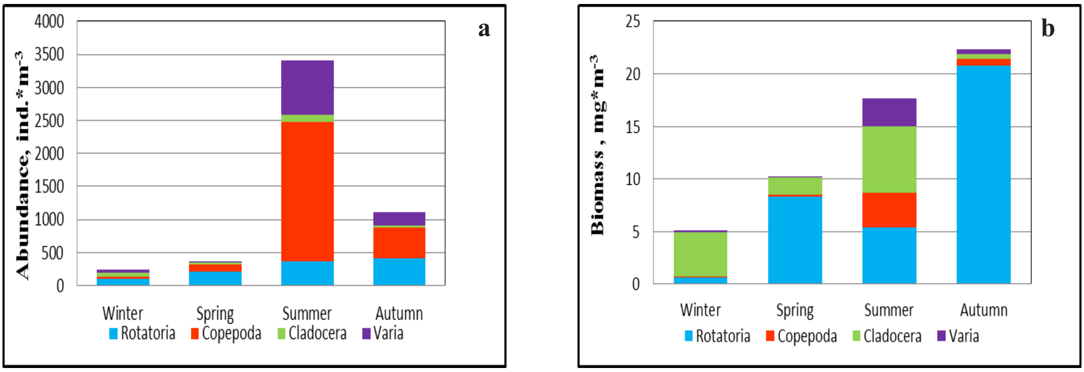


Figure 4. The abundance and biomass of different groups of zooplankton in the Dniester delta in 2020

In regard to the seasonal changes of zooplankton community in the Dniester delta and inferior Dniester, the species diversity and quantitative characteristics in winter were low. Only rotifers of the genus *Notholka* Gosse, 1886, *Brachionus* Pallas, 1766 were abundant. In addition, the cladocerans *Chydorus sphaericus* (O.F. Müller, 1776) was numerous in the Dniester delta and in the Inferior Dniester river sector during the winter season. There were also rotifers of the genera, Asplanchna Gosse, 1850, *Filinia* Bory de St. Vincent, 1824, *Lecane* Nitzsch, 1827 as well as nauplii of copepods. In spring, there was an increase in the diversity and biomass. Copepods of the genera *Acanthocyclops* Kiefer, 1927, *Eucyclops* Claus, 1893 and Eudiaptomus Kiefer, 1932 appeared in the samples. The variety of rotifers in Dniester delta increased, the first maximum of development occurred in June. Also, in June there was a maximum of Cladocera variety, which together with rotifers formed most of mesozooplankton biomass. The second maximum occurred in September and was formed mainly by rotifers and copepods. In October, abundance began to decline and in November became typical for winter. The group of cladocerans in the lower Dniester sector were underdeveloped and were not constantly present during the investigation period, which usually, make a significant contribution to the formation of zooplankton biomass.

In Dniester delta rotifers formed the basis of species diversity throughout the entire observation period. In the spring, they also were the dominant group by abundance. During 2019-2020

[19, 20] rotifers dominated by abundance in most months and made a significant contribution to biomass. In other years, in different months, the main contribution to the abundance and biomass was made by copepods and cladocerans. Other organisms during the entire observation period did not contribute significantly to the species diversity and for most of the observation period did not make a significant contribution to the abundance and biomass. When comparing the obtained data with historical data, one can note the absence of significant changes in the current structure of the community since the 70s, when the river was already regulated. The proportion of different groups of organisms changed only insignificantly, the saprobity indices improved slightly, and the biomass practically did not change. However, when comparing actual data with the changes that occurred place before 1950s, a tendency of decreasing of the role of rotifers in the community was observed, that might be related to a decrease in the water level of the delta. This assumption was forwarded by Naberezhny [18], who mentioned a change in the ratio towards rotifers in years with high water level. Nevertheless, no clear relationship was found between the proportion of rotifers and runoff volumes. There are also no significant correlations between the indicators of zooplankton and phytoplankton biomass, as well as any measured hydrophysical and hydrochemical parameters (Fig. 5), with the exception of water temperature, for which a correlation with r = 0.55 to r = 0.63 was revealed with different indicators of zooplankton. It is noteworthy that a weak negative correlation was observed with most chemical parameters such as salinity, pH, oxygen, phosphorus, nitrogen. It might be difficult to identify the main factor determining the development of zooplankton. To understand the processes occurring in the zooplankton community, it is necessary to analyze the complex impact of hydrological and hydrochemical factors.

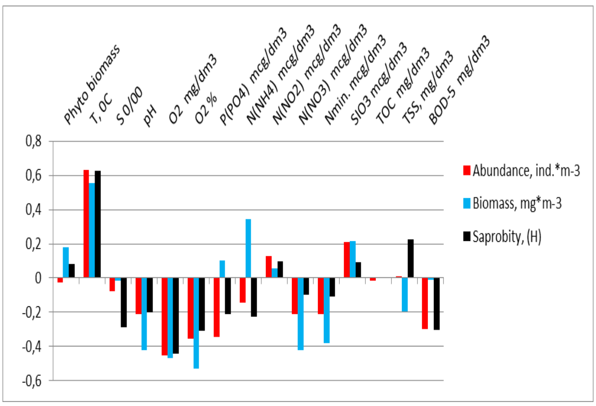


Figure 5. Correlations between zooplankton parameters and hydrochemical indicators

During 2020, significant fluctuations of the saprobic index in the Lower Dniester in the seasonal aspect and in different sectors of it were observed. In the lower sector of the Dniester river, species with a preference for the β-mesosaprobic zone predominated. The values of the saprobic index varied during the winter in the limits of 2.29-2.50 and in the spring in the limits of 1.30-1.65, in summer between 1.72-1.84 and in autumn between 1.28-1.79. During the Lower Dniester, the value of the saprobic index is presented in figure 6 and varied in the limits of 1.60 - 2.29. The maximum value at Sucleia station, due to the presence of the genus Brachionus Pallas 1766, attests to the higher content of organic substances and pollution of the given area.

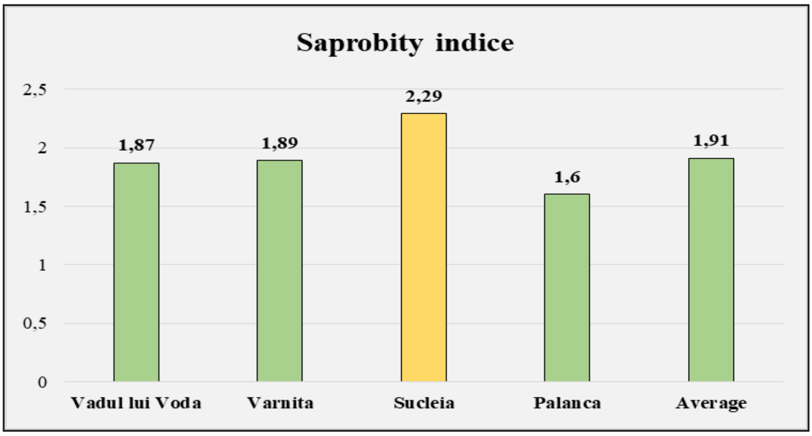


Figure 6. Variation of saprobic index and water quality class in the lower Dniester river sector, 2020.

The average value of saprobic index in the Lower Dniester was 1.91 (Fig. 6), that falls within the II class limit of water quality as "good". Saprobity index in the Dniester delta by zooplankton indicators varied from 1.56 in February to 2.44 in June (fig. 7), on average per year of 1.62, which corresponds to β-mesosaprobic zone (moderately polluted waters). It is noteworthy that the values of index were slightly better compared with 70th of XX century, when it was 1.88 [18].

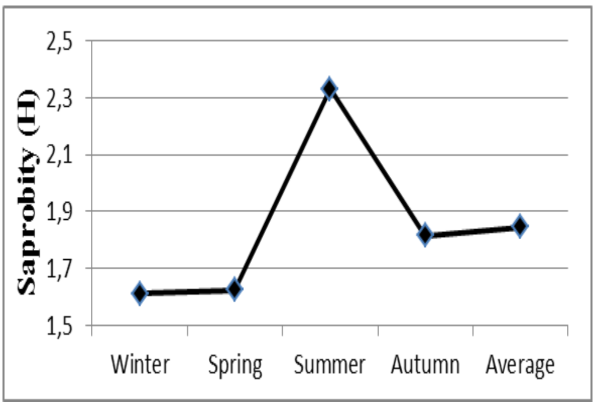


Figure 7. Seasonal variation of saprobity index for zooplankton in the Dniester delta in 2020

The ecological status of the investigated ecosystems, according to the parameters of the zooplankton communities corresponds to the b-mesosaprobe area, and the water quality according to the values of the saprobity index is attributed to classes II - III and is characterized as good - moderately polluted [5].

Conclusions

The state of the Dniester River was studied in detail in the middle of the last century; however, climate change and active hydropower construction and the changes caused by them require updating of the data. When comparing the modern with the historical ones, a moderate improvement in the state of the zooplankton community was noted during 2018-2020, in comparison with years 70s and 80s, when the river was already regulated. The proportion of different groups of zooplankton organisms changed insignificantly, the saprobity indices improved slightly, and the average zooplankton biomass did not change. However, when considering the changes occurring since the 1950s, there was a clear tendency towards a decrease in the role of rotifers in the community. This may be due to a decrease in water level in the delta. A clear relationship between the share of rotifers and runoff volumes was not found, but an inverse relationship between the total abundance and runoff volumes was visible. In general, there is a tendency to improve the state of the river in comparison with the 70s, when the maximum eutrophication of the water area was observed. However, no significant correlations were found between hydrophysical and hydrochemical parameters and planktonic communities. To understand the processes occurring in planktonic communities, it is necessary to do a comprehensive analysis of the complex impact of hydrological and hydrochemical factors on planktonic communities.

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ZOOBENTHOS OF THE DNIESTER RIVER ON THE TERRITORY OF THE REPUBLIC OF MOLDOVA FOR THE PERIOD 2018-2021

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Abstract. The data on the influence of dams on the structural and functional parameters of the macrobenthos of the Dniester River on the territory of the Republic of Moldova are presented. A decrease in the biodiversity and number of groups most sensitive to negative environmental changes (mayflies, stoneflies and caddis flies) was noted. The results described in this article, indicate that the total number of zoobenthos species and the number of most sensitive species to negative environmental changes can serve as suitable indicators of the impact of hydropower facilities on the Dniester River.

Introduction

The study on the influence of the regulation of the Dniester riverbed by the dams of the Dnestrovsk HPPs and Dubossary HPPs [4] on benthic communities contributes with recommendations for decision-making on the conservation of biodiversity of the river and sustainable use of aquatic ecosystems. This is especially important for transboundary rivers, of which water resources are used for hydropower, water supply, irrigation, navigation, recreation and environmental protection.

Material and methods

Samples were collected from the Dniester riverbed seasonally and monthly, from 2018 to May 2021, at 11 sampling points: Naslavcea, Vălcineț, Soroca, Camenca, Erjovo, Goieni, Cocieri, Vadul-lui-Vodă, Varnița, Sucleia and Palanca. In total, more than 250 samples of benthic fauna were collected and processed. At the sampling points: Camenca, Erjovo, Goieni, Cocieri, samples were collected from both the left and right banks, at Soroca sampling point, samples were collected downstream of the city and Soroca 1, a few kilometers away from the city.

Quantitative samples were taken using an Ekman bottom grab with a sampling area of 0.025 m2, and a rectangular dredge with a sampling area of 8 m2; for qualitative samples, a net and manual collection from various substrates were used [1, 2]. Samples were fixed with 37% formalin or 96% alcohol. The identification of species was carried out to the smallest possible taxon level in the laboratory using identification keys [5, 10]. Species were identified using an Axio Imager A.2 microscope (Zeiss) and a SteREO Discovery.V8 binocular microscope (Zeiss). The abundance and biomass were recalculated as ind./m2 and g /m2, respectively.

The Shannon biodiversity index and *Jaccard* coefficient for macrobenthos communities were calculated [1]. Statistical analysis was performed with the use of Excel и STATISTICA 10.1.

Results and discussion

The abundance, biomass and species composition of macrobenthos in the Dniester River and Dubăsari reservoir were determined. According to the obtained data, the average lowest multiannual (2018-2021) abundance of total zoobenthos and zoobenthos without molluscs were noted at sampling points located downstream of the dams of reservoirs, namely at Naslavcea and Vadul-lui-Vodă (Figs 1.1 and 1.2). These indices accounted for 3055 and 3461 ind./m2 of total zoobenthos, and 2370 and 2585 ind./m2 of zoobenthos without molluscs, respectively.

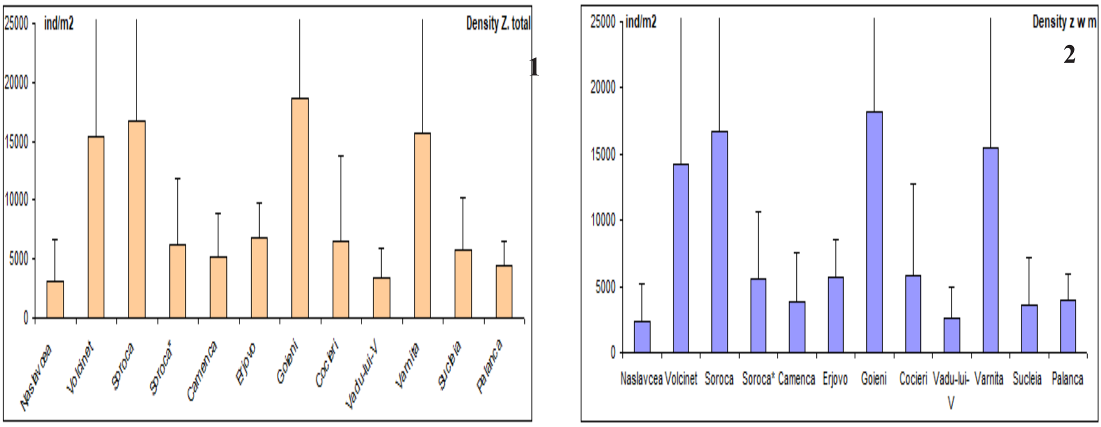

Figure 1. The mean (+ SD) density of total zoobenthos (1.1) and zoobenthos without molluscs (1.2) from Dniester River during 2018-2021

Multiannual (2018-2021) lowest average biomass of zoobenthos without molluscs was noted at Naslavcea, sampling point, located directly downstream of the dam of the Dniester cascade hydroelectric power plants, namely HPP 2, which amounted to 3.1 g/m2 and Cocieri, on the dam section of the Dubossary reservoir which amounted to 3.9 g/m2, respectively (figs. 2.1 and 2.2).

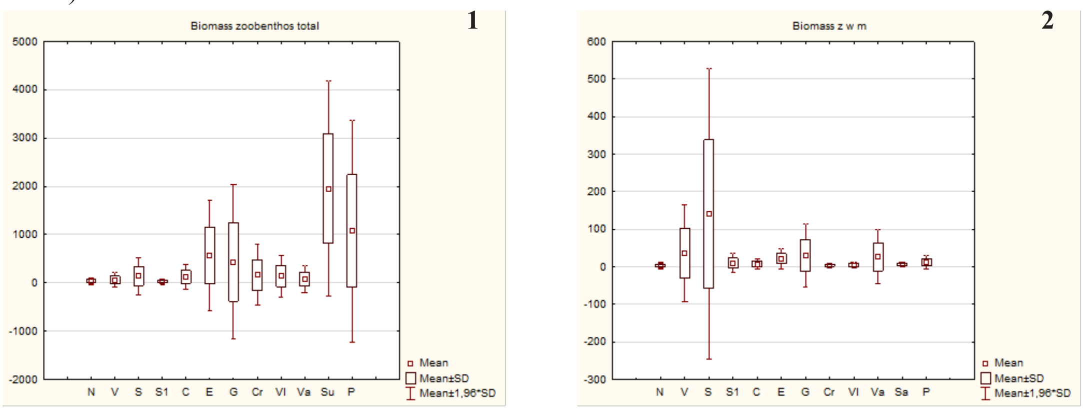

Figure 2. The mean (± SD) biomass of total zoobenthos (2.1) and zoobenthos without molluscs (2.2) from Dniester River during 2018-2021. N-Naslavcea, V-Vălcineț, S-Soroca, S*- Soroca 1, C-Camenca, E-Erjovo, G- Goieni, Cr-Cocieri, Vl-Vadul-lui-Vodă, Va-Varnița, Sa-Sucleia, P-Palanca.

The lowest long-term mean biomass (2018-2021) of total zoobenthos were recorded at Soroca 1 - 27.3 g / m2 and Naslavcha - 34.9 g / m2.

The overall biodiversity assessment of invertebrates in Dniester River and Dubossary reservoir for the period 2018-2021, revealed a number of 259 taxa, the largest number (138) was recorded at Erjovo sampling point. The taxonomic structure and distribution of species along the river, indicated the smallest number of taxa at Soroca sampling point, downstream of the city, with only 25 taxa and at Naslavcea – with 45 taxa (fig. 3). At these sampling points, as well as at Sucleia, no representatives of the groups most sensitive to negative environmental changes such as: mayflies, stoneflies and caddis flies, the so-called EPT taxa (Ephemeroptera, Plecoptera, Trichoptera) were registered (fig. 3). This would be related to the unfavorable environmental conditions in these sections of the river.

It should be noted that in the Dniester River, the highest biodiversity of EPT taxa in 2015-2019 was recorded at Camenca and Goieni sampling points [6, 7]. At Camenca station, 7 species of Ephemeroptera and Trichoptera were recorded and at Goieni - 4 species of Trichoptera and 7 species of Ephemeroptera were found. During the period 2018-2021, 3 taxa of Trichoptera and 3 of Ephemeroptera were recorded at Camenca station and 12 taxa Trichoptera and 5 Ephemeroptera at Goieni station (fig. 3). It is important to note that on October 25, 2019, in Camenca, near the Dubossary reservoir, a new species for the fauna of Moldova the mayflies *Ephemera lineata* Eaton, 1870 (striped mayfly) was recorded [9].

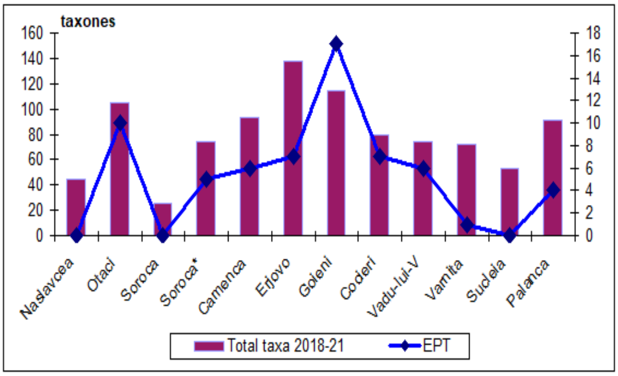

Figure 3. The number of taxa of benthic invertebrates in Dniester river and Dubosari reservoir for the period 2018-2021.

Springflies (Plecoptera) were recorded only once in 2012, at the sampling point Vălcineț and in May 2021 near the village Vîșcauți, in a stream, at the confluence with the Dniester River. Comparing composition of species of benthic macroinvertebrate communities using the Jaccard index, it could be seen that such sites as Naslavcea and Soroca were considerable different from other sites in terms of species composition (fig. 4). These differences, first of all, could be caused by the sharp changing water level in Naslavcea and the discharge of untreated wastewater in Soroca.which negatively affected the development of benthic invertebrates.

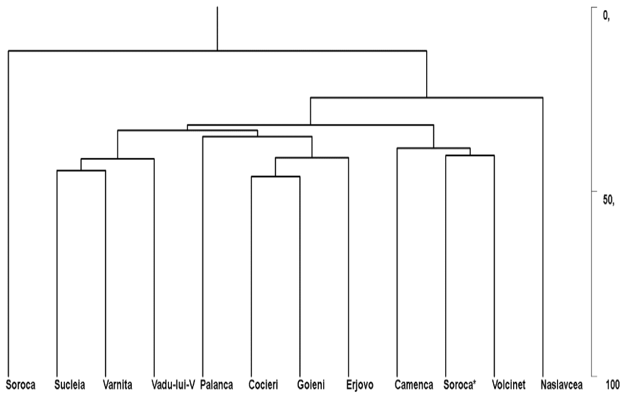

Figure 4. The dendrogram of species similarity between communities of benthic macroinvertebrates in the Dniester and Dubăsari reservoir for the period 2018-2021.

In accordance to Shannon index the highest indices were recorded at Erjovo, Goieni and Vălcinets, the lowest at Soroca and Naslavcea (tab.1).

Table 1. Shannon Biodiversity Index for benthic macroinvertebrate communities in the Dniester and Dubăsari reservoir for the period 2018-2021.

Index	Naslavcea	Valcinet	Soroca	Soroca*	Camenca	Erjovo	Goieni	Cocieri	V-l-V	Varnita	Sucleia	Palanca
Shannon	1,65	2,02	1,4	1,9	2	2,1	2,1	1,9	1,87	1,85	1,7	1,96

Comparing historical data on the composition of macrobenthos, it can be observed that species sensitive to negative environmental changes have disappeared, such as: *Oligoneuriella rheana* (Imhoff, 1852), *Ecdyonurus*, Dniester endemic *Behningia lestagei* Motas & Bacesco, 1937 and others. Almost complete extinction of mayflie *Palingenia longicauda* (Olivier, 1791) (Ap.II, Bern Convention, 1998) and *Unio crassus* Philipsson, 1788, a rare protected Natura 2000 species of bivalve molluscs, which is currently found only in the upper part of the Dubossary reservoir with dense overgrowth of Dreissena (fig.5).

Figure 5. Dense overgrowth of Dreissena on the rare protected Natura 2000 species *Unio crassus*

At the same time, alien species not only appeared in the macrobenthos communities, but became common: *Branchiura sowerbyi* Beddard, 1892, Dreissena rostriformis bugensis Andrusov, 1897, F*errissia fragilis* (Tryon, 1863), *Macrobrachium nipponense* (De Haan, 1849).

Artificial ecosystems such as reservoirs are most susceptible to the penetration and rapid spread of alien species [3].

Thus, the total number of species and the number of species of groups most sensitive to negative environmental changes such as mayflies, stoneflies and caddis flies (EPT) can serve as indicators of the impact of hydropower facilities on the Dniester River.

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BENTHIC AND PERIPHYTIC INVERTEBRATE CONTOUR GROUPS IN TECHNO-ECOSYSTEMS OF POWER PLANTS OF UKRAINE

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Abstract. Based on many years of research experience of water techno-ecosystems of thermal and nuclear power plants a brief review of the main patterns of formation of the composition, cenotic structure, elements of the functional organization of benthos and periphyton communities was made. It was shown that the composition of zoobenthos and zooperiphyton in some cooling ponds, other technical water bodies and watercourses was quite rich. In addition, due to the rather intensive invasive process, the list of taxa is constantly expanding. Species of tropical and subtropical origin have been recorded. Of particular importance is the invasion of species that may be the cause of bio-hindrances in the operation of power plant equipment. Techno-ecosystems have been studied to varying degrees. One of the most studied for a long time are the cooling ponds of Khmelnitsky and Chernobyl nuclear power plants. Hydrobiological research and monitoring at the first one has been carried out for more than 20 years. It was found that the influence of biotic invasion (invasion of Dreissenidae) may have a significant impact, comparable to extreme technogenic factors, on both the ecosystem and technical water supply facilities. The stages of contourisation and decontourisation processes in the Khmelnitsky NPP techno-ecosystem were established. At the Chernobyl NPP cooling pond, studies were carried out during all periods of the existence of the reservoir and the power plant, until the process of uncontrolled pond descent and transforming it into a unique wetland. Based on the obtained data, practical recommendations relating to the organization of hydrobiological and environmental monitoring, as well as reducing biological hindrances and improving the reliability of power plant equipment have been developed.

Introduction

A feature of water techno-ecosystems is their dependence on the regime of technogenic impact, the predominance of technogenic factors, technogenic properties of biotopes, this complex of factors and conditions also determines the characteristics of ecological processes. Research of power plants techno-ecosystems of Ukraine showed successional processes occurring in cooling ponds (CP) and other water bodies are violated by both technogenic and biotic (invasions) factors and have not sustained, but rather, pulsating character (Hydrobiology.., 1991; NPP techno-ecosystem..., 2011).

The increased interest in the study of the hydrobiological regime of cooling ponds was associated with the beginning of the active construction of power plants (1960–1970s). At present, studies of technoecosystems of power plants have undergone certain transformations, both in direction and in scale (Protasov, 2021).

The aim of this study was to summarize the available long-term research material of important components of ecosystems of technical water bodies, contour groupings – zoobenthos and zooperiphyton.

Materials and methods

The studies were carried out at the operating CPs of Ukraine – Khmelnytsky (KhNPP), Zaporozhye (ZNPP), South-Ukrainian (SUNPP), at the CP of large thermal power plants –

Krivrozhskaya, Zmievskaya, etc., as well as at the CP of the Chernobyl NPP (ChNPP), the CP of which in 2014–2018 was drained and transformed into a technogenic wetland. The generally accepted research methods of invertebrates were used, as well underwater research were conducted. Archival materials, own hydrobiological database were used. The characteristics of some of the studied techno-ecosystems are presented in the table 1.

Table 1. Characteristics of techno-ecosystems of Ukrainian NPPs

Techno-ecosystems	CP area, km^2	CP volume, mln. m^3	Maximum temperature,°C*	Average depth, m	Typical solid substrates
KhNPP	20	120	36	6,0	concrete
ZNPP	8,2	47	37	5,7	stone dump
SUNPP	8,6	86	41	10,0	stone dump
ChNPP	21,7	149	36	6,9	stone dump, concrete**

* Noted in the study period. ** before the descent

Results and discussions

Changes in the composition of invertebrate communities, their abundance, the structure of dominance are determined by factors of technogenic and biotic nature. Great importance is the nature and mode of operation of TPPs and NPPs. Among the technogenic factors in techno-ecosystems, the temperature factor is the most significant. The most intense thermal regime observed in CP ZNPP and SUNPP, summer temperature was more then 41°C at the area of discharge water.

It should be noted quite high richness of contour groupings of tehno-ekosistems – over the period of many years of research, about 300 taxa of invertebrates were recorded (Protasov, Silaeva, 2012). Most of them were found in both benthos and periphyton communities. Sedentary invertebrates such as Spogia, Bryozoa, Enthoprocta, Coelenterata can be distinguished as specific for periphyton. However, this specificity is pretty relative (Protasov, 2011), and «typical» periphyton organisms were often found in benthic samples they settle on the shells of mollusks, random solid substrata.

With a significant and permanent technogenic impact, the degradation of the bottom communities occurs, at the same time, a low level of development of invertebrates can be maintained for a significant period of time, the ecosystem goes into a «pseudo-climax» state. So, in the first year after filling the SUNPP CP (1980), dramatic change in conditions (from a small river to a cooling pond) led to a decrease in richness – from 14 (in Tashlyk river) to 2. In three to four years *Hydra sp*., Nematoda, Amphipoda, Cumacea, Odonata, Ephemeroptera, Triphoptera, Ceratopogonidae were noted in the reservoir (upper reaches), *D. polymorpha* was ubiquitous. In 1986, 48 taxa from 15 groups were recorded in the zoobenthos (Novoselova et al., 2020). After a significant summer temperature increase in 1986, the species composition of zoobenthos sharply decreased (7 taxa – in 1988, up to 12–13 taxa in 1989–1992). After complete elimination (not registered in 1988) *Dreissena* was recorded locally in 1989–1992, but since 1997 it has completely disappeared. From this period to the present, the zoobenthos of the CP was extremely poor (9–15 taxa).

Oligochetes *Limnodrilus claparedeanus* Ratzel, и *L. hoffmaisteri* Claparede, *Leptochironomus tener* (Kieffer) were the most tolerant to the effect of temperature in zoobenthos, these species inhabit areas of the SUNPP CP with high heating.

Taxonomic richness of zoobenthos of ZNPP CP also was low– from 10–11 (1995) to 29 taxa (in 2017). *Hydra sp*., Nematoda, Oligochaeta, Hirudinea sp., Ostracoda gen. sp., Trichoptera, Chironomidae, Gamamridae, Ephemeroptera, Gastropoda were noted.

Taxonomic distribution was heterogeneous – intake (IC) and discharge (DC) channels differed by a specific taxonomic composition relative to the CP. For example, *Branchiura sowerbii* Beddard and *Psectrocladius dilatatus* Wulp were recorded only in IC of ZNPP.

In taxonomic terms, the zoobenthos of CP of KhNPP is one of the richest from another CP. During the observation period (mostly summer and early autumn seasons of research 1998–2019) in the zoobenthos of CP of KhNPP 177 taxa of invertebrates from 24 groups were recorded.

During the first period of research (when only one NPP unit was in operation) 87 taxa (from 30 to 61 taxa for separate seasons and years) were found in zoobenthos (NPP techno-ecosystem..., 2011). In the second period (2005–2010), after the *D. polymorpha* invasion, the total number of benthic taxa increased to 128 taxa (from 42 to 82 taxa), and during the third period (2012–2019) after the invasion of *D. bugensis*, in general, remained at a close level – 117 taxa (from 40 to 76 taxa for certain years). The taxonomic composition of zoobenthos over the study periods was similar.

Unstable water level in CP when the water level declines, it leads to the formation of vast shallow waters, which determined the increase in the total richness of benthos due to insects. They are mainly represented by psammophilic and near-water invertebrates (Simuliidae sp., *Pseudosmittia sp.*, Tabanidae sp., Tipulidae sp., Dolichopodidae sp., Stratiomyidae sp., Psychomyiidae sp., Haliplidae sp.). KhNPP CP is only one in Ukraine, in which Gammaridae were absent.

In the taxonomic composition of zoobenthos and zooperiphyton Chironomidae and Oligochaeta were dominated. Thus, in the KhNPP CP their share in the total number of taxa of zoobenthos was 24–42 and 22–33% respectively.

Long-term studies of zooperiphyton in the KhNPP techno-ecosystem have shown both taxonomic richness one and benthos was similar. It was quite high, in the first (1998–2001) – 69 taxa and the second period (2005–2012) – 95 taxa. The total list of zooperiphyton taxa in KhNPP CP included 125 taxa (NPP techno-ecosystem..., 2011). In the third period (2012–2019), after the invasion of *D. bugensis* and a decrease in the abundance of *D. polymorpha*, the number of periphitic taxa decreased, from 40 in *D. bugensis* communities (2013) to 8 taxa (2013 and 2015) in D. *bugensis* and *E. carteri* communities.

Techno-ecosystems undergo significant, often unpredictable, anthropogenic influences, leading radical changes in ecosystems. Thus, with an uncontrolled descent of the CP of ChN-PP the species composition of the zoobenthos, like the periphyton, has undergone significant changes. During the operation period 1979–1986 more then 70 species of invertebrates were registered in the contour subsystem. After stop of NPP work removal of technogenic load led to a significant increase in taxonomic richness (in 2002 was noted 143 taxa). In the period 2012–2013, before the descent of the CP, there were 95 taxa invertebrates in the contour subsystem. No significant changes in the taxonomic composition of invertebrates during the descent period (2016–2018) were recorded – there were 118 taxa in zoobenthos and 85 in zooperiphyton. The dominance of oligochaetes and chironomid larvae was note. By the autumn of 2018 the low water level in the former CP has stabilized, now the CP is a complex of separate floodplain-type water bodies. The elimination of Dreissenidae from benthic groups led to a reduction in the total number of species and the almost complete disappearance of Amphipoda. With a decrease in the water level, the number of non-living substrates for the development of invertebrates of zooperiphyton significantly decreased, substrates were found extremely mosaic and in insignificant quantities. However, such changes significantly did not affect the taxonomic richness of the zooperiphyton.

Very important phenomenon in technoecosystems is invasive process. The invasion of alien species is important, in this case, in two aspects. Firstly, cooling ponds may cause further invasions. Alien species are resistant to adverse conditions, in particular, the temperature factor. CPs are refugia for the alien species. Secondly, invaders, as a rule, act as significant agents of biological hindrances. The invasive process was observed in all studied techno-ecosystems. In CP of KhNPP new invasive species from different taxonomic groups of invertebrates are constantly recorded. Invaders are different, including tropical origin: *Eunapius carteri* (Bowerbank) (since 2013), *Bratislavia daday* (Michaelsen) (since 2013), *Stenocypris sp.* (since 2018), *Limnomysis benedeni* Czerniavsky (since 2012), *Corophium robustum* G.O.S. (since 2019), *Dreissena polymorpha* Pall. (since 2003), *D. bugensis* Andr. (since 2012), *Ferrissia sp.* (since 2008). Most of them have naturalized. Bat, some species were registered in CP only once and have not met again, for example *Tyrrhenocythere amnicola donetziensis* (Dubowsky), *Theodoxus euxinus* (Clessin), *Planorbella sp*.

Abundance of contour hydrobionts was very different. In CP of ZNPP in 1995 and 2011, the abundance indicators were at the level of 67–7450 ind./m^2, biomass – 0,004–2,68 g/m^2, with the dominance of oligochaetes and chironomid larvae. The invasion of subtropical Gastropoda *Melanoides tuberculata* (Müller) and Terebia granifera (Lamarck) into techno-ecosystem determined biomass increase on average up to 44,06 g/m^2 (maximum to 180,10 g/m2).

In the SUNPP CP, a significant increase in biomass (up to 350 g/m^2) occurred after the invasion of *M. tuberculata* in this water body. In the first years of its existence (1985–1986), a community with the dominance of *D. polymorpha* and a biomass of about 300 g/m^2 has formed in the benthos. But with a constant high thermal effect, the zebra mussel has eliminated. Communities with low biomass (near 1 g/m^2) which existed for over a decade have formed.

In the CP of KhNPP in the first period, the indices of zoobenthos abundance were within

5,2–9,1 thousand ind./m2 and 5,45–471,25 g/m^2 (including large bivalve molluscs that lived in local areas), dominated by oligochaetes and chironomid larvae. After the invasion of D. polymorpha, the numbers increased on average to 21,0 thousand ind./m^2, total biomass increased on average for CP to 1,1–2,9 kg/m^2 and this level was observed until 2010. The maximum biomass of zoobenthos in the local area was 25,7 kg/m^2. During the third period, the biomass is in the range of 13,06–697,82 g/m^2.

In the first period (1998–2001), the indices of the numbers of zooperiphyton in the reservoir ranged from 93 (depth 2–3 m) to 129000 ind./m^2 (depth 0,4–0,5 m). Almost at all investigated stations, there was a well-pronounced trend to decrease in numbers with depth.

In the second period (2004–2012), after the introduction of *D. polymorpha*, a spatial displacement of the maximum abundance from area at the water's edge into the depth have occurred. Biomass has increased by 3 orders (Protasov, Silaeva, 2012). If at a depth of 4–5 m in 2005 at the dam the biomass was 14,1 kg/m^2 and in the intake channel – 19,7 kg/m^2, then in 2009 the biomass of zooperiphyton decreased to 2–6 kg/m^2.

The third period (2012–2019) was associated with the invasion of the second species of Dreissena – *D. Bugensis* into the reservoir. In 2012, the numbers of zooperiphyton decreased with depth (123056 ind./m² at a depth of 0,5 m to 13800 ind./m² at 6,0 m). The biomass in the intake channel was the largest at 5 m, (the share of Dreissena was 97.0%) and exceeded 100 g/m^2. From 2014 to 2016 indices of the periphyton abundance in the IC and at the dam decreased (IC – 3835,4–1024,4 g/m^2) and on the dam 711,2 g/m^2 (2015) to 1,63 g/m^2 (2017).

At present, populations of two species of Dreissena coexist in the KhNPP cooling pond, with D. bugensis prevailing in the periphyton.

Studies have shown a significant role in techno-ecosystems, in addition to technogenic factors, biotic factors also play a role. Thus, the invasion of Dreissena into the CP of KhNPP in the contour subsystem led to the formation of spatially complex of consortium-type communities with high biomass, relatively stable over of years. However, with long-term observation it was found that the period of stability continued for relatively short time (figure). In the main technogenic biotopes – at the dam and in the intake channel – the distribution of Dreissena in depth was characterized by an increase in biomass by 4–6 m. Therefore, we present the average values of the zebra mussel biomass at these depths.

And in CP ChNPP the development of Dreissenidae determined the quantitative indices of the invertebrates of the contour subsystem.In the period before the descent (2012–2013), the level of zooperiphion development in the CP of ChNPP was high – 11,70–190,3 thousand ind./m^2 and 1,29–17,78 kg/m^2. In 2012–2013, the quantitative indicators of benthos were 960–57480 ind./m^2, 0,43–9148,52 g/m^2 (biomass without molluscs 0,43–50,37 g/m^2). The biomass in the contour subsystem was determined by *D. bugensis,* which invited into the water body in the 1990.

Fast draining of the CP stone dump during the descent led to the fast decline in the total stock of zooperiphyton. The biomass of the zooperiphyton in 2016–2017 decreased by 2 times in comparison with the period before descent, and by 2018 – decreased by almost an order of magnitude (relative to 2016–2017). Reducing in the abundance of zoobenthos during the descent (2016–2018) occurred relatively gradually. Only by 2018 elimination of dreissenids determined a significant decrease in the abundance of zoobenthos – numbers tripled, biomass – in 122 times.

Thus, the cardinal transformation of the technogenic reservoir led to a decrease in the quantitative indicators of the invertebrates of the contour subsystem.

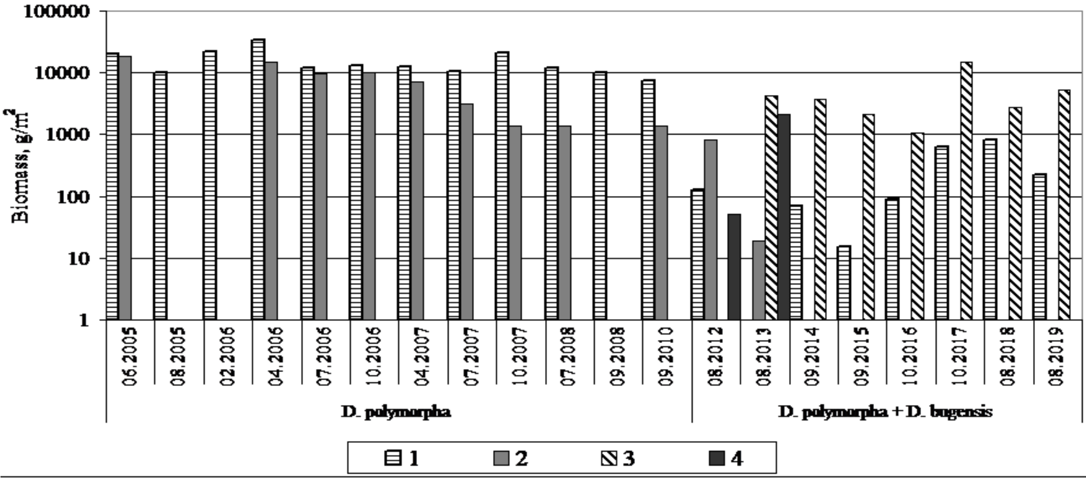

Figure 1. Dynamics of Dreissenidae biomass in the techno-ecosystem KhNPP: 1 – D. polymorpha in IC, 2 – D. polymorpha on the dam, 3 – D. bugensis in IC, 4 – D. bugensis on the dam. The average values at a depth of 4–6 m are presented

It is possible to conclude the richness of invertebrate of contour groups in techno-ecosystems is quite large. At the early stages of research of cooling ponds, it was supposed that the fauna in them should be poor (Ecology…, 1975). In the CP, there is a constant invasive process, which replenishes the composition of groups. In the investigated techno-ecosystems, an increase in the number of taxa and quantitative indicators of invertebrates was associated with the invasion of *D. polymorpha*. However, during the invasion of the second species of Dreissenids (*D. bugensis*) in the CP of KhNPP, neither an increase in richness nor indicators of abundance was noted. At the same time, the second species took a dominant position in biomass, this phenomenon was also noted in the ChNPP CP (Model groups..., 2002).

Filtration activity of Dreissena caused the transformation of the entire ecosystem of the KhNPP CP due to contouring processes (NPP techno-ecosystem..., 2011; Protasov et al., 2021). With the influence of Dreissena increases, in phytoplankton the dominants and the structure of dominance changed, the indices of abundance and taxonomic richness decreased to their minimum values at the peak of contouring. After reducing the impact and adaptation processes in the ecosystem there was an increase in the quantitative indicators and richness of phytoplankton. The reduction of individual phylums of algae in the phytoplankton composition proceeded in the reverse sequence with respect to their disappearance under the pressure of *Dreissena*.

Further studies of the contour groupings of techno-ecosystems should be carried out in the following directions – study of the taxonomic composition, especially of such groups as Oligochaetes, Chironomid larvae. It is important to study the invasive process, since CPs can be refugia of the distribution of invasive species into natural water bodies. Particular attention should be paid to contour groupings in terms of their ability to create biological hindrances in technical water supply systems.

Our long-term studies show for practical purposes, a modern system of hydrobiological monitoring should be created in techno ecosystems.

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INVASIVE SPECIES IN THE CONTEXT OF CLIMATE CHANGE: THE CASE OF MAGALLANA GIGAS (THUNBERG, 1793) IN THE BLACK SEA

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Abstract. The current climate changes are having a significant impact on species distribution. While some species are affected, others are thriving in these new conditions, expanding their range. This is also the case of some invasive species who seem to benefit from these changes. The invasive Pacific oyster Magallana gigas is among these species. The current situation of the Pacific oyster in the Black Sea is discussed, with new data from the Romanian waters.

Introduction

The constant global warming trend has a great impact on the distribution of species worldwide. Some native species spread their areal, while others are greatly affected and are faced with extinction. The warming climate is also a favourable factor in the dispersal and acclimatisation of some invasive species to new habitats [4, 10].

Not many species can acclimate to the Black Sea conditions, but there are some species with a wide tolerance to salinity, temperature and pH that have been successful in these conditions. Among these species, the ones with the biggest impact on Black Sea ecosystems were the invertebrates *Mnemyopsis leidyi, Rapana venosa, Mya arenaria, Anadara kagoshimensis* etc. These species have managed to drastically impact the ecosystems on different levels: the ctenophore M. leidyi has seriously affected the zooplankton stock which led to the collapse of the planktivorous fish [11]; the veined rapana whelk R. venosa is the one responsible for the disappearance of the native flat oyster *Ostrea edulis* [6]; the softshell clam *M. arenaria* has produced major changes in the benthic ecosystem by replacing the native dominant species *Lentidium mediterraneum* [5].

The most recent invasive species settled in the Black Sea is represented by the Pacific oyster *Magallana gigas* (Thunberg, 1793). Here we present the past several years status of an established population discovered in the Romanian Black Sea.

Matherials and methods

A small wild population of *M. gigas* was discovered in 2017, in Agigea harbor, near Constanța [8]. The population was assessed at least once a year since its discovery, during diving sessions. Individuals were measured and surface of the colony assessed using the same methods described in previous studies [8].

Results and discussion

The *M. gigas* colony found in the Romanian Black Sea is most likely the result of the offshore attempts of acclimating the species to the Black Sea conditions for farming purposes. Since its discovery in 2017, there was no evident growth of the colony, but it has remained stable with living individuals growing. The largest specimen found in 2017 was of 18.6 cm in length, which suggested that the probable age of the colony was of about 5 years. Until now, no evidence of reproduction was observed in the colony. This year, however, small individuals of less than 5 cm in length were observed. This indicates that the colony is reproducing and, considering the growing rate of 4–5 cm in the first year, this shows that they are about one year old [3].

Figure 1. Small *M. gigas* individuals, of less than 5 cm in length, found in 2021 attached to other shells in Agigea colony (scale in cm)

In the context of the global climate change with higher average temperatures, M. gigas has fairly good chances of reproducing and forming stable colonies in the Black Sea. A recent study has also demonstrated that the range expansion of the Pacific oyster is driven by climate warming, predicting its presence all along the Northwest European Shelf [7]. Isolated individuals have been constantly found in the Black Sea even since 1989, and in the last 10 years, wild small populations have started to be found along the eastern, northern and western coasts of the Black Sea [9]. The species seems to follow the same trend in the Bulgarian Black Sea, where more and more specimens are beginning to be found, including small viable colonies of up to 20 individuals [9]. Recently, quite dense young colonies of M. gigas were discovered in the southern Black Sea, along the Turkish coast [1].

The status of *M. gigas* in the Black Sea is not yet clear, as it could fill in an ecosystem niche left empty by the disappearance of the native flat oyster Ostrea edulis or it could affect the present ecosystems in other positive or negative ways. At the moment, according to the framework proposed [2], *M. gigas* in the Black Sea falls into the C3 category with individuals surviving in locations where introduced with reproduction occurring, and some wild self-sustaining populations. Considering the current climate trends and the high reproductive potential and plasticity of the species, *M. gigas* could easily start establishing secondary colonies in the future and transition to a fully invasive species. The possible impact of the Pacific oyster, with its highly adaptive potential, on the Black Sea ecosystems depends greatly on different complex factors and it is still unknown so far.

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STUDY OF ACID-NEUTRALIZING CAPACITY OF THE DNIESTER RIVER UNDER WINTER LOW-WATER CONDITIONS

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Abstract. The resistance to acidification of the Dniester water was tested by the method of potentiometric titration. Water samples were collected on the right bank of the Dniester River within the territory of the Republic of Moldova in February 2020. The acid-neutralising capacity values obtained (ANC) were compared with the critical acid load (CAL), which makes it possible to assess the habitat conditions for any group of hydrobionts whose resistance to acidification is known

Introduction

Anthropogenic acidification of the environment became a spread phenomenon in the twentieth century and continues today, affecting not only the hydrosphere, but also the lithosphere and biota. Surface water ecosystems are most exposed, as acidification affects all their components, including water, bottom sediments and hydrobionts. Acidic precipitations alter the geochemical cycles of chemicals elements both in the catchment area and in the water body. An increase in acidity has negative consequences for the aquatic ecosystem, such as change in the ionic composition of water, the transition of metals from bound to free forms and, ultimately, a decrease in the buffer capacity of a water body.

The full buffer capacity is ensured by all components of water ecosystem: dissolved ions, suspended substances, bottom sediments, aquatic organisms, etc. In the world practice, various indicators are used to assess water acidification: pH dynamics, acidity, alkalinity, the ratio of concentrations of different ions and others. A method was proposed (Henriksen et al., 1992) for calculating the acid-neutralizing capacity (ANC) based on the content in water of such ions as $Ca^{2}+$, $Mg^{2}+$, Na+, K+, $SO_4{}^{2}-$, $NO^{3}-$, $HCO-_3-$, H+ and $Al^{3}+$. This calculation method does not take into account the effects of such components of the aquatic habitat as suspended solids, polypeptides, polyphenols and organic acids (humic, fulvic), which are often present in surface waters. To assess the resistance to anthropogenic acidification of a water body, a method of potentiometric titration with graphic processing of the results obtained can also be used [1].

The Dniester River is one of the largest sources of fresh water in the Eastern Europe, with an average flow volume of about 10 km3/year. This is a transboundary river, a 652 km long section of which (Middle and Lower Dniester) is located on the territory of the Republic of Moldova or serves as a border with Ukraine. After the construction of the Dubossary dam and later the Novo-Dnestrovsc hydropower complex (Ukraine), the natural hydrological regime of the river was disrupted. As a result, three sections of the Dniester River within the territory of the Republic of Moldova have different hydrological and hydrochemical characteristics: the section downstream of the Dniester HPP-2, the Dubossary reservoir and the Lower Dniester downstream of the Dubossary dam. Climate change and flow regulation are not the only factors affecting the ecological state of the Dniester River. There is also modification of temperature and oxygen regimes, industrial and domestic pollution, exploitation of floodplain areas and destruction of natural habitats as a result of the extraction of sand and gravel.

The aim of the study was to analyze the acid-neutralizing capacity of the Dniester River water under conditions of alteration of the natural hydrological regime and climatic changes.

Materials and methods

Samples were collected at the Moldovan section of the Dniester River, which includes the sector downstream of the Dniester HPP-2 dam (stations Naslavcea, Valcinet, Soroca) and the sector downstream of the Dubossary dam (stations Vadul lui Voda and Varnita). “In situ”, water temperature and hydrogen ion concentration (pH) were measured using a CONSORT C5030 multi-parameter analyser with built-in temperature sensor. In laboratory, potentiometric titration was performed using a pH-meter SARTORIUS PB-11, a magnetic mini-stirrer HI 190M and a BLAUBRAND automatic burette 25/0.05. Processing the results of titration was carried out in MS Excel program.As noted above, the *full buffer capacity* of water body to acidification is ensured by all components of water ecosystem (dissolved substances, suspended solids, bottom sediments, hydrobionts, etc.). The *partial buffer capacity* of water to acid neutralizing due to dissolved and suspended substances (ANC, mmol/L) was determined by the titration curve at the pH inflection point (fig. 1) using the method [1]. The water samples were not preserved, filtered, diluted or concentrated. This method used for the acid-neutralising capacity of a water body is direct and does not require correction for the presence of suspended solids in the water. Also, there is no need to determine the entire composition of dissolved inorganic and organic substances involved in the neutralization of acidic pollution from rainfall and industrial wastewater.

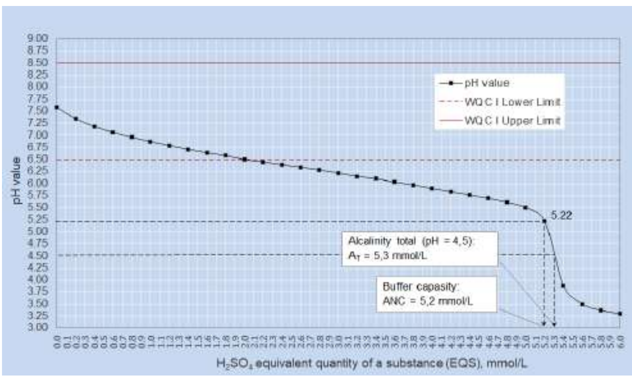

Figure 1. Assessing ANC and alkalinity by potentiometric titration curve (station Ustia, Feb 2020)

The sum of all titratable bases characterizes such hydrochemical parameter as alkalinity of water. Depending on the endpoint pH used, the composite alkalinity (AP, pH ≥ 8.3) or the *total alkalinity* (AT, pH = 4.5) is identified [2]. Because the total alkalinity of many surface waters is primarily a function of carbonates, bicarbonates and hydroxides, it also can be analyzed by the potentiometric titration curve at the endpoint pH = 4.5. When titrating to this pH value, all alkaline compounds in the water sample are used up.

Results and discussion

The main components of a surface water body, which determine the values of such interrelated parameters as pH, alkalinity and ANC (acid-neutralizing capacity), are carbonates (CO_3^{-2}), bicarbonates (HCO_3-) and hydroxide ions (OH-). But not only the carbonate buffer system ensures the acid-neutralizing capacity of the aquatic habitat. Ammonia, borate, phosphate and silicate buffer systems, as well as organic anions, can also affect the resistance to acidification of a water body. The biological effect of acidification begins at a pH value < 6.5 (water quality Lower Limit for freshwater ecosystems). The critical pH value below which the irreversible biological effects can occur is considered to be 5.6 [2, 3].

There are not many publications devoted to the study of the acid-neutralizing capacity of the Dniester River on the territory of Moldova. In the article by A. Lis et al. [4], a number of parameters characterising the buffer properties of the Dniester River and its tributaries were analysed: pH, acidity, alkalinity, acid-base constant, etc. The acid-neutralizing capacity of the Dniester water were determined by potentiometric titration. The authors note insignificant seasonal fluctuations of tested parameters. Regarding resistance to acidification, the monitoring stations are ranked as follows: Cosauti > Naslavcea > Boshernita > Dubossary Reservoir.

In the work of N. Goryacheva et al. [5], the acid-neutralizing capacity of the Dniester River was determined by Henriksen's method with calculation of the sum of hydrocarbonates and anions of organic acids ($ANC_1 = HCO_3- + A_{org-}$) and the ionic balance of cations and anions ($ANC_2 = Ca^{2+} + Mg^{2+} + Na^+ + K^+ - SO_4^{2-} - Cl^- - NO_3^-$). During the period 2008 – 2009, a steady tendency towards water acidification downstream of the Buffer reservoir (Naslavcea station, ANC = 2,42) was observed. It was noted that the acid-neutralizing capacity of the Dniester water increased along the longitudinal profile of the river (Cosauti station from Soroca District, ANC = 2,57; Boshernita station from Rezina District, ANC = 2,94), reaching its maximum values in the zone of the Dubossary dam (ANC = 3,15). It was of interest to study how the buffer properties of the Dniester River have changed over the past decade, which was marked by global climate changes, aggravating the anthropogenic impact on natural ecosystems.

In our study, samples were collected from the surface water layer 0.8 – 1.0 (m) in the February 2020. In winter, the hydrological conditions of the Dniester River in the Republic of Moldova are characterized by low water level. Therefore, the concentration of industrial and domestic pollutants in water can be more critical than in summer. On the other hand, at low water temperatures, the productivity of hydrobionts is reduced. Thus, the influence of the biotic communities on the buffer capacity of the aquatic habitat is minimised during winter period. The temperature and acidification conditions (pH) of the Dniester water at the time of sampling are presented in figure 2.

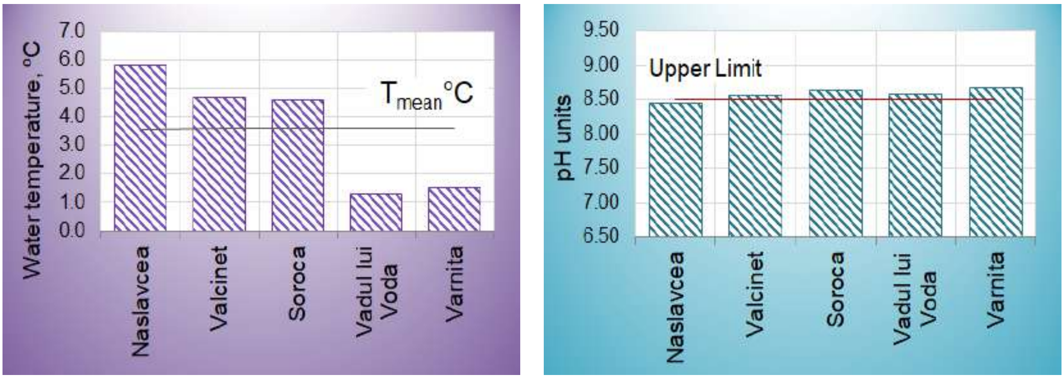

Figure 2. Dynamics of water temperature and pH values at the Dniester River monitoring stations

The concentration of hydrogen ions in the Dniester water varied in a narrow diapason 8.5 – 8.7 (pH units), which includes the Upper Limit for the surface Water Quality Class I (pH = 8.5), regulated by the Decision of the Government of the Republic of Moldova No. 890 [6]. The average water temperature of the collected samples was 3.6°C. The presented spatial dynamics of this parameter (as well as long-term investigations by the Laboratory of Hydrobiology and Ecotoxicology of the Institute of Zoology [7]) confirm the impact of the Dniester Hydropower Complex on the temperature regime of the Dniester River: winter water temperatures in the section downstream of HPP-2 (Naslavcea - Soroca) are higher than natural ones, while summer temperatures are lower.

Sampling over 400 km long river section and their delivery to the Laboratory takes at least 12 hours, therefore the potentiometric titration was carried out the next day. Until titration, the samples were stored in the dark at 3.6°C (average water temperature at the time of sampling). In winter biocenoses, the metabolic processes of hydrobionts are slowed down (respiration, growth, nutrition), but they do not stop even at this temperature. In the process of respiration and biochemical oxidation of substances dissolved in water, oxygen is consumed and carbon dioxide is released, which is the reason for a slight decrease of pH values in the water samples during the storage period (tab. 1).

Table 1. Decrease of pH in water samples due to metabolic processes

pH value:	Naslavcea	Valcinet	Soroca	Vadul lui Voda	Varnita
In situ	8.45	8.55	8.64	8.57	8.68
In lab	8.00	8.02	7.95	8.10	8.02

When determining the resistance to acidification “in situ”, the potentiometric titration curve will correspond to the *instantaneous buffer capacity* of the water body. Such results can be obtained using portable digital titrators. In our case, the burette method was implemented under standard laboratory conditions.

To determine the *partial buffer capacity*, 100 mL of an unfiltered water sample was taken, transferred to a beaker, placed on a magnetic stirrer and titrated with a solution of H2SO4 0.02 mol/L. Sulfuric acid was chosen because it is predominant in acidic atmospheric precipitation. Some of titration curves with the values of ANC and total alkalinity are presented in fig. 3 – 5.

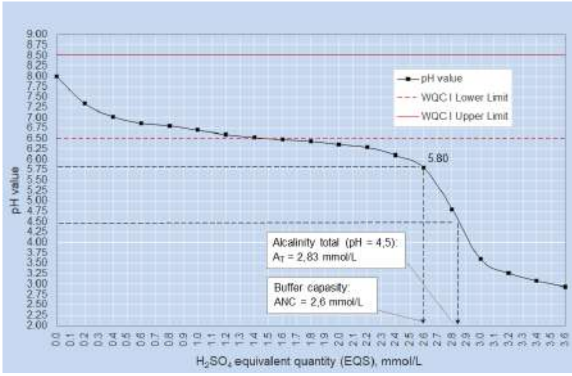

Figure 3. Assessing ANC and total alkalinity for station Naslavcea (February 2020)

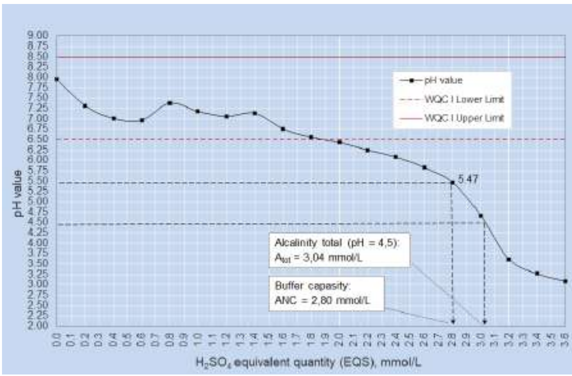

Figure 4. Assessing ANC and total alkalinity for station Soroca (February 2020)

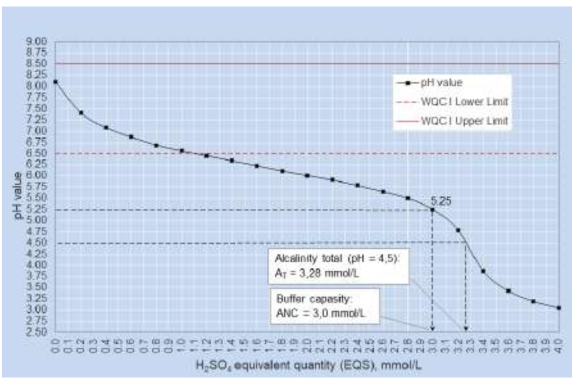

Figure 5. Assessing ANC and total alkalinity for station Vadul lui Voda (February 2020)

As noted above and can be seen from the presented graphs, all tested samples at the beginning of potentiometric titration had a pH < 8.3. This means that the free carbonates present in water at the time of sampling have already undergone the first hydrolysis stage: $CO_3^{2-} + H_2O = HCO_3^- + OH^-$. The buffer properties of the standard solution at this stage of titration with a strong acid are determined by the capacity of the buffer system H_2CO_3 / HCO_{3-}. Natural surface waters also contain other buffer systems, and the more there are, the more resistant to acidification the water body is, the more acid is required to reach the "inflection point" on the titration curve. The presented titration curves demonstrate that the acid-neutralizing capacity of the Dniester River (fig. 3 – 5) is lower than its tributaries (Fig. 1), in which, as a rule, the concentration of suspended solids, organic and inorganic components is higher. For practical purposes of observation monitoring, it is not even necessary to determine the "inflection point": it is enough to estimate the "point of Critical Acid Load" at pH = 5.6. A summary of the assessment results of the Dniester water buffer capacity parameters is presented in table 2.

Table 2. Buffer capacity parameters of the Dniester water in the winter low-water conditions

Parameter	Naslavcea	Valcinet	Soroca	V lui V	Varnita
pH	4.5				
A_T, mmol/L	2.83	2.86	3.04	3.28	3.30
pH	5.6				
Critical Acid Load, mmol/L	2.65	2.50	2.75	2.70	2.75
pH	5.80	5.42	5.47	5.25	5.54
ANC, mmol/L	2.60	2.60	2.80	3.00	3.00

Conclusions

The acid-neutralizing capacity of the Dniester River under the winter low-water conditions varies in the range of 2.60 – 3.00 (mmol/L). The vector of ANC gradient "min - max" coincides with the longitudinal profile of the river: the minimum value is in the section downstream of the HPP-2 (Naslavcea – Soroca), the maximum is downstream of the Dubossary dam (Vadul lui Voda – Varnita). The results were unexpected in the sense that the ANC values for all monitoring stations are in close proximity to the Critical Acid Load (CAL), estimated at pH = 5.6. Stations Naslavcea and Soroca (with all the difference in their hydrological and ecological characteristics) have the same lowest stability reserve to acidification: CAL – ANC = 0.05 mmol/L. The highest is at Vadul lui Voda station: 0.30 mmol/L. Thus, determining the acid-neutralizing capacity of the water body and comparing result with the Critical Acid Load makes it possible to assess habitat conditions for any group of hydrobionts whose resistance to acidification is known.

Determination of the Critical Acid Load of a water body can be used also in aquaculture for technological control of the cultivation conditions for hydrobionts with different thresholds of adaptation to habitat acidity.

The ability of an aquatic ecosystem to self-recover after anthropogenic impact is directly related to its buffering capacity. Today, the anthropogenic load on ecosystems is aggravated by climate change, and adaptation mechanisms do not have enough time to ensure the resistance of biotic communities to these pressures. Under such conditions, the biota loses its ability to stabilize the habitat, and the ecosystem becomes less productive. Rational use of water resources was the agenda of the last century. Today's attitude to natural ecosystems should be not just rational, but nature-saving.

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DYNAMICS OF MINERAL NITROGEN COMPOUNDS IN THE WATERS OF THE DNIESTER RIVER

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Abstract. This article presents the results of studying the content of mineral (ammonium ions, nitrites and nitrates) and organic nitrogen compounds in water samples collected from the Dniester river in 2020. In the study are examines the seasonal dynamics of the forms of mineral nitrogen, total nitrogen and the correlation between organic and mineral nitrogen. Limits of ammonium ion concentration in the Dniester river varied between 0.002 mg N/l and 0.93 mg N/l, nitrite ions 0.002 mg N/l and 0.05 mg N/l, nitrate ions 0.002 mg N/l - 1.36 mg N/l.

Introduction

Dniester is one of the largest rivers of Europe. It flows from the north-western part of the Eastern Carpathians, the mountain slope Rozlici, its length being equal to 1352 km, the basin area - 72,100 km, including the Moldovan territory of 657 km and 19,000 km, respectively. The average annual flow reaches up to 10 km [5].

For the Dniester River, as well as for all surface waters, the content of nitrates, nitrites and ammonium nitrogen are important indicators of the chemical composition of water, which are taken into account when assessing the ecological impact and regulating water quality. Knowing the concentrations of mineral nitrogen compounds and the ratio between organic and mineral nitrogen, helps to identify in time the existence of conditions for the development of anthropogenic eutrophication [2]. In aquatic ecosystems, mineral nitrogen compounds (ammonium ions, nitrites and nitrates) are mutually dependent and can change from one form to another, so it is important to monitor them as a whole.

Mineral nitrogen compounds are often determinants in the development of aquatic organisms, in the intensity of the production-destructive processes of primary producers of hydrobionts and different groups of aquatic microorganisms and the level of trophicity of ecosystems.

Mineral nitrogen compounds are biogenic substances, but under certain conditions generated by anthropogenic activity (use of fertilizers, discharge of wastewater) can become toxic substances. [4]

Monitoring these compounds and other biogenic substances allows estimating changes in the aquatic environment, assessing the intensity of migration and the chemical circuit, assessing their impact on hydrobionts, as well as establishing the legitimacy of hydrobiocenosis and preventing adverse consequences on ecosystems.

Materials and methods

Water samples were collected from the Dniester river in the collection points Naslavcea, Valcinet, Soroca, Camenca, Erjova, Goieni, Cocieri, Vadul lui Voda, Varnița, Sucleia, Palanca, during 2020 in February, May, June (flood period), July, October (fig. 1).

Figure 1. Sample collection points from the Dniester river: Naslavcea, Valcinet, Soroca, Camenca, Erjova, Goian, Cocieri, Vadul lui Voda, Varnita, Sucleia, Palanca

The content of nitrates, nitrites and ammonium ions was determined by the spectrophotometric method [1].

The principle of the method for determining nitrites consists in the formation of a diazonic compound of color from pink to red, the intensity increasing with increasing concentration.50 ml of analyte water are taken into the flask (50 ml), 0.1 g of Griess reagent is added. Mix well and leave for 40 min. for color development. After 40 min. the optical density is measured at the spectrophotometer (λ = 540 nm) in 10 mm cuvettes in relation to the water to be analyzed.

The method of determining nitrates consists in determining the color intensity of the nitro derivatives formed by the chemical reaction between salicylic acid and nitrates in water in an acidic medium. Take 10 ml of the test sample in the beaker (50 ml). Add 1 ml of sodium salicylate (freshly prepared). Place the sample beaker in the oven (or water bath) on evaporation and keep to dry. After beaker cooling to room temperature, add 1 ml of concentrated sulfuric acid (ρ = 1.84 g/l), moisten the walls of the beaker well and leave for 10-15 minutes. Meanwhile, by slow rotating movements of the beaker, the acid "drips" well on the walls of the beaker for a total dissolution. After 15 minutes, wash the walls of the beaker well with distilled water and transfer to a 50 ml volumetric flask. Add 7 ml of NaOH (10N) to the beaker and make up to volume with distilled water. Put the lid on and mix well. After 10 min. measure the optical density of the solution in cuvettes of 10 mm at λ = 400 nm in relation to the analyzed water. In the case of water with a high nitrate content, 1 ml of test sample will initially be taken.

The principle of determination of ammonium ions consists in the reaction of ammonium ions, in basic medium, with potassium tetraiodomercurate (K2 [HgI4]) which forms a yellow-brown complex (oxymercur ammonium iodide). The color intensity is proportional to the ammonium ion content of the sample to be analyzed. Take 50 ml of the test sample into the volumetric flask (50 ml), add 1 ml of Seignette salt and mix well. Then add 1 ml of Nessler reagent. The obtained solution is mixed well and left for 7-10 minutes to develop the color. After 10 min the optical density of the solution is measured in cuvettes of 10 mm at at $\lambda = 400$ nm in relation to the analyzed water.

The UV-VIS Specord 210 Plus spectrophotometer (Analytik Jena, Germany) was used to measure the optical densities.

Results and discussions

Maximum concentrations of ammonium ions were obtained in February in Varnita. In most of the samples from Naslavcea to Vadul lui Voda in February and October, non-essential changes of ammonium ion concentrations are observed. In Varnita throughout the year there is a high level of ammonium ions, in February about 6 times more than upstream, in May dozens of times, October 3 times, a fact observed downstream in Sucleia in February and Palanca in May. This can be argued as a result of diversions from the Bic river, a tributary of the Dniester river, whose mouth is located upstream of Varnita and the concentrations of ammonium ions being 10 times higher than in Varnita in May. In June (flood period) there is no major difference between concentrations (fig. 2).

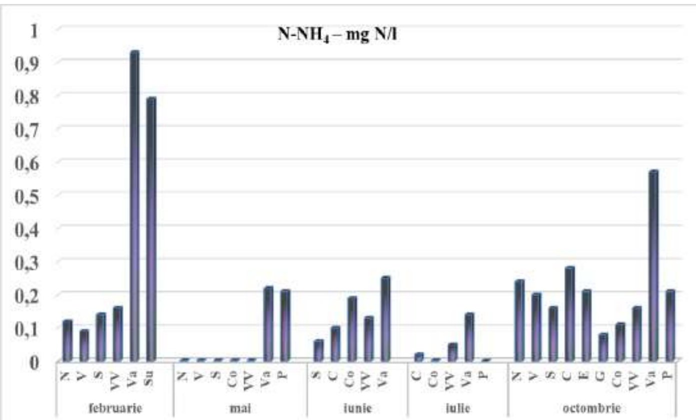

Figure 2. Dynamics of ammonium cation concentration (N-NH4+) in the Dniester waters in mg N/l (N-Naslavcea, V-Valcinet, S-Soroca, C-Camenca, E-Erjova, G- Goian, Co-Cocieri, VV-Vadul lui Voda, Va-Varnita, Su-Sucleia, P-Palanca)

According to the Regulation on environmental quality requirements for surface waters [3], according to the content of ammonium ions, Dniester river refers to quality classes I and II, very good and good water respectively, only in February in Sucleia and in October in Varnita - it referred to quality class III (moderately polluted), reaching in February in Varnita even the class IV - polluted.

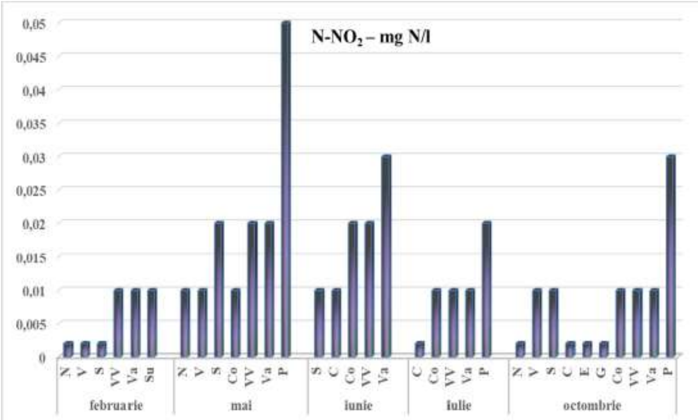

Figure 3. Dynamics of nitrite anion concentration (N-NO2-) in the Dniester waters in mg N/l (N-Naslavcea, V-Valcinet, S-Soroca, C-Camenca, E-Erjova, G-Goian, Co-Cocieri, VV-Vadul lui Voda, Va-Varnita, Su-Sucleia, P-Palanca)

Nitrites are the intermediate step between ammonium ions and nitrates. In the waters of the Dniester river in 2020 the nitrite concentrations were low, the vast majority being up to 0.02 mg N/l. The maximum values were determined in Palanca in May and October and in Varnita in June (fig. 3).

According to the quality requirements for surface waters, the waters of the Dniester River referred to classes I (very good water) and II (good water) of quality [3].

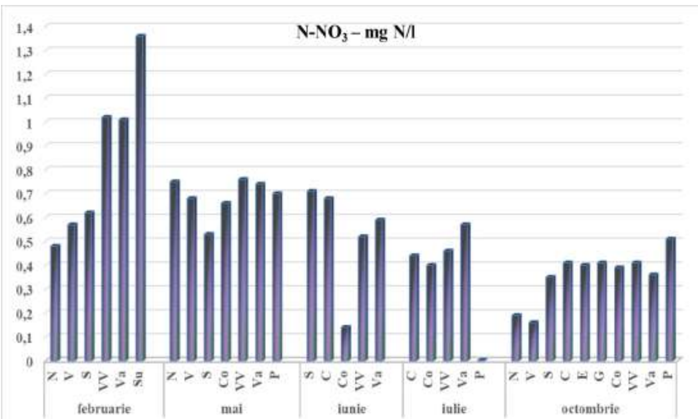

Figure 4. Dynamics of nitrate anion concentration (N-NO3-) in the Dniester waters in mg N/l (N-Naslavcea, V-Valcinet, S-Soroca, C-Camenca, E-Erjova, G-Goian, Co-Cocieri, VV-Vadul lui Voda, Va-Varnita, Su-Sucleia, P-Palanca)

According to the Regulation on environmental quality requirements for surface waters [3], according to the content of ammonium ions, Dniester river refers to quality classes I and II, very good and good water respectively, only in February in Sucleia and in October in Varnita - it referred to quality class III (moderately polluted), reaching in February in Varnita even the class IV - polluted.

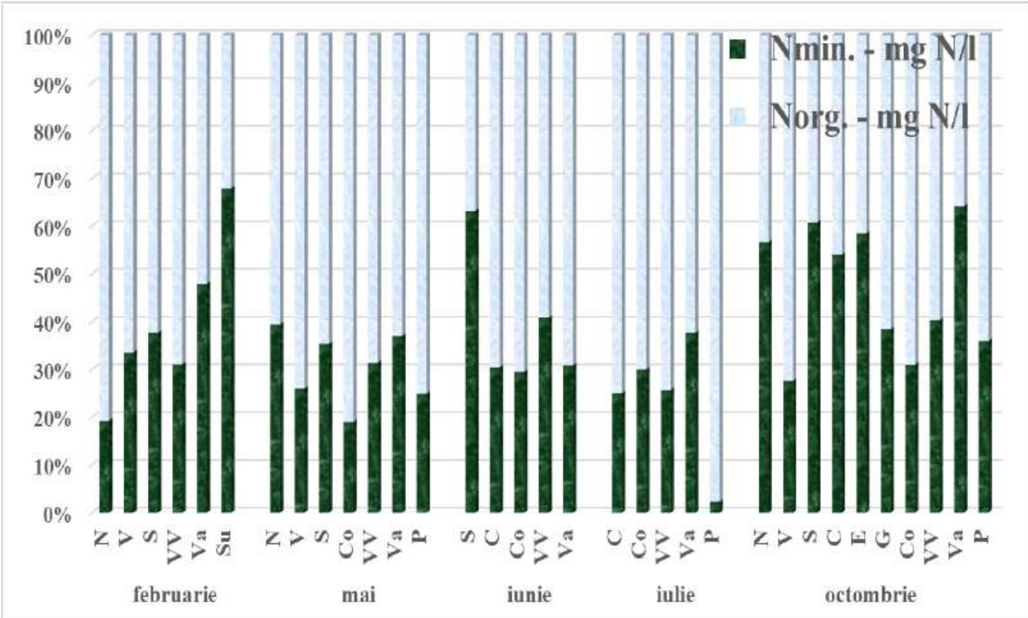

Figure 5. Dynamics of the ratio between Nmin. with Norg. (N-Naslavcea, V-Valcinet, S-Soroca, C-Camenca, E-Erjova, G-Goian, Co-Cocieri, VV-Vadul lui Voda, Va-Varnita, Su-Sucleia, P-Palanca)

Practically along all the Dniester river and throughout the year there is a dominance of organic nitrogen. In February, May, June and July we notice that the organic nitrogen content represents from 53% to 82%, and in Palanca in July 99% and only in the points Sucleia in February and Soroca in June (flood period) predominate the mineral forms of azote. In October, there is an increase in the share of mineral nitrogen from 29% to 63% of the total nitrogen content, the majority being in half of the sample collection points (fig. 5).

Total nitrogen concentrations during the year were in the range of 0.86 - 4.07 mg N / l. According to the quality requirements for surface waters, the concentrations of total nitrogen the Dniester river correspond to class I (very good water) and class II (good water) of quality and only in Varnita in February the waters refer to class III of quality - moderately polluted [3].

Conclusions

1. The concentrations of mineral nitrogen forms in the Dniester waters largely correspond to quality classes I and II, very good and good waters.
2. Nitrate concentrations largely prevailed throughout the year over ammonium ions and nitrites.
3. The Dniester river in 2020 in most cases was, largely favorable for the development of aquatic organisms.
4. Organic nitrogen predominates in most cases throughout the year over mineral forms of nitrogen.

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ANALYSIS OF THE ECOLOGICAL CONDITION OF WATER AND BOTTOM SUBSTRATES OF THE LOWER DNIESTER BY THE METHOD OF BIOTESTING ON MICROALGAE

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Abstract. In the autumn-spring periods 2018 - 2020. biotesting of water and bottom sediments of the lower Dniester and Ukrainian coastal waters of the Dniester region of the Black Sea was carried out by biotesting on a laboratory culture of planktonic algae Desmodesmus communis. The assessment of the ecological and toxicological situation and a comparative analysis of indicators of the level of pollution of the ecosystem of the "lower reaches of the Dniester" testify to the chronic pollution of this ecosystem by dangerous toxicants, acute toxicity occurs only during peak periods, while chronic toxicity is constantly manifested. It was found that in the overwhelming majority of water areas with different nature of anthropogenic factors and anthropogenic load, there were no toxic substances. Hazardous pollutants were more often found in the surface water layer of the zones affected by urban wastewaters on the Ukrainian coast of the Dniester region of the Black Sea.

Introduction

The biomonitoring of the lower Dniester was carried out in the fall (October) 2018 and (September) 2020, as well as in the spring (March) 2019. Research in the Ukrainian coastal waters of the Dniester region of the Black Sea was carried out in the fall (September) 2020. The urgency of assessing this region environment by the method of biotesting is due to the need to use in monitoring of modern integrated and cost-effective research aimed at obtaining information on changes in the ecological condition of clean waters and those under anthropogenic influence. We studied the quality of the Dniester water and sediments of the Dniester Delta by biotesting on laboratory culture of unicellular algae made within the project BSB165 "Creation of a system of innovative transboundary monitoring of transformations of river ecosystems of the Black Sea under the influence of hydropower and climate change".

Materials and methods

Modeling of influence of water and extracted pollutants from bottom sediments on the environment in the laboratory was performed on the microalgae population to assess the quality of the water environment and bottom sediments. Biotesting was performed according to the generally accepted method of biotesting in laboratory culture of unicellular algae. The criterion of toxicity were changes in algae cells, reflecting the number and reproductive capacity of algae [4], [5] [9],[10],[13]. The studies were performed with water samples, mainly from the surface layer from sampling sites, and bottom sediments taken in the autumn (october) of 2018 and spring (march) of 2019.

The laboratory culture of planktonic algae *Desmodesmus communis* (E.Hegewald) E. Hegewald was used as a test object. The test function was the reproducibility of the test object. The studied concentrations were 1.0 ml • l-1-10.0 ml • l-1 and 1.0 g • l-1-10.0 g • l-1 [2], [3].

We have developed an express tool for assessing the quality of the aquatic environment (water and sediments). The result of the assessment of environmental quality of water and sediments is defined and included in accordance with its own weight (selected by the expert) in a qualitative response: stimulating -> 100%; inactive - 75% - 100%; toxic - 50% - 75%; acute-toxic - 25% -50%; lethal - <25%.The final assessment of the quality of the aquatic environment ranges from - <25% to> 100% of the control values and corresponds to 5 classes [14] (Table 1).

Table 1. Scale of environmental quality of water and bottom sediments by biotesting on microalgae

% of control	Color code	Ecological status class	Characteristics of aquatic environment
> 100 %	Blue	High	Stimulating
75 % - 100 %	Green	Good	Inactive
50 % - 74 %	Yellow	Moderate	Toxic
25 % - 49 %	Orange	Poor	Acutely toxic
- < 25 %	Red	Bad	Lethal

Results and discussion

The ecological and toxicological situation plays an ever-increasing role in the formation and functioning of the aquatic ecosystems of the Dniester.

Since the Dniester flows in a densely populated region saturated with industry, with intensively chemicalized agriculture, its water quality throughout the river is largely determined by industrial, urban and agricultural runoff from the territories of Moldova and Ukraine, which together form the toxicogenic river runoff [6], [7], [12], [15]. The ecological and toxicological indicators observed in the lower reaches of the Dniester are the result of complex processes of interaction between fresh water, land, atmosphere and the Black Sea. Toxicants are distributed in various parts of the ecosystem, forming its ecological and toxicological status.

As a result of regulation of the Dniester in the middle and lower sections of the river, the characteristic spring floods necessary for the normal functioning of the river ecosystem have practically disappeared. The Dniester is turning from a mountain-lowland river into a lake-type reservoir. In recent years, the water level in the middle and lower sections of the Dniester has been formed due to small tributaries and groundwater in the middle and lower sections of the river. The thermal and gas regimes have changed, the physical properties of water, the composition of suspended solids, the physical absorption processes of self-purification of water are minimal, have decreased tenfold, the processes of secondary pollution have significantly increased [15].

An important role in the retention and detoxification of pollutants entering the lower reaches of the Dniester is played by floodplains that accumulate pesticides, heavy metals and other persistent toxicants, which, when plants die off, again enter the water masses and cause their secondary pollution [1].

Created on the Dniester and Dubossary reservoirs to a large extent delay or transform toxicogenic runoff, therefore, the quantity and quality of water in the lower reaches of the Dniester is largely determined by the nature and scale of releases from the latter and the degree of contamination of the water coming from it.

According to the literature, biotesting of treated wastewater from industry and cities entering the Dniester showed that the degree of toxicity depends on pollutants, that they are all acutely toxic and lose this property only at a high degree of dilution. Although a significant part of these pollutants is deposited in intermediate reservoirs (Dniester and Dubossary), diluted to the required level or partially, the toxic runoff of each water release from the Dubossary reservoir provides rather intensive "feeding" with toxicants as in the lower reaches of the Dniester [8].

Comparative analysis of the levels of pollution of the ecosystem of the lower Dniester allows us to conclude that this ecosystem is chronically polluted by the most dangerous toxicants of the level of alpha, meso- and polytoxicity [6], [12]. In recent years, the assessment of the qualitative characteristics of the Dniester waters makes it possible to class them as III pollution class (moderately polluted waters) [3].

In the scientific literature it is stated that in 1986-1987, chronic experiments were carried out at the Maiaki section to study the toxicity of the Dniester water of 5 species of cladocerans. The main biological parameters of test objects in a number of generations were evaluated, which ultimately affect the growth rates, fertility, quality of offspring, potential and actual productivity of the test objects. It was established that the Dniester water is chronic toxic, which is due to the presence of low concentrations of toxicants It is especially important that in this case the winter eggs (efippiums) formed in females during fertilization, which are usually very resistant to toxicants, also turn out to be inferior after the exposure of maternal individuals in the Dniester water and it turned out to be impossible to bring out the next generations from them under favorable conditions, the offspring did not exceed 15-0%. To identify the role of wastewater discharged by enterprises in Ukraine and Moldova, and wastewater treatment plants in cities, biotests were carried out on the same species of cladocera and two species of copepod crustaceans. It has been established that all city and waste waters discharged into the Dniester after treatment retain acute toxicity, which can be eliminated only with multiple dilutions.

The conclusions obtained on the tested test objects can, of course, be transferred to other species of crustaceans living in the estuary, in particular to crayfish and representatives of the relict Caspian fauna (mysids, gammarids, korofiids, kumacea, etc.), which indicates the prospects for its gradation and extinction under conditions of increasing toxic pollution.

Biotesting carried out by us on freshwater algae showed that in autumn (October 2018) and spring (March 2019) toxic water entered the lower reaches of the Dniester; its acute toxicity is not always manifested, but only in the ‘’ peak ‘’ periods, while chronic toxicity occurs constantly, which is associated with the presence of relatively low concentrations of solutes exhibiting a cumulative toxic effect.

In autumn (September) 2020 in the area of the village Maiaki were found to have toxicity in the lowest concentrations of the studied samples of water and bottom sediments. In water, toxicity was higher (50.88% of control values) than in bottom sediments (69.37% of control values). The maximum concentrations of the tested samples of water and bottom sediments contained substances that stimulate the reproduction processes of the test-object, and the bottom sediments contained 14 times more stimulants of the reproduction processes than in water These water and bottom massifs belong to ecological classes - “ high”.

In the autumn of 2020, studies were also carried out in the Ukrainian coastal waters of the Dniester region of the Black Sea: recreational zones, zones of influence of urban wastewater. Experimental results show that most of the tested waters did not contain toxic substances. These dangerous pollutants were more often found in the surface water layer of urban wastewater impact zones.

In autumn, substances that during biotesting had a toxic effect on the reproduction of *D. communis*, were in the surface layer of the water massif of the Dacha Kovalevsky district (53.92-58.82% of the control). From the point of view of an ecological condition this area is estimated as “moderate”, as well as the area of sanatorium of a name of Chkalov (51,96%), Cape Small Fountain Beach (55.88% of control) - “high - mederate”. The surface water layer of the Zatoka beach massif belonged to the ecological class of “good” waters; beaches of Arcadia and Dolphin, belonged to the class of waters - “high”. The locations of these beaches contained substances that significantly enhanced the reproductive capacity of the seaweed of the object under study.

In autumn, when studying the quality of the extracted pollutants from bottom sediments sampled in the coastal zone more often than during biotesting of water quality, a significant stimulating effect of pollutants on the reproduction of *D. communis* was observed (especially in the sediments of the beach of the Chkalov sanatorium). The presence of toxic substances was also revealed in the bottom sediments of the coastal strip. The proliferation of algae cells of the studied object was below the control values in the minimum and maximum concentrations of the removed samples of contamination of the bottom sediments of the studied areas. Thus, in the samples with the minimum concentration taken in the waters of Zatoka beaches, the number of algae cells was 64.73% of the control, Dacha Kovalevsky - 37.44%. These bottom massifs belong to ecological classes - “moderate - high” and “acutely toxic (poor) - high”.In variants with the maximum concentrations of samples of bottom sediments of the beach of Cape Small Fountain, the number of *D. communis* cells was - 55.88% of control. The ecological quality of this array corresponds to the class - “high - mederate”. Bottom sediments of Dolphin Beach in maximum concentrations have significant toxicity and are characterized as belonging to the ecological class “high lethal(bad)”. During the experiments, the obtained values of pH changes of the control and experimental media, where the studied object was cultivated, are not enough for conclusions. Indicators of changes in the alkalinity of the experimental environment are due to many factors: the processes of algae life and changes in pollutants.

Conclusions

An assessment of the ecological and toxicological situation and a comparative analysis of the indicators of the level of pollution of the ecosystem of the “lower reaches of the Dniester” allows us to conclude that this ecosystem is chronically polluted by dangerous toxicants.

Biotesting, carried out in 2018 - 2020, showed that water saturated with various pollutants constantly flows into the lower reaches of the Dniester; acute toxicity occurs only during peak periods, while chronic toxicity occurs continuously. In the fall (September) of 2020, in the area of the Mayaki village, the presence of toxicity was detected in the lowest concentrations of the studied water and bottom sediments samples, and the toxicity in the water was higher (50.88% of the control values) than in the bottom sediments. The maximum concentrations of the studied samples of water and bottom sediments contained substances that stimulate the reproduction of the test object, and the bottom sediments contained 14 times more stimulators of reproductive processes than in water.

The results of biomonitoring of the Ukrainian coast of the Dniester region of the Black Sea in the fall of 2020 show that most of the tested waters did not contain toxic substances. These hazardous pollutants were more often found in the surface water layer of urban wastewater impact zones. In the surface water layer of the Arkadia beach massifs and in the bottom sediments of the Arcadia beach zone, the Chkalov sanatorium contained substances that significantly increase the reproductive capacity of the alga D. communis. The bottom sediments of the Dolphin Beach contained substances with significant toxicity, which can be attributed to the ecological class “bad (lethal)”.

The obtained research results and assessment of the ecological and toxicological situation of the studied ecosystem, the processes of reproduction of hydrobiological resources, the possibility of self-purification of the aquatic environment under the influence of hydropower indicate the need to continue continuous environmental monitoring in the study area using a promising and indicative method of biotesting on freshwater algae cultures.

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CASPIOSOMA CASPIUM (KESSLER, 1877) IN THE LOWER DNIESTER RIVER

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Abstract. The result of multiannual research that supports the concept of biological progression of Gobiidae family representatives on the territory of the Republic of Moldova in current ecological conditions are revealed in this paper. A new species was found for the Dniester riverbed - Caspiosoma caspium (Kessler, 1877), in the spring of 2021, being captured in number of 16 specimens, this previously being identified only in the Cuciurgan refrigerated lake in 1969, in a single specimen..

Introduction

In the current ecological conditions, unfortunately, we find profound negative changes in the ichthyofauna of natural aquatic ecosystems in the Republic of Moldova. Often create premises for the emergence and proliferation of large species small of marine or deltaic origin which, previously, were missing within the limits of the republic or were considered very rare, against the background of unregulated fishing with selective effect (when large species are extracted, regulating small numbers), rapid climate change and multiple fragmentations of riverbeds by dams [1]. One of these is the caspian goby - *Caspiosoma caspium* (Kessler, 1877).

Caspian goby is a relict species of Pontic-Caspian origin, protected by law, included in the Red Book of the Republic of Moldova ed. III with endangered species status (EN) [2] and the Red Book of Ukraine [14], protected at European level (LC) [13].

Figure 1. *Caspiosoma caspium* **(Kessler, 1877) – Caspian goby**

According to its taxonomic affiliation, it is unique in the Republic of Moldova from the genus Caspiosoma, it belongs to the class *Actinopterygii*, the order *Perciformes,* the family *Gobiidae*.

The species can be found only in the Cuciurgan refrigerated lake, according to some authors

[2, 3, 4, 9, 12]. It was identified only once in 1969 in the ecosystem of the Cuciurgan reservoir (Dniester basin), where one specimen only was reported [11].

Abroad it is found in the deltas of rivers flowing into the northwestern part of the Black Sea, the Azov Sea and the Caspian Sea [6, 15, 16]. Also, lately, as a result of the regularization of watercourses, there has been a tendency to expand the upstream distribution area on large rivers. The spread of the species in the Lower Volga River is a typical example. [8].

Materials and methods

The capture of ichthyological material was carried out in the spring of 2021, in the lower Dniester River, the Purcari-Palanca section. In scientific fishing, the seine net was used with a length of 5 m, height = 2 m, the size of eyes = 10x10mm, of the bag = 5x5mm. Classical ecological and ichthyological methods were used to taxonomic determination and analysis of ichthyological material [6, 15].

The obtained data were statistically processed using the Excel program - 2007. The values of the analytical and synthetic ecological indices express the following meanings [5]:

D1 subrecedents: <1,1%		
D2 recedents: 1,1%-2%	**C1** accidental: < 25%	**W1** accidental: <0,1%
D3 subdominants: 2,1%-5%	**C2** accessory:25,1%-50%	**W2-W3** accessory:0,1%-5%
D4 dominants: 5,1%-10%	**C3** constants: 50,1%-75%	**W4-W5** characteristic:5,1%-100%
D5 eudominants: >10%	**C4** euconstants:75,1%-100%	

***Note:** **D-**dominance, **C-**constancy, **W-**ecological significance index

Results and discussions

According to scientific control fishermen in the spring of 2021 in the Lower Dniester River made by researchers of the Laboratory of Ichthyology and Aquaculture of the Institute of Zoology, have been identified 16 specimens of this species. The captured specimens were processed metrically and gravimetrically, having the following values: n=16, L med. = 3,43±0,16 cm (C.V.=18,32%), l med.=2,91±0,109 cm (C.V.=14,65%) and P med. = 0,33±0,017 g (C.V.=20,98%). The age structure was not determined due to the preservation of intact bodily integrity (species without scales) and their alive returning to their living environment. Three specimens have been preserved for the laboratory collection.

The structure of catches and the contribution of each species to the formation of the fishing community is presented below. Thus, the caspian goby included into the category of subdominant species (D 3-2,4%), accessories (C2-30%), and attendant (W2-0,7%).

As a result, after 50 years of uncertainty from the first registration, we can see not only a reappearance of the species on the territory of the Republic, but also an obvious expansion of it in the Lower Dniester Riverbed [11].We can, repeatedly, confirm the tendencies of continuous modification of the fish fauna in the Republic of Moldova based on the finding of the presence of this species. As a result, the analysis of ichthyocenotic successions in the last 50 years shows a continuous increase in the share of representatives of the *Gobiidae* family in the country.

Among the most essential causes, which led to the biological progression of ponto-caspian gobyes on the territory of the Republic, we can mention: 1) anthropogenic factors - fragmentation and regularization of lotic ecosystems with limnification effect, clogging, district heating and mineralization; unregulated fishing of natural predators and strong competitors 2) climatic factors- the trend of global warming and the intensification of natural cataclysms 3) of biocenotic order - spreading of lotic biotopes with macrophytes that serve as refuge areas for the representatives of this family, the proliferation of bivalve mollusks whose shells serve as reproductive substrate for spawning, the deficit of ichthyophagous species, the increase of available food resources 4) idioadaptive order - small body size, hidden benthic lifestyle, pronounced mimicry, care for offspring and high survival of offspring, early maturation (0+, 1+), portioned reproduction, broad trophic spectrum - from malocophagy to optional ichthyophagy; they are eurythermic, mixohaline and eurioxybionte species; missing of larval stages [1].

Conclusions

Presence identification of this species confirms the conception regarding the biological progression of the *Gobiidae* family (Ponto-Caspian relics) in current ecological conditions.

The model way of spread and numerical dynamics of the species is similar to Black Sea tadpole-goby - *Benthophilus nudus* Berg, 1898, which also recently demonstrates an obvious biological progression in the Dniester and Prut Rivers.

The biological progression of the Ponto-Caspian relics of the *Gobiidae* family is a strong indicator of accentuated climate change. Thus, current global warming and the progression of some relict species can be correlated with the interglacial phases in climate change.

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MORPHOHISTOLOGICAL STUDIES OF THE GONADS OF HYBRID POLYPLOID LOACH COBITIS FROM THE LOWER DNIESTER

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Abstract. In the lower Dniester, polyploid females of the hybrid complex Cobitis taenia are a portion spawning species with asynchronous development of germ cells during the entire breeding period. The spawning season in this water basin begins in the second decade of May and ends in July. In the population, in parallel with females, hermaphrodite individuals are also present, in which the ovary zone is functional, and the testis zone is sterile. A decrease in the relative mass of the gonads of subsequent generations was noted, which leads to a consistent decrease in the value of the gonadosomatic index, in females, before the second and third spawning.

Introduction

In the genus Cobitis (Cobitidae), inhabitants of rivers and water bodies of Eurasia, along with bisexual diploid species, clonal forms were found, represented, as a rule, by triploid females [1]. To date, about 85 forms have been found among fish, which are represented only by females that reproduce by gynogenesis. In natural gynogenesis, males are needed, whose sperm stimulate the development of eggs, while true fertilization does not occur. In this regard, gynogenetic forms of fish live together with one or two closely related bisexual species, as a result of hybridization of which they arose [12]. In rare cases, clonal forms for their reproduction use males of the third less closely related bisexual species. Thus, the gynogenesis of clonal forms leads to the emergence of unisexual-bisexual (clonal-bisexual) complexes.

In this study, the ovarian histology of hybrid polyploid loach of the *C. taenia* complex of the lower Dniester during the reproductive cycle was investigated.

Material and methods

The collection of ichthyological material was carried out in 2020 from the lower Dniester during the pre-spawning and spawning periods from April to May. The collected material was processed in accordance with the methods generally accepted in ichthyology and fish ecology [4]. All caught females of *Cobitis tanaitica* in the amount of 37 individuals were subjected to general biological analysis with the determination of linear mass indicators, gonadosomatic index (GSI). For histological studies, gonadal samples were fixed in 4% formalin, followed by histological processing according to the generally accepted method. Sections 7 μm thick were stained by the Mallory method [6]. The stages of maturity of the gonads were determined according to the standard recommendations [6], and the degree of development of oocytes according to the classification of Kazansky [2]. The gonadosomatic index was calculated as the ratio of the mass of the gonads to the mass of the body without viscera in percent. Oocytes, in the phase of complete vitellogenesis, were measured with an eyepiece micrometer. All digital data were processed statistically [3]. Microphotographs were made using a microscope Lomo, Mikmed-2 with a video camera.

Results and discussions

Since during the entire spring-summer season, only females and hermaphrodites of *Cobitis taenia* were caught in the catches, it can be assumed that in the lower Dniester, the fish reproduce gynogenetically and produce triploid eggs, which are stimulated to develop sperm by Cobitis males [11]. The length and body weight of sexually mature females ranges from 6.8 to 9.2 cm and from 2.19 to 6.76 g, respectively.

In the pre-spawning period, in April at water temperature of 120C, the control catches included hermaphrodite individuals and females with gonads at maturity stage IV with older generation oocytes in the phase of intense vitellogenesis. Their diameter corresponded to 861.33 ± 4.76 µm. The ovaries of hermaphrodites are also at the IV stage of maturity and contain yolk eggs with a diameter of 857.60 ± 7.01 microns, and the testicles contain seminal ampoules along the periphery of which cysts with spermatogonia and spermatocytes are located. These individuals have one gonad, but the zones of the ovary and testis were always limited and no signs of the transition of the testis to the ovary were found (fig. 1). In the testis zone, signs of degeneration are observed, expressed in the formation of pyknotic cells. Similar signs of destructive changes in the development of testes were also noted among the diploid hybrid males of *C.elongatoides* and *C. taenia* [8], as well as in tetraploid Cobitis individuals [9]. As the authors point out, the first signs of degeneration (cell pyknosis) appear in spermatocytes. In the family Cobitidae, cases of hermaphroditism have been reported [10]. Also, the presence in one gonad of the hermaphrodite zone of the ovary and the zone of the testis, separated from each other was noted.

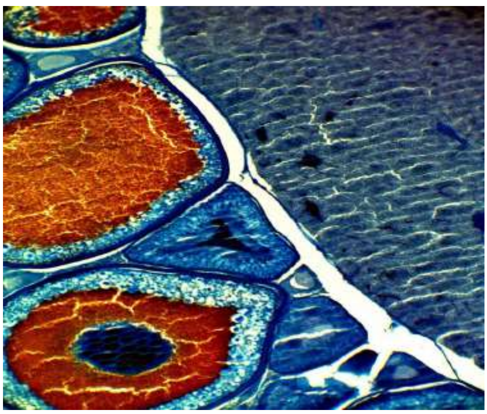

Figure 1. Fragment of hermaphrodite gonad

With a slight difference in body length and weight, the average value of GSI in hermaphrodites is slightly higher than in females during this period, but their difference in values is not significant P≤0.95 (tab.1).

The spawning season for the loach in the lower Dniester begins in the second decade of

May at a water temperature of 140C. During the spawning period, the ovaries of some females were at the IV-V stage of maturity, with oocytes of the older generation in the maturation phase, with the diameter of 1056.0±19.67. As the temperature of the water in the Dniester rises, the amount of food consumed by the fish increases, which contributes to an increase in oocyte size and ovarian weight.

During the research period, the catches also contained spawning females with gonads at maturity stages VI-IV2, as evidenced by the presence of empty follicular membranes and oocytes in the phases of intense vitellogenesis, the beginning of yolk accumulation and vacuolization. From the data given in the table, it can be seen that a higher GSI value in females is observed before the first spawning, while in individuals before the second and third spawning, this indicator decreases (tab.1).

Table 1. Biological characteristics of the loach from lower Dniester

Period, month	Sex	Maturity stage	Body length, cm	Body weight, g	GSI%
IIIrd decade of April	Females	IV	7,62±0,31	3,36±0,33	9,00±1,18
	Hermaphrodites	IV	7,95±0,34	3,53±0,51	12,31±1,21
IInd decade of May	Females	IV-V	9,20±0,41	6,76±0,39	29,19±1,84
	Females	VI-IV_2	7,0±0,28	2,85±0,17	7,88±1,59
Ist decade of June	Females	V_2-VI	7,93±0,57	3,28±0,63	10,95±1,12
	Females	IV_2- V	8,03±0,25	3,19±0,18	23,76±2,03
Ist decade of July	Females	V_3-VI	7,25±0,29	2,91±0,19	7,25±1,57
	Females	IV_3- V	7,30±0,23	3,21±0,34	15,41±1,23
	Hermaphrodites	VI -III	6,8±0,21	2,19±0,14	9,04±1,34

After the spawning of the first portion of eggs, the ovaries contain, along with empty follicular membranes, oocytes at different phases of the period of trophoplasmic growth, which indicates the asynchronous nature of their development. In early June, before the second spawning in the season, the second generation of oocytes completes the accumulation of yolk and passes into the maturation phase, and their gonads at the IV2-V stage of maturity. By this time, some of the females have already spawned a second time. The onset of ovulation of mature germ cells of the second generation occur in the first decade of June, with a thermal regime of the lower Dniester of 170C.

The gonads of females caught in the first ten days of July have the maturity stages IV3-V. The water temperature during this period does not exceed 180C. Among the caught fish, a hermaphrodite specimen was found, laying eggs, with an ovary at the VI –III stage of maturity. This individual has completed the spawning season. After the third spawning, empty follicular membranes remain in the ovary of the hermaphrodite, resorbing oocytes of the next year's generation in the phases of protoplasmic growth, vacuolization, and not spawned yolk oocytes in the process of deep resorption (fig. 2). Such an individual will miss the next spawning season.

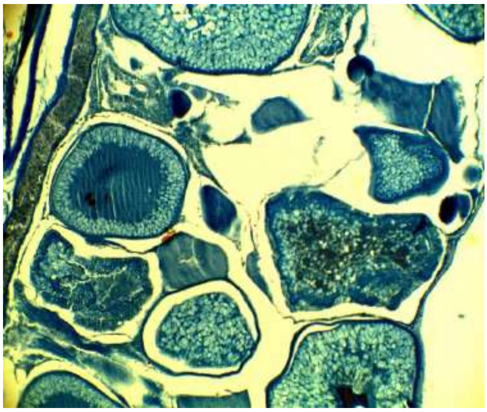

Figure 2. Fragment of hermaphrodite oocyte that has completed the spawning season

It must be noted that the hermaphrodite testes in July, as well as in April, still contain cysts with sexual products in the early stages of spermatogenesis in the process of degeneration, which causes sterility of the seminal tissue (fig. 3). The sterility of the gonads is a consequence of profound disturbances in the development of the reproductive system, which probably appears due to gynogenetic reproduction. For the first time, male sterility was noted in the hybrid fish *C. taenia* and *C. Elongatoides* [8]. It is also pointed out that males are heterozygous sex.

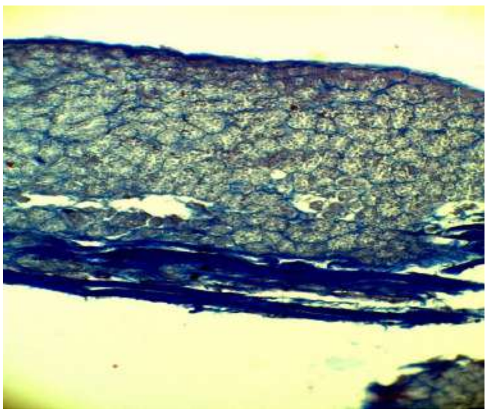

Figure 3. Testes of hermaphrodite in July

Before the third spawning in the spawning season, females with degenerating reproductive cells were identified. On histological preparations, destructive changes in yolk oocytes are expressed in the disappearance of nuclei, fragmentation of the oocyte's own membrane, and partial homogenization of the yolk. Oocytes also underwent resorption in the phases of vitellogenesis, which is accompanied by the absence of cell turgor, fragmentation of the oocyte's membrane, destruction of vacuoles and fusion of their contents into a homogeneous mass, as well as oocytes of the reserve fund in the initial phase of vitellogenesis (fig. 4).

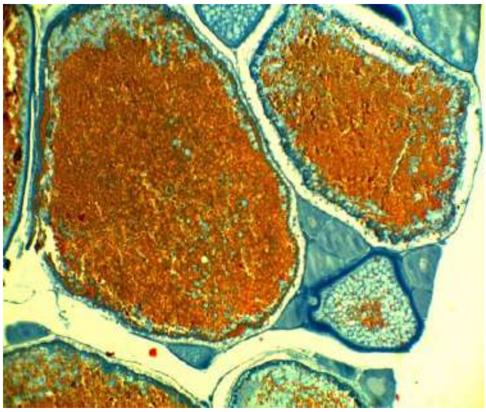

Figure 4. Resorption of yolk in third generation oocytes

According to the studies carried out, it was found that during May-July, at a temperature regime of the lower Dniester within the range of 14-180C, three generations of oocytes are formed and prepared for spawning (tab.1). A similar spawning time, from May to July, was observed in C. taenia from Lake Klavoi, but at a water temperature exceeding 18,50 C [7].

Conclusions

In the lower Dniester, polyploid females of the hybrid complex *Cobitis taenia* are a portion spawning species with asynchronous development of germ cells during the entire breeding period. The spawning season in this reservoir begins in the second decade of May and ends in July. In the population, in parallel with females, hermaphrodite individuals are also present, in which the ovary zone is functional, and the testis zone is sterile.

With three portions of spawning in females, a decrease in the relative mass of the gonads of subsequent generations is noted, which leads to a consistent decrease in the value of the gonadosomatic index before the second and third spawning. Lower values of this indicator, after the next spawning, are associated with a decrease in the number of yolk oocytes before spawning of the current generation.

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CONCENTRATION OF ORGANOCHLORINE PESTICIDES IN WATER AND BOTTOM SEDIMENTS OF THE DNIESTER RIVER ECOSYSTEM

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Abstract. The main goal of this study is assessing the degree of water and bottom sediment pollution in the lower Dniester by organochlorine pesticides. During the study period, the waters of the lower Dniester were in satisfactory state, an excess of EQS was registered only for the γ-isomer of HCH (Lindane), for other pollutants, an excess of EQS was not noted. The main pollutants were accumulated in bottom sediments, which is associated with the input of organic matter, bio-sedimentation. The concentration of γ-isomer HCH (lindane) in bottom sediments exceeded EQS by 5 and 20 times in spring and summer, respectively, DDT exceeded EQS by more than 4 times in summer, POPs (dieldrin and heptachlor) in summer exceeded EQS by 10.8 and 2.5 times, respectively.

Introduction

State assessment and variability of water and bottom sediment pollution by toxic substances and pollutants in the lower Dniester is an important indicator of the integral transboundary load from the entire basin on the ecosystems of the lower part of the river, the Dniester estuary and the adjacent Black Sea coastal area. Therefore, the main goal of this study is assessing the degree of water and bottom sediment pollution in the lower Dniester by organochlorine pesticides according to the Water Framework Directive 2000/60/EC [1, 2]. In the lower Dniester, the most polluted part is between the cities of Dubossary and Bendery. Most pollutants come to this territory from the runoff of the Reut and Byk rivers, in which basins there are located the main large and medium industrial and communal enterprises of Moldova, together with majority of water users [3].

Ukrainian part of the lower Dniester, together with the transboundary transport of pollutants, is also affected by industrial and municipal enterprises of the Odessa region, as well as food industry enterprises. Irrigation systems are also a significant pollutant, the largest of which is the Dniester irrigation system with a high concentration of easily oxidizing organic substances in its waters.

Materials and methods

Water and bottom sediments were sampled in the lower Dniester under the project BSB165 HydroEcoNex, near the village Mayaki, at a distance of 15.4 km from the confluence of the Dniester river into the Dniester estuary. The present paper covers the period of study from December 2018 to August 2019.

To analyze the concentration of pollutants in bottom sediments, the samples were preliminarily dried in a vacuum freezer, homogenized, and weighed by the analytical weighing scales. Chlorinated hydrocarbons (OCPs) were analyzed by capillary gas chromatography, using an Agilent 7890B gas chromatograph with a capillary column and an electron capture detector according to the measurement procedure (MVI) No. 10/09-09 Sea waters approved by the State Enterprise "Odessa Regional Center for Standardization, Metrology and Certification". The certification was carried out according State Standard GOST 8.010-99.

Results and discussion

Organochlorine pesticides (OCPs) are a group of synthetic poisons created primarily for insect control. A number of organochlorine pesticides (α, β and γ isomers of HCH, DDT and its metabolites DDE, DDD, aldrin, dieldrin, heptachlor, hexachlorobenzene, etc.) belong to the dangerous group of persistent organic pollutants (POPs). The most toxic OCPs are dichlorodiphenyltrichloroethane DDT and HCH (lindane), which were first by production and use in agriculture until the 1980s [4]. The widespread use of OCPs in agriculture and other industries in the last century led to their accumulation in environment, in water bodies and soils, and their migration along the food chains from lower to higher levels of the ecosystem. In aquatic ecosystems, take place a sorption of organochlorine compounds by suspensions, with subsequent sedimentation and burial in bottom sediments.

During the observation period, γ-isomer of HCH (lindane) was registered in all water samples. Its concentrations were in the range of 0.17–2.12 ng/l, and in February exceeded the ecological quality standard by more than 10 times (EQS = 0.20 ng/l). The lindane concentration averaged 0.60 ng/l. Concentrations of the β-isomer of HCH varied from analytical zero <0.05 ng/l to 1.55 ng/l, the average value was 0.55 ng/l. In all water samples, α-HCH isomer was not detected.

The presence of DDT (dichlorodiphenyltrichloroethane) was observed in all water samples. Its concentration varied within the range of 3.15–17.95 ng/l and did not exceed the ecological quality standard (EQS = 25.0 ng/l). The average DDT concentration in the Dniester waters during the study period was 9.58 ng/l. The presence of its transformation product DDE (dichlorodiphenyldichloroethene) was not observed. The presence of DDD (dichlorodiphenyldichloroethane) was observed in only three out of nine water samples with a maximum concentration of 1.97 ng/l in February. The total concentration of DDT and its metabolites was in the range of 3.28-19.42 ng/l and did not exceed EQS. The average concentrations in water of lindane and the DDTsum (DDT + its transformation products) decreased in spring (April-May) (Fig. 1).

A decrease in the concentrations of HCHg and DDTsum in spring may be caused by their dilution due to increase of water runoff during the flood period.

In the lower Dniester waters, organochlorine compounds of POPs group (aldrin, dieldrin, heptachlor, hexachlorobenzene) were not detected during this observation period.

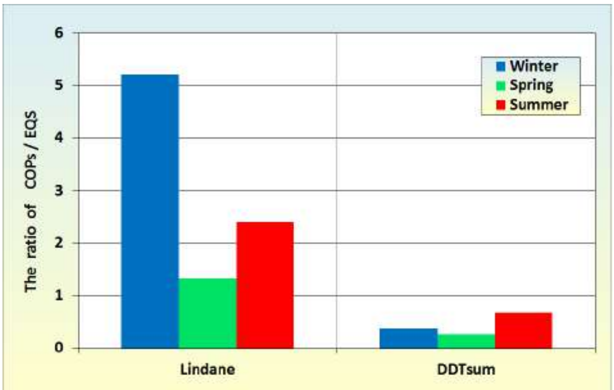

Figure 1. The ratio of OCPs concentration to their EQS (average per season) in the waters of lower Dniester in 2019

The transfer of organochlorine compounds to bottom sediments is largely due to biosedimentation - accumulation and sedimentation with suspended organic material. In bottom sediments in the lower Dniester, the concentration of the γ-isomer HCH (lindane) was 0.25 mkg/kg in March, 1.02 mkg/kg in June, thus, exceeded the environmental quality standard by 5 and 20 times respectively (EQS = 0.05 mkg/kg); β-isomer of HCH was noted only in March, with the concentration of 0.24 mkg/kg; α-isomer of HCH was not detected in bottom sediments, the same as in water. It is noteworthy that earlier studies [5] of bottom sediments in the lower Dniester also revealed the presence of the β-isomer HCH in the samples in a similar concentration range from 0.04 to 0.28 mkg/kg. The α-HCH isomer was also detected in the samples of bottom sediments, the average concentrations varied from 0.05 to 0.059 mkg/kg.

The DDT (dichlorodiphenyltrichloroethane) concentration in bottom sediments in March was 1.52 mkg/kg, and in June 10.90 mkg/kg, exceeding more than 4 times the environmental quality standard (EQS = 2.5 mkg/kg) (Fig. 2).

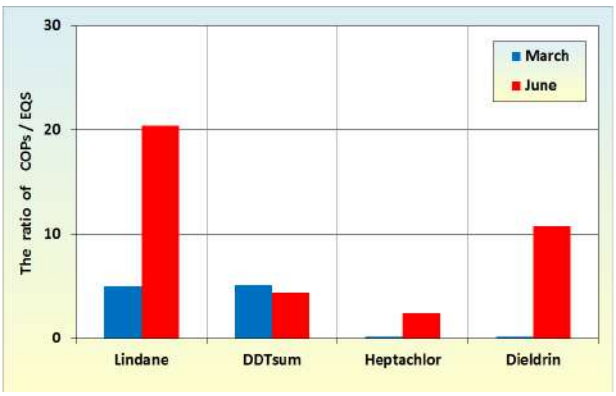

Figure 2. The ratio of OCPs concentration to their EQS in bottom sediments of lower Dniester in March and June 2019

In March, the presence of DDD (dichlorodiphenyldichloroethane) in the concentration of 9.68 mkg/kg was noted in the bottom sediments, which is almost 4 times higher than the EQS.

DDE (dichlorodiphenyldichloroethene) was detected in the sediments in March at 1.59 mkg/kg. In previous studies of bottom sediments in the Moldavian part of the lower Dniester from Vadul-lui-Voda to Palanca, the concentrations of DDT and its transformation products DDD and DDE were several times lower than our data and amounted to 0.08, 1.68 and 1.14 mkg/kg, respectively [6].

In general, our results were much higher than the total concentrations of DDT and HCH in bottom sediments established for the lower Dniester in 2017-2018, which averaged 7.9 mkg/kg and 0.19 mkg/kg, respectively [5].

In bottom sediments, among the pesticides of POPs group (aldrin, dieldrin, heptachlor, hexachlorobenzene), dieldrin and heptachlor were detected in June at concentrations of 5.4 mkg/kg and 6.13 mkg/kg, respectively, with an excess of EQS of 10.8 and 2.5 times.

Conclusions

During the study period, the waters of the lower Dniester were in satisfactory state, an excess of EQS was registered only for the γ-isomer of HCH (Lindane), for other pollutants, an excess of EQS was not noted. The main pollutants were accumulated in bottom sediments, which is associated with the input of organic matter, bio-sedimentation. The concentration of γ-isomer HCH (lindane) in bottom sediments exceeded EQS by 5 and 20 times in spring and summer, respectively, DDT exceeded EQS by more than 4 times in summer, POPs (dieldrin and heptachlor) in summer exceeded EQS by 10.8 and 2.5 times, respectively.

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THE STRUCTURE OF FISH COMMUNITIES IN BISTRIȚA RIVER, UPSTREAM THE CONFLUENCE WITH SIRET RIVER (ROMÂNIA)

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Abstract. The researches were carried out in 2020 on the main course of Bistrița River, upstream the confluence with Siret River. The biological material was sampled in 10 sampling sites placed on the main course of Bistrița River. Seventeen fish species were identified, one of them being a non-native one: Pseudorasbora parva. We found that the chub and the Prussian carp are characteristic species. Species diversity is quite high, being identified even 12 fish species in some of the sampling sites. There is an unequal distribution of individuals by species. The main threats in the research area are the organic inputs and the increase of turbidity. However, there are no great imbalances, and there is a good capacity for self-support of aquatic communities.

Introduction

Fish communities are extremely important links in food chains of aquatic ecosystems since they include all food categories and occupy various habitat niches. Moreover, ichthyofauna is one of the most precious natural resources for human interest.The human impact in the last decades has affected both aquatic ecosystems and groups of aquatic organisms. Having in mind the importance of water for socio-economic systems and the importance of ichthyofauna for human beings and the environment, it is imperative to know as accurately as possible the state of limnic ecosystems and their structural and functional changes.

This can be achieved by biomonitoring of some groups of characteristic organisms, indicators for a certain habitat.Fish are one of the most important group of organisms used for the assessment of the limnic ecosystems state for the following reasons: they live in almost all types of waters, they have a lifespan of several years, they do not show significant changes in their population structure, and through the position of final consumer in food chains, they include the changes appeared in the lower trophic levels.

The interest for the presence or absence of some fish species or fish communities, for determining their density and abundance, respectively for knowing the structure and diversity of fish communities is justified by the importance of this group of organisms in lotic ecosystems, as well as their usefulness in biomonitoring studies.

Material and methods

In this study, the research were carried out on the Bistrița River, upstream the confluence with Siret River, on which a number of 10 sampling stations were established, as shown in table 1 and figure 1.

The field stage was carried out in August - September 2020. Ichthyofauna was sampled from each sampling site using an FEG 5000 electric fishing gear. The samples were placed in vessels with fresh water to keep the fish alive. The individuals were determined, counted, and weighed, and then released in the water.

Table 1. The sampling sites on the Bistrița River, upstream the confluence with the Siret River

No.	Sampling site	River	GPS Coord.	(GMS)	(GZ)	Alt. (m)
1	Upstream CET Bacău	Bistrița	N	46°32’28.3”	46.541194	147
			E	26°56’42.3”	26.945083	
2	CET Bacău side	Bistrița	N	46°32’01.7”	46.533805	145
			E	26°56’43.3”	26.945361	
3	Ruși Ciutea village side	Bistrița	N	46°31’11.0”	46.519722	141
			E	26°57’06.8”	26.950188	
4	Downstream aval bridge, Ruși Ciutea village side	Bistrița	N	46°31’10.9”	46.519694	141
			E	26°57’06.9”	26.950191	
5	SOFERT side, Ruși Ciutea village side	Bistrița	N	46°31’00.1”	46.516694	141
			E	26°57’09.8”	26.950272	
6	SOFERT side, Ruși Ciutea village side, right shore	Bistrița	N	46°30’55.7”	46.515472	140
			E	26°57’02.3”	26.950638	
7	Downstream SOFERT, Siretu village side	Bistrița	N	46°30’47.9”	46.513305	140
			E	26°57’02.9”	26.950805	
8	3+126 km upstream bridge	Bistrița	N	46°30’16.5”	46.504583	137
			E	26°57’19.6”	26.955444	
9	3+126 km downstream bridge	Bistrița	N	46°30’14.4”	46.504000	137
			E	26°57’21.2”	26.955888	
10	3 +126 km downstream bridge, 9 sampling site side	Bistrița	N	46°30’15.5”	46.504305	137
			E	26°57’20.3”	26.955638	

Figure 1. The sampling sites on the Bistrița River, upstream the confluence with the Siret River

Results and discussions

The seventeen fish species which were identified in Bistrița River, upstream the confluence with the Siret River are presented in the table 2.

Table 2. The fish species identified in Bistrița River, upstream the confluence with the Siret River

No.	Scientific name	Common name
1	*Esox lucius* L.,1758	pike
2	*Squalius cephalus* L, 1758	chub
3	*Scardinus erythrophthalmus* L., 1758	rudd
4	*Aspius aspius* L., 1758	asp
5	*Alburnus alburnus* L., 1758	bleak
6	*Abramis brama* L., 1758	freshwater bream
7	*Vimba vimba* L., 1758	vimba bream
8	*Rhodeus amarus* Bloch, 1782	European bitterling
9	*Gobio obtusirostris* Valenciennes, 1842	-
10	*Pseudorasbora parva* (Temminck & Schlegel, 1846)	stone moroko
11	*Carassius gibelio* Bloch, 1782	Prussian carp
12	*Cobitis taenia* L., 1758	Spined loach
13	*Sabanejewia balcanica* (Karaman, 1922)	Balcan spined loach
14	*Perca fluviatilis* L., 1758	European perch
15	Neogobius fluviatilis (Pallas, 1814)	monkey goby
16	Babka gymnotrachelus (Kessler, 1857)	racer goby
17	*Proterorhinus marmoratus* (Pallas, 1814)	tubenose goby

Frequency of the fish species. Two of the seventeen fish species (Prussian carp and European bitterling) were identified in all the 10 sampling sites, with a frequency of 100%. Other two species with a high frequency are the chub and the stone moroko (90%). The bleak was recorded with a frequency of 80%, and other three species have had a frequency of 70% (spined loach, *Gobio obtusirostris* and rudd). European perch was identified in 4 sampling sites (40%), the pike and the monkey goby were identified in 3 of the 10 sampling sites (30%). The rest of 5 species were identified in less than 3 sampling sites, with a very low frequency (fig. 2).

Numerical stock (ind/100 m^2) and weight stock (g/100 m^2) of the fish species

The fish species that have recorded significant stock in research area are: Squalius cephalus, *Scardinius erythrophthalmus, Alburnus alburnus, Rhodeus amarus, Gobio obtusirostris, Pseudorasbora parva, Carassius gibelio, Cobitis taenia, Proterorhinus marmoratus*.

Numerical stock (ind/100 m^2) and weight stock (g/100 m^2) of the fish species are presented in table 3 and table 4.

The chub (*Squalius cephalus*) was identified in 9 of the 10 sampling sites. The numerical stock of the chub in the 9 sampling sites ranges between 1,27 and 128 ind./100 m^2, while the weight stock is significantly higher, ranging between 1,90 and 1453,27 g/100 m^2 since the chub

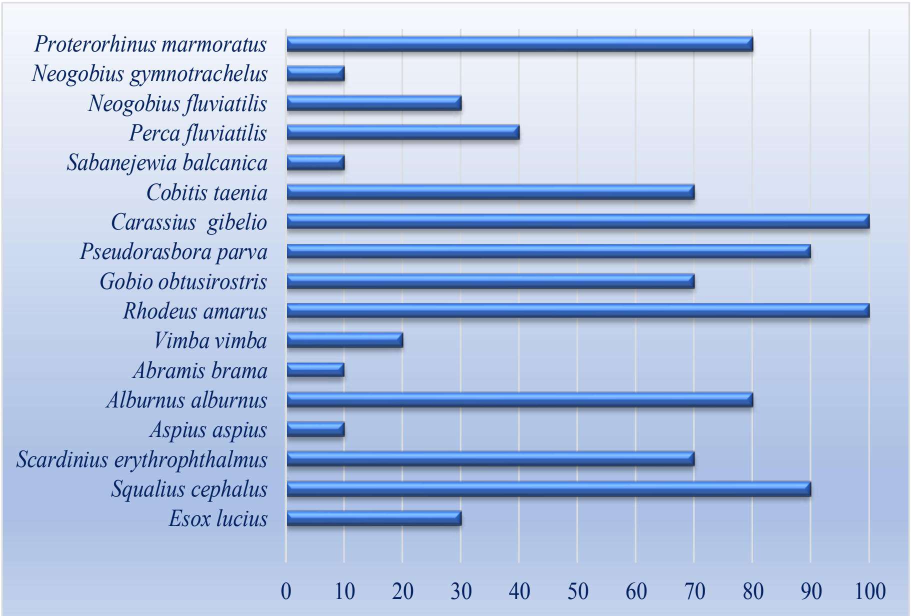

Figure 2. Frequency of the fish species in Bistrița River, upstream the confluence with Siret River

is a relatively large species.

The rudd (*Scardinius erythrophthalmus*) was identified in 7 sampling sites. The numerical stock of the chub in the 7 sampling sites ranges between 8,00 and 382,35 ind./100 m^2 while the weight stock ranges between 6,80 and 313,65 g/100 m^2.

The bleak (*Alburnus alburnus*) was identified in 8 sampling sites. The numerical stock of the bleak in sampling sites ranges between 1,27 and 208,67 ind./100 m^2 while the weight stock ranges between 3,04 and 523,60 g/100 m^2.

The European bitterling (*Rhodeus amarus*) was identified in all the 10 sampling sites. The numerical stock of the bleak in sampling sites ranges between 0,56 and 5,33 ind./100 m^2 while the weight stock ranges between 1,17 and 14,20 g/100 m^2.

Gobio obtusirostris was identified in 7 of the 10 sampling sites. Its numerical stock in sampling sites ranges between 1,11 and 39,26 ind./100 m^2 while the weight stock ranges between 1,44 and 302,89 g/100 m^2.

The stone moroko (*Pseudorasbora parva*) was identified in 9 sampling sites. The lowest value of the numerical stock of the stone moroko in sampling sites was 1,33 and the highest value was 40,00 ind./100 m2 while the weight stock ranges between 3,30 and 110,40 g/100 m^2

Table 3. Numerical stock (ind/100 m^2) of the fish species in Bistrița River, upstream the confluence with Siret River

No. of sampling sites	*Squalius cephalus*	*Scardinius erythrophthalmus*	*Alburnus alburnus*	*Rhodeus amarus*	*Gobio obtusirostris*	*Pseudorasbora parva*	*Carassius gibelio*	*Cobitis taenia*	*Proterorhinus marmoratus*
1	22,96	14,81	2,96	2,22	39,26	1,48	9,63	3,70	4,44
2	50,67	8,00		1,33	20,00	1,33	1,33	5,33	5,33
3	45,88	382,35	76,47	2,35	27,06		9,41	1,18	3,53
4	128,00	66,00	208,67	5,33	35,33	3,33	12,67	1,33	2,00
5	1,27	29,21	1,27	0,63	22,86	2,54	41,27	0,63	2,54
6	32,92	35,00	6,25	1,67	5,00	9,17	40,83	1,25	0,83
7				0,56	1,11	8,89	26,11		
8	3,81	13,33	68,57	3,81		3,81	32,38	1,90	
9	23,20		48,80	4,80		33,60	87,20		0,80
10	10,67		109,33	5,33		40,00	128,00		2,67

Table 4. Weight stock (g/100 m^2) of the fish species in Bistrița River, upstream the confluence with Siret River

No. of sampling sites	*Squalius cephalus*	*Scardinius erythrophthalmus*	*Alburnus alburnus*	*Rhodeus amarus*	*Gobio obtusirostris*	*Pseudorasbora parva*	*Carassius gibelio*	*Cobitis taenia*	*Proterorhinus marmoratus*
1	739,85	19,33	3,04	6,22	302,89	7,93	35,93	7,48	3,78
2	711,73	6,80		4,27	141,60	6,93	8,27	8,27	4,80
3	602,24	313,65	113,65	8,00	215,29		97,76	2,59	3,65
4	1453,27	107,00	523,60	14,20	294,07	16,13	106,20	4,53	1,27
5	24,38	21,33	0,89	2,67	78,86	3,30	294,29	1,71	2,48
6	570,96	168,08	9,08	6,08	47,04	31,67	364,21	4,83	1,08
7				1,17	1,44	26,11	601,17		
8	1,90	12,19	12,95	6,10		8,00	2582,29	3,24	
9	11,12		25,28	6,72		58,72	1118,40		0,72
10	32,27		50,13	9,07		110,40	2815,47		5,87

The Prussian carp (*Carassius gibelio*) was identified in all of the 10 sampling sites. The numerical stock of the Prussian carp in sampling sites ranges between 1,33 and 128,00 ind./100 m^2 while the weight stock ranges between 8,27 and 2815,47 g/100 m^2.

The spined loach (*Cobitis taenia*) was identified in 7 sampling sites. The numerical stock of the spined loach in sampling sites is quite low, ranging between 0,63 and 5,33 ind./100 m^2. Its weight stock ranges between 1,71 and 8,27 g/100 m2.

Finally, the tubenose goby (*Proterorhinus marmoratus*) was identified in 8 of the sampling sites. The lowest value of the numerical stock of the tubenose goby in sampling sites was just 0,80 and the highest value was 5,53 ind./100 m^2 while the weight stock ranges between 0,72 and 5,87 g/100 m^2.

Biodiversity indices. Table 5 display the values of some of biodiversity indices which were calculated to characterize the fish communities in the research area: species richness (Margalef and Menhinick), heterogeneity (Simpson and Shanon-Wiener) and equitability (eveness) in sampling sites.

According to Margalef and Menhinick indices, the greatest species richness was highlighted in the following sampling sites: 6 - Bistrița, SOFERT side, Ruși Ciutea village side, right shore and 8 - Bistrița, 3+126 km upstream bridge.

Heterogeneity indices values indicate that, based on similar species frequencies, the highest biodiversity for the fish communities was recorded in the sampling site 6 - Bistrița, SOFERT side, Ruși Ciutea village side, right shore. In the sampling sites 6 (Bistrița, SOFERT side, Ruși Ciutea village side, right shore), 4 (Bistrița, Downstream aval bridge, Ruși Ciutea village side) and 1 (Bistrița, upstream CET Bacău) we found the highest species richness (12, 12, and 11 species respectively).

Table 5. Biodiversity indices in sampling sites in Bistrița River, upstream the confluence with Siret River

Sampling sites	No. of species	Species richness		Heterogeneity		Equitability
		Margalef	Menhinick	Simpson	Shanon-Wiener	
1. Bistrița, upstream CET Bacău	11	3,881	0,567	0,645	0,242	0,101
2. Bistrița, CET Bacău side	9	3,803	0,799	0,702	0,126	0,057
3. Bistrița, Ruși Ciutea village side	8	2,591	0,357	0,553	0,290	0,139
4. Bistrița, Downstream aval bridge, Ruși Ciutea village side	12	3,796	0,427	0,757	0,345	0,139
5. Bistrița, SOFERT side, Ruși Ciutea village side	10	3,724	0,619	0,783	0,203	0,088
6. Bistrița, SOFERT side, Ruși Ciutea village side, right shore	12	4,086	0,541	0,834	0,288	0,116

7. Bistrița, Downstream SOFERT, Siretu village side	5	2,168	0,598	0,493	0,081	0,050
8. Bistrița, 3+126 km upstream bridge	9	3,897	0,847	0,720	0,117	0,053
9. Bistrița, 3+126 km downstream bridge	8	2,787	0,444	0,768	0,231	0,111
10. Bistrița, 3 +126 km downstream bridge, 9 sampling site side	7	2,812	0,600	0,740	0,363	0,186

Equitability values range from 0.050 to 0.186, but far from the value of 1 which would imply a fair distribution of individuals by species. The highest value of equitability was recorded for the sampling site 10 (Bistrița, 3 +126 km downstream bridge, 9 sampling site side), even if there were identified just 7 fish species.

Conclusions

An amount of 17 fish species were identified in the 10 sampling sites placed on Bistrița River, upstream of the confluence with Siret River. One of the 17 fish species is a non-native species: *Pseudorasbora parva*.

The chub (*Squalius cephalus*) and the Prussian carp Carassius gibelio) are the two main characteristic species in the research area, being dominant both numerically and by weight.

At the same time, the European bitterling (*Rhodeus amarus*) and the stone moroko (*Pseudorasbora parva*) are other two species with a great frequency, even constant and dominant species.

Species diversity is quite high, being identified even 12 fish species in some of the sampling sites (4 - Bistrița, Downstream aval bridge, Ruși Ciutea village side and 6 - Bistrița, SOFERT side, Ruși Ciutea village side, right shore). However, heterogeneity indices and equitability indicate an unequal distribution of individuals by species.

The main threats in the research area are the organic inputs and the increase of turbidity.

Although some threats have also been identified since the research area is in a highway construction zone, the fact that 17 fish species were found suggests that the fish communities are diverse, and there are still favorable environmental conditions.

Having in mind that the highway construction involves many changes in habitat conditions, we can say that there are no great imbalances, and there is a capacity for self-support of aquatic communities.

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ABOUT THE EUROPEAN EEL ANGUILLA ANGUILLA (LINNAEUS, 1758) OF THE DNIESTER RIVER

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Abstract: The presence of the European eel Anguilla anguilla in the Dniester River has been reliably established. In May 2021, an European eel 94.5 cm long and 1400 g in weight was caught in the Dniester River near Tiraspol.

Introduction

The European eel Anguilla anguilla is one of the rarest fish of the r. Dniester. In 2008, it was included in the IUCN Red Data Book as a species “on the brink of extinction”, with the Critically Endangered (CR) status, the eel is included in the Red Data Books of Moldova [1] and Transnistria [3], absent in the Red Data Book of Ukraine [4].

In the volume “Fishes, amphibians, reptiles” from the series “Animal world of Moldova” [2] in the article by E.N. Tomnatik dedicated to eel, noted:

“The European eel sometimes reaches 150 cm in length and 6 kg in weight. Males are much smaller: their length is 51 cm, and their weight is 200-250 g”.

Regarding its presence in the reservoirs of Moldova, the following information is presented here: *“The first mentions of eels in reservoirs of Moldova belong to K.F. Kessler (1857), who pointed to the capture of an eel about 90 cm in size in the Dniester above Yampol, L.S. Berg (1916) noted that eels in the lower reaches of the river were not uncommon, but there were no cases of catching this fish in Moldova ”.*

“An specimen of eel from the Turunchuk sleeve, 115 cm long, weighed 3 kg. More often in the Dniester and Dubossary reservoir there were eels weighing 1337-1920, the length of which ranged from 87.5-99.7 cm”.

In the book *“Commercial fish of the North-Western Black Sea region” [5], the following information is given: “In the Dniester delta, single small-sized specimens (2-3) of eels were still found in the spring of 1977 in a venture on burnt reaches. often at the mouths of the Dniester and Turunchuk before the construction of a new powerful Novodnistrovskaya hydroelectric power station in 1983, and after that eels became very rare, but, nevertheless, 1 large eel 80 cm long, probably the last time, was caught by fishermen at the mouth of the Dniester in April 2001 of the year”.*

T.D. Sharapanovskaya, director of the national park Yagorlyk, mentioned that one specimen of the eel was caught in the Dniester by fishermen in July 2005 near the pumping station of the village Butor.

Thus, there is practically no information in the literature on the registration of this species in the Dniester in recent decades; therefore, reliable data on the presence of eels in the Dniester are of great importance.

Results and discussions

On May 12, 2021, in the Dniester near the city of Tiraspol near the ferry, a local fisherman caught an eel on a bunch of worms with a bottom tackle. At the time of withdrawal from the water, the fish had already died and could not be released into the river. The fisherman handed over the eel to the Department of Zoology and General Biology of the Faculty of Natural Geography of Pridnestrovian State University, where the fish was photographed, measured and weighed (fig. 1).

Figure 1. Eel caught in the Dniester (photo by the authors)

The eel had the following morphometric and weight characteristics: body length 94.5 cm; body height 6.2 cm; head length from end of snout to pectoral fin 11.4 cm; weight 1400 g.

Conclusion

It has been reliably established that the European eel *Anguilla anguilla* (Linnaeus, 1758) is very rare, but occurs in the lower reaches of the Dniester River.

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THE ANTIPROLIFERATIVE, ANTIOXIDANT ACTIVITIES AND TOXICITY OF MIXED-LIGAND AMINE-CONTAINING COPPER(II) COORDINATION COMPOUNDS WITH 2-(2-HYDROXYBENZYLIDENE)-N-(PROP-2-EN-1-YL)HYDRAZINECARBOTHIOAMIDE

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Abstract. Five compounds 2-(2-Hydroxybenzylidene)-N-(prop-2-en1-yl)hydrazinecarbothioamide (H2L), bis[μ2-2-({2-[(prop-2-en-1-yl) carbamothioyl] hydrazinylidene}methyl) phenolato-S,N,O:O] diaquadicopper (II) nitrate (1), bis [μ2-2-({2-[(prop-2-en-1-yl) carbamothioyl] hydrazinylidene}methyl)phenolato-S,N,O:O]d iimidazoldicopper(II) nitrate (2), bis[μ2-2-({2-[(prop-2-en-1-yl)carbamothioyl]-hydrazinylidene}methyl)phenolato-S,N,O:O]bis-(3,5-dibromopyridine)dicopper(II) nitrate hexahydrate (3), bis[μ2-2-({2-[(prop-2-en-1-yl)carbamothioyl]-hydrazinylidene}methyl) phenolato-S,N,O:O]bis(4-methylpyridine)dicopper(II) nitrate hexahydrate (4) were synthesized. The antiproliferative properties of these compounds towards cancer cell lines HeLa, RD and normal cell line MDCK have been investigated. The tested compounds demonstrated high antioxidant and antiproliferative, selective activities towards cancer cells. Direct toxic evaluation of compounds was performed by Daphnia magna bioassay.

Introduction

Thiosemicarbazones and coordination compounds of transition metals with them are biologically active compounds. Many of them exhibit antiproliferative and antioxidant activities [1, 2]. In most cases, the coordination of thiosemicarbazones to copper(II) ions leads to the most significant increase in biological activity compared to ions of other 3d metals. A number of studies have shown that the introduction of various amines into the inner sphere of copper(II) thiosemicarbazonates leads to a change in their biological properties. In this regard, it is of interest to study synthesized mixed-ligand amino-containing copper (II) coordination compounds with thiosemicarbazones [1].

Material and methods

1.1. In vitro antiproliferative activity assay

Cells lines RD (human muscle rhabdomyosarcoma spindle and large multinucleated cells, ATCC CCL-136), MDCK (Madin Darby Canine Kidney epithelial normal cells, ATCC CCL-34) for experiments were taken after cryopreservation, in liquid nitrogen vapor phase at −180°C to −196°C in freeze medium: complete growth medium supplemented with 5% (v/v) DMSO. For the formation of a healthy monolayer on the substrate, cells were cultured for at least three weeks, passaged every 2-3 days, followed by trypsinization of adhesive cell clusters and replacement of growth media, inactivated fetal bovine serum was added as a growth factor. Cells in logarithmic growth phase were used for experiments. Viability of cells was assessed by dye 0.2% trypan blue.

Investigation of the antiproliferative activity of the synthesized compounds in relation to cells lines HeLa and MDCK was carried out by resazurin assay. Resazurin is a non-fluorescent indicator dye, which is converted to highly red fluorescent resorufin via reduction reactions of metabolically active cells. The amount of fluorescence produced is proportional to the number of living cells. Resazurin was dissolved in physiological buffers (resulting in a deep blue colored solution) and added directly to cells in culture in a homogeneous format. Usually, in the presence of NADPH dehydrogenase or NADH dehydrogenase as the enzyme, NADPH or NADH is the reductant that converts resazurin. Cells of lines were trypsinized from subconfluent cultures by adding 3 ml of trypsin-EDTA 0.05% (Invitrogen) to 50 ml of culture flasks with confluent cells followed by 5-15 min incubation at 37°C with regular gentle shaking and counted under an inverted microscope. The trypsin reaction was stopped by adding 10 ml of appropriate culture medium containing 10% FBS. The cell suspension was centrifuged at 750 rpm for 10 min at 25°C. The cell pellet was suspended in 2 ml of medium with 10% FBS and thoroughly mixed. Cells were counted and brought to a concentration of 1×105 cells/ml. The resulting cell suspension was seeded into triplicate wells of a 96-well microtiter plat (100μL/well) and incubated at 37°C, 5% CO2. After an initial 2-3 h period to allow cell attachment, 10 μL of the tested compounds and reference controls were directly added to the medium resulting. The plate was further incubated for 24 h at 37°C, 5% CO2. The tested compounds and reference controls were dissolved in DMSO to prepare the stock solution of 10 mM, which were used as reference at final concentrations ranging from 10, 1, 0.1 μM in medium. The compounds were incubated with cell suspension at 37°C, 5% CO2 for 24 h. Following each treatment, 20 μL resazurin indicator solution was added to each well and incubated at 37°C, 5% CO2 for 4 h. Subsequently, the absorbance was read by hybrid reader (Synergy H1, BioTek) with 570 nm and 600 nm filters. The percentage of cell proliferation inhibition was calculated according to the formula: $\% \text{ inhibition} = 100 - \frac{Abs_{570\text{ nm}(sample)} - Abs_{600\text{ nm}(sample)}}{Abs_{570\text{ nm}(control)} - Abs_{600\text{ nm}(control)}} \times 100$

1.2. In vitro antioxidant activity assay

The antioxidant activity by the ABTS•+ method was assessed according to the method described by Re et al. [9] with modifications. ABTS•+ assay is an excellent tool for determining the antioxidant activity of hydrogen-donating antioxidants and of chain-breaking antioxidants. The ABTS•+ radical was formed through the reaction of ABTS (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)) solution 7 mM with potassium persulfate (K2S2O8) solution 140 mM, incubated at 25°C in the dark for 12-20 hours at room temperature. The resulting solution was further diluted by mixing with acetate buffered saline (0.02 M, pH 6.5) to obtain an absorbance of 0.70 ± 0.01 units at 734 nm.

The dilutions of the tested compounds were prepared in DMSO at concentrations ranging from 1 to 100 μM. After that, 20 μL of each tested compound dilution were transferred in a 96-wells microtiter plate and 180 μL of working solution of ABTS•+ were dispensed with dispense module of hybrid reader (BioTek). The decrease in absorbance at 734 nm was measured exactly after 30 min of incubation at 25°C. DMSO was used as negative control. Blank samples were run by solvent without ABTS•+. All tests were performed in triplicate and the obtained results were averaged. The percent of inhibition (I %) of free radical cation production of ABTS•+ was calculated by using the following equation:

$$I(\%) = \frac{Abs_{734\ nm_0} - Abs_{734\ nm_1}}{Abs_{734\ nm_0}} \times 100, \text{ where}$$

Abs_{734nm0} is the absorbance of the control solution; $Abs_{734\ nm1}$ is the absorbance in the presence of sample solutions or standards for positive controls.

1.3. In vivo acute toxicity assay

The general toxicity of the new tested compounds was evaluated using *Subphylum: Crustacea Order: Cladocera. Species: Daphnia magna* (Straus, 1820). The *Daphnia magna* originated from a culture maintained parthenogenetically at the Institute of Zoology, Center of Research of Biological Invasions, Laboratory of Systematics and Molecular Phylogeny. The test design was based on GOST R 56236-2014 (ISO 6341: 2012). This International Standard specifies a procedure for the determination of the acute toxicity of chemical substances to the water flea *Daphnia magna* (Straus, 1820). This method is applicable to chemical substances, which are soluble under the conditions of the test, or can be maintained as a stable suspension or dispersion under the conditions of the test. The test specified in this International Standard involves the determination of the immobilization of the *Daphnia magna* after 24 h and 48 h exposure to the test sample under the conditions specified in this International Standard. The Daphnia magna acute mobility inhibition assay was performed using juvenile individuals of *Daphnia magna* aged up to 24 h, originating from ephippia.

The test-organisms *Daphnia magna* were fed with *Chlorella vulgaris* (Beijerinck 1890). These unicellular algae were grown using aseptic technology to exclude contamination of the culture by bacteria, algae or protozoa. The Chlorella vulgaris were cultivated in Prat's growth medium containing KNO_3 (1 Mm), $MgSO_4.7H_2O$ (40 μM), $K_2HPO_4.3H_2O$ (400 μM), $FeCl_3.6H_2O$ (3.6 μM) in H_2O distilled (adjusted the pH to 7.0, autoclaved and stored at 5°C).

The *Daphnia magna* were maintained in aerated aqueous straw infusion growth media supplemented with $CaCl_2$ (11.76 g/l), $NaHCO_3$ (2.59 g/L), KCl (0.23 g/L), $MgSO_4.7H_2O$ (4.93 g/L). (pH~7.5±0.2; O2 ≥6.0 mg/L)

Juveniles were selected according to their size and kept in fresh medium for 24 h. The *Daphnia magna* were cultured in Costar® 24-well culture clear sterile multiple well plates covered by a lid to prevent the possibility of contamination and evaporation but at the same time to allow gaseous exchange between air and culture medium. Each well contained 10 daphnids in 1000 μL final volume of each dilution of the tested compounds.

The bioassay was then repeated at the concentrations ranging from 0.1 to 100 Mm (0.1, 1, 10, and 100 μM) in order to determine LC50 for each compound, including the positive control. The final test solutions contained up to 0.1% DMSO and had a final volume of 1 mL. A 0.1% solution of DMSO in aerated medium (pH~7.5±0.2; O2 ≥6.0 mg/L) was used as a negative control and compounds were used as positive controls. Throughout the experiment, the juvenile daphnids were incubated at 22±20C, using a 16 h /8 h light/dark cycle (500–1000 lx). The mobility (viability) of the test organisms was observed after the 24 h and 48 h exposure. The experiment was performed in triplicate. The daphnids were considered immobilized only if they did not swim during the 15 s which follow gentle agitation of the test and control solutions, even if they could still move their antennae. The percentage of viability (V (%)) of *Daphnia magna* was calculated according to the formula: $\mathbf{V(\%)} = \frac{\mathbf{N_{(sample)}}}{\mathbf{N_{(control)}}} \times \mathbf{100}$, where N - Number of viability of *Daphnia magna.*

1.3.Statistical analysis

The cell proliferation assay results were reported as the percent inhibition of the test and control substances. As an indicator of efficiency of the experimental compounds on proliferation of cell lines, the half maximal inhibitory concentration was used. According to FDA documents, IC50 is an indicator of the concentration of medicinal substance required for 50% inhibition of the tested reaction in vitro. The toxicity activity of compounds was evaluated as the median lethal concentration values (LC50) were calculated from the dose-response equation determined by the least squares fit method, using the GraphPad Prism software. All data are presented as means ± standard deviation (SD).

Results and discussion

The 2- (2- Hydroxybenzylidene) -N- (prop -2 - en1 -yl) hydrazinecarbothioamide **(H_2L)**, bis [μ2-2- ({2-[(prop-2-en-1-yl) carbamothioyl]-hydrazinylidene }methyl)phenolato-S,N,O:O]-diaquadicopper (II) nitrate (**1**), bis[μ2-2-({2-[(prop-2-en-1-yl)carbamothioyl] hydrazinylidene}-methyl)phenolato-S,N,O:O]diimidazoldicopper (II) Nnitrate (**2**), bis [μ2-2-({2-[(prop-2-en-1-yl)carbamothioyl]hydrazinylidene}methyl) phenolato-S,N,O:O]bis-(3,5-dibromopyridine)-dicopper(II)Nnitrate hexahydrate (**3**), bis [μ2-2-({2-[(prop-2-en-1-yl)carbamothioyl]-hydrazinylidene}methyl)phenolato-S,N,O:O] bis-(4 -methylpyridine)dicopper(II) nitrate hexahydrate (**4**) were synthesized in Research Laboratory of Advanced Materials in Biopharmaceutics and Technics of the Moldova State University by acad. A. Gulea et al.

The thiosemicarbazone **H_2L** and complexes **1-4** were synthesized as described in the literature [1]. The thiosemicarbazone **H_2L** was characterized by NMR (1H and 13C) spectroscopy. The complexes **1-4** were characterized by electronic, FT-IR and EPR spectroscopy, molar conductivity, magnetic susceptibility measurements and elemental analysis. Also, the crystal structure of complexes were determined by single-crystal X-ray diffraction analysis. Melting points, IR, and NMR spectra of the tested compounds correspond to the literature data [1].

The antiproliferative activity experiments were displayed in a dose-dependent manner and showed concentration dependence between the inhibitory effects of the tested compounds at the micromolar concentration range. It is known from the literature that, as a rule, anticancer chemotherapeutic drugs have a high cytotoxic effect on normal cells, which leads to serious side effects that can be fatal. On this basis, we have exploited normal kidney epithelial cells of line MDCK for selective cytotoxicity evaluation.

Table 1 shows concentrations IC50 of half-maximal inhibition, which represent a measure of efficiency of antiproliferative action of the investigated substances in relation to human muscle rhabdomyosarcoma spindle and large multinucleated cells of line RD and to model line of normal mammalian cells MDCK, and also indices of selectivity of anticancer activity, indicating how many times the activity against cancer cells is higher than in normal cells MDCK.

Study of the antiproliferative activity of the **H_2L** ligand and compounds 1-4 against the cancer cells RD showed that the **H_2L** ligand shows almost no activity, suppressing the growth and reproduction of cancer cells by 14.0±1.2 % at a concentration of 10 μM. The tested complexes 1-4 and the FDA-approved anticancer drug doxorubicin (DOXO) possess antiproliferative activity on RD cells with IC50 values of 0.6±0.1, 0.8±0.3, 1.03±0.03, 1.3±0.2 and 16.2±0.3 μM, respectively. Thus, the inhibitory activity of the tested complexes on RD cells proliferation manifest higher than the corresponding values of the DOXO.

The tested compounds **H_2L, 1-4** and DOXO inhibit the formation and growth of MDCK cells, with IC50 values of ≥10, 6.0±0.4, 12.0±0.6, 9.0±0.9, 3.5±0.5 and 7.1±0.5 μM, respectively.

The selectivity index SI of complexes **1-4** and DOXO are 10, 15, 9, 3 and 0.4, respectively. Thus, the complexes **1-4** are 12.5–27 times more active than doxorubicin against RD cells. In addition, complexes **1-4** are superior to doxorubicin in selectivity for this cancer cell line.

In order to exclude the eventual presence of concomitant adverse effects associated with oxidative stress, the tested compounds were tested by ABTS•+ assay. The antioxidant potency of the tested compounds was compared to the DOXO and the reference antioxidant control trolox. It is known, that doxorubicin-induced cardiomyopathy carries a poor prognosis and is frequently fatal. Doxorubicin induces toxic damage to the mitochondria of cardiomyocytes contributing to increased oxidative stress.

Table 1. Antproliferative activity of the tested compounds H2L, 1-4 and the positive control DOXO on cells of lines RD, MDCK and selectivity activity

Compound	MDCK	RD	
	IC_{50} (μM)	IC_{50} (μM)	SI
DOXO	7.1±0.5	16.2±0.3	0.4
H_2L	≥10	≥10	-
1	6.0±0.4	0.6±0.1	10
2	12.0±0.6	0.8±0.3	15
3	9.0±0.9	1.03±0.03	9
4	3.5±0.5	1.3±0.2	3

The results of studying the antioxidant activity of trolox, DOXO, $\mathbf{H_2L}$ ligand and complexes **1–4** against ABTS•+ cation radicals are presented in figure 1 in the form of semi-maximal inhibition concentrations IC50. The uncoordinated thiosemicarbazone H_2L shows higher activity than trolox. Coordination of $\mathbf{H_2L}$ to the copper(II) ion (complex 1) leads to a decrease in antioxidant activity, whereas the introduction of amines into the inner sphere (complexes **2–4**) re-increases antioxidant activity, and the activity of complexes with amines exceeds not only that of complex 1, but also the activity of the initial thiosemicarbazone $\mathbf{H_2L}$. Analyzing the results of ABTS•+ method, it was observed that the tested compounds **2–4** showed the best antioxidant activity compared with trolox and DOXO.

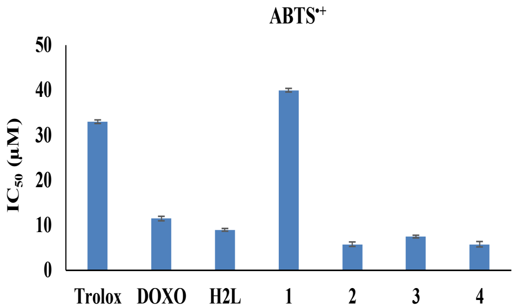

Figure 1. The influence of the tested compounds and reference controls for ABTS•+

Toxicity studies are an important stage in the development of drugs, being a prerequisite before starting their use in preclinical and clinical trials. Since the fundamental principle of toxicity studies is the protection of animals, including those participating in studies, it is currently recommended that in all possible cases, studies should be conducted on in vivo invertebrate organisms, avoiding the inclusion of laboratory animals in studies. Daphnia magna is a cladoceran organism used frequently along with Artemia salina (Linnaeus, 1758) in the cytotoxicity and biological activity evaluation of synthesis compounds [2].

The toxicity of the tested compounds was evaluated using the *Daphnia magna* bioassay in the Institute of Zoology, Center of Research of Biological Invasions by acad. I. Toderas et al. The results of *Daphnia magna* bioassay are given in table 2. The tested compounds have manifested the general toxicity against *Daphnia magna* after 24h and 48h of exposure, according to the sequence: $\mathbf{3 \geq 1 \geq 4 \geq 2}$ **and** $\mathbf{1 \geq 3 \geq 2 \geq 4}$, respectively. The complexes 1–4 are less toxic for Daphnia magna than for cells of lines RD, HeLa [1] and MDCK. The tested copper complexes showed promising antiproliferative activity and low toxicity on *Daphnia magna*.

Table 2. Toxicity on Daphnia magna of complexes 1–4.

Compound	Incubation Period	
	24 h	**48 h**
	LC_{50} (μM)	**LC_{50}** (μM)
1	12.8±4.1	8.9±1.3
2	3.3±0.1	3.5±2.7
3	14.0±3.2	4.4±0.1
4	7.9±4.2	3.1±0.1

The above experimental data indicate the prospects for further search for selective anti-cancer substances with antioxidant activity and low toxicity among copper mixed-ligand amino-containing coordination compounds.

Conclusions

In conclusion, our results may be useful in designing novel antiproliferative agents.The ligand **H_2L** and the complexes **1–4** have been screened for their in vitro antiproliferative, antioxidant activities and toxicity. Inhibitors of cancer cell proliferation complexes **1–4** characterized by high selective activity, low toxicity and higher efficiency compared to DOXO have been identified, which opens up prospect of their employment as anticancer agents.The tested compounds **H_2L** and **1–4** have manifested higher antioxidant activity against ABTS•+ radical compared to the reference compounds DOXO and trolox. The synthesized complexes **1–4** are less toxic for Daphnia magna than for cells of lines RD and MDCK. Thus, the copper complexes **1–4** showed promising antiproliferative activity against cancer cells and low toxicity on Daphnia magna.

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LONG-TERM DYNAMICS OF TOTAL BACTERIOPLANKTON IN THE DUBOSSARY RESERVOIR OF THE REPUBLIC OF MOLDOVA

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Abstract. The results of long-term (1982-2019) studies of the quantitative development of bacterioplankton in the flow-channel Dubossary reservoir in the Republic of Moldova and analysis of the reasons for its change are shown.

Introduction

Bacteria are one of the most numerous and diverse groups of aquatic organisms that have the most active metabolism and play an important role in the biochemical processes of transformation of biogenic and other elements and organic matter in general. The results of many years of research earnestly indicate that aquatic bacteria are an indispensable and essential component of trophic chains, playing an important role in the structural organization of freshwater ecosystems. Due to their species diversity and high physiological activity, they perform the function of an ecosystem stabilizer, responding extremely quickly to the slightest changes of the environmental conditions [2].

Research on reservoirs in the temporal aspect has been going on since a long time. On the territory of the CIS (formerly the USSR), they have been held for more than 80 years, starting from the 30s of the last century. At the same time, the study of the ways and prospects of the transformations of the water bodies undergoing permanent changes under anthropogenic impact still remain actual.

The initial research on the microbiological status of the flow-channel Dubossary reservoir was started in 1957, 3 years later, after its filling. However, the research has not yet been systematic, although the main indicators of the state of the aquatic bacteriocenosis, namely, the quantitative development of the general microflora, its biomass and the number of individual physiological groups have already been studied [8]. In the subsequent years, the studies were expanded by studying the production potential of the total bacterioplankton, as well as its sanitary and microbiological regime [5, 10, 13, 14, 15].

In the early 80s, the Dniester River experienced considerable environmental transformations due to construction on the territory of Ukraine of the Novo-Dnestrovsk HPP, a pumped storage reservoir (HPP 2 and the accidental discharge into the river of the concentrated effluents from the Stebnikov chemical plant (Ukraine). At the same time, the most serious transformations were related to the impact on the ecosystem of the Dubossary reservoir as a result of an uncontrolled growth of submerged aquatic vegetation. Before the regulation of the Dniester River by the dams of the aforementioned hydroelectric power plants, an increased number of microflora, especially during the flooding period, was associated with suspended particles, the amount of which was quite high. With a decrease in their number of suspended particles due to sedimentation processes, the number of bacteria was reduced significantly. It should be noted that aggregated bacteria closely linked to these particles had much more favorable conditions for their existence, which is why their growth rate was significantly higher than that of single planktonic bacteria.

All these reasons served as the basis for carrying out detailed and systematic studies of the bacterioplankton of the Dubossary reservoir. For a comparative assessment, all the data obtained were conditionally divided into several periods characterizing the stages of the formation of the Dubossary reservoir and changes in the anthropic press: 1957-1959, 1986-1990, 1991-2005, 2006-2010, 2011-2014. All of these periods are characterized by specific conditions of hydrological, physico-chemical and hydrobiological regimes, which left a noticeable footprint on the quantitative indicators and physiological activity of bacterioplankton.

Material and methods

All the indicators described in the current article were determined by generally accepted microbiological methods [3]. Sampling was carried out in accordance with the national standard SM SR ISO 5667-6 and the Guidelines for the collection of samples of hydrochemical and hydrobiological samples [1]. In general, during the growing seasons of 1982-1919, more than 300 water samples were collected and analyzed. The total number (Ntot) of bacterioplankton and the amount of its saprophytic group (Nsapr) were determined. Inoculations were carried out on various elective media using generally accepted methods, in particular, with the SM SR EN ISO 6222: 2014 standard. The results obtained were compared with similar data obtained in previous years by other authors [8]. Certain aspects of the studies below were reflected in the studies carried out in the subsequent years [4, 5, 12, 13] and others). The data obtained were statistically processed using Exel, Statistica.

Results and discussion

The quantitative dynamics of aquatic bacteria in the Dubossary reservoir is determined by a complex of natural (morphometry, hydrology, hydrochemistry, hydrobiology) and anthropic factors (wastewater, runoff from the surrounding area). The complex dynamics of these indicators is the reason for the high interannual variability in the amount of bacterioplankton. In some years, fluctuations in the number are so great that the minimum indicators differ from the maximum up to 16 times (fig. 1).

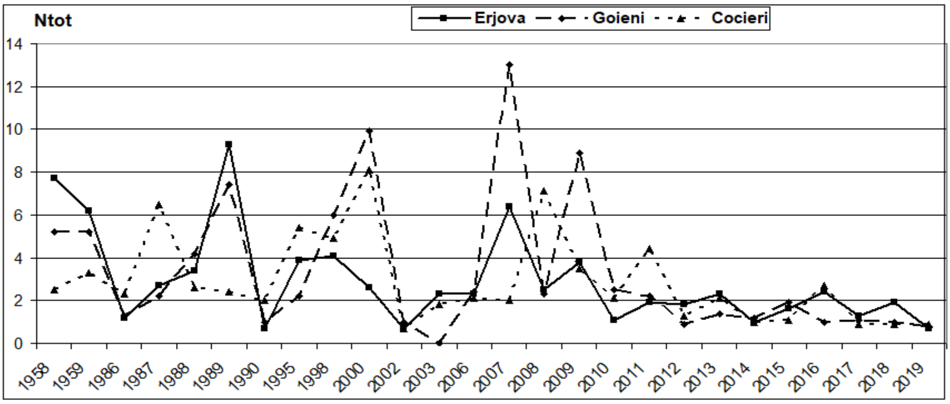

Figure 1. Dynamics of the average growing number of bacterioplankton (Ntot x 106 cells / ml) at individual stations of the reservoir in 1958-2019 years.

The abundance of total bacterioplankton in the reservoir 4 years after its filling varied in different seasons of 1958-1959. from 2.5 to 7.7 x 106 cells / ml, and the average for the growing season (April-October, 270 days) was 5.0 ± 1.9 × 106 cells / ml (fig. 2).

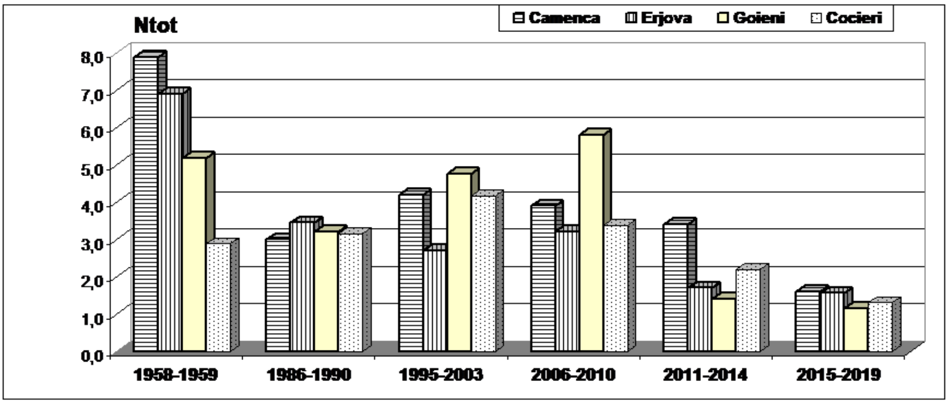

Figure 2. Interannual dynamics of the total number of bacterioplankton (Ntot x 106 cells / ml) in different periods 1958-2019 (average for the growing season)

Despite the fact that the water entering the reservoir (St. Camenca) was characterized by a higher content of bacteria - 8.0 ± 5.9 × 106 cells / ml, the differences (P = 0.05) are still insignificant. In subsequent periods, noticeable fluctuations in the number of bacterioplankton were recorded, however, in the last period (2015-2019), it significantly decreased at all the studied sections and varied in a narrow range of 1.2 - 1.6 × 106 cells / ml, amounting to, on average, 1.22 ± 0.47 × 106 cells / ml, which indicates the transformation of the reservoir from a eutrophic to a mesotrophic reservoir. Observations of the numerical development of bacterioplankton, carried out during the recent research period (1986-2019), in all studied areas demonstrate stabilization of bacterioplankton the level of 2.6-3.3 × 106 cells / ml. (fig. 3), which clearly confirms the uniformization of the conditions for the existence of the bacterial population. Moreover, due to a significant decrease in the speed of water flow throughout the entire section of the Dniester river, downstream of the aforementioned dams of hydropower stations, as well as the massive development of submerged aquatic vegetation, the river undergoes limnophilization and leveling of microbiological indicators, both in the section flowing into the entrance to the reservoir (St. Camenca) and in the reservoir itself.

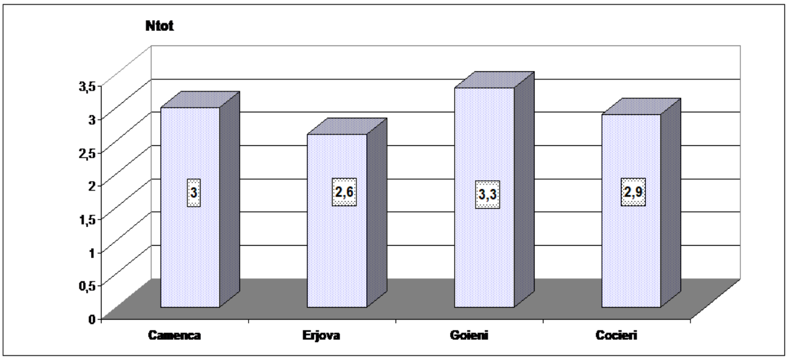

Figure 3. Average long-term (1986-2019) number of bacterioplankton (Ntot x 106 cel / ml) at the entrance to the Dubossary reservoir (St. Camenka) and in the reservoir (St. Erjova-Cocieri)

Seasonal changes in solar radiation and dynamics of water masses usually determine the existence of winter, spring-summer and autumn periods. The peculiarities of the functioning of hydroelectric power plants on the territory of Ukraine led to the fact that in the upper and even middle sections of the Dubossary reservoir in winter, the temperature practically corresponds to the spring level. As a rule, this results in significant changes in the entire hydrobiocenosis. However, for bacterioplankton, in contrast to other groups, these changes are not so pronounced. As before, the trend of an insignificant increase in the number of bacteria in the summer period persists (fig. 4).

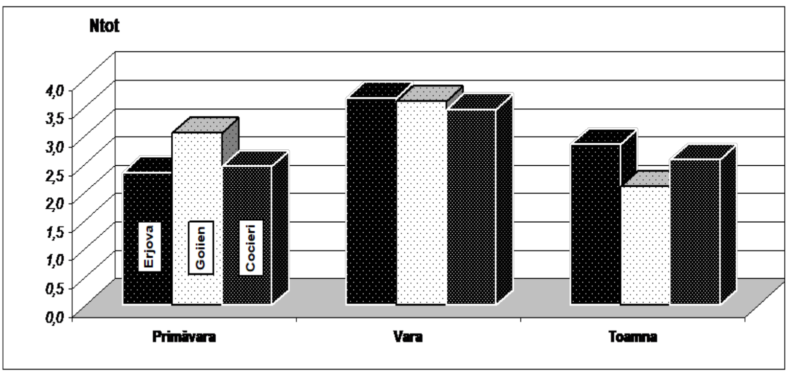

Figure 4. Seasonal population dynamics (Ntot x 106 cells / ml) at different stations Dubossary reservoir in the period 1957-2019.

However, temperature is not always the dominant factor. This impact is often indirect, through local enrichment of water with autochthonous and allochthonous organic matter [7], which leads to significant variations in the numerical indicators of bacterioplankton development. This is especially true [6, 11, 9] for such an important indicator group of bacterioplankton as saprophytic bacteria (Nsapr). The dynamics of the quantitative development of this group of bacteria, as well as the dynamics of the total bacterioplankton, is very variable not only within the same season, but also in different years (fig. 5).

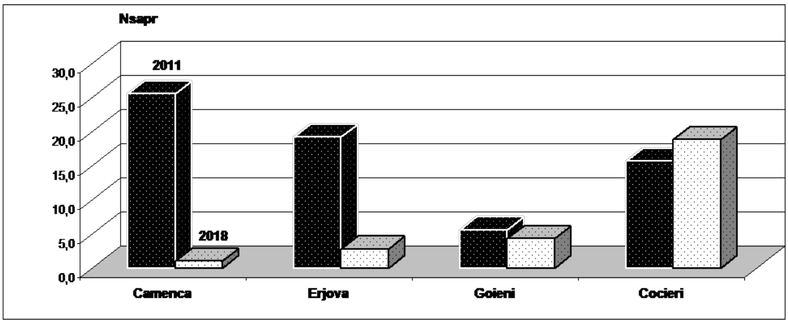

Figure 5. The number of saprophytic bacteria (Nsapr, × 103 CFU / ml *) in summer 2011 and 2018 at St. Camenca and in the Dubossary reservoir. (* CFU – colonies forming units)

Similar changes are observed in the interannual aspect (fig. 6).

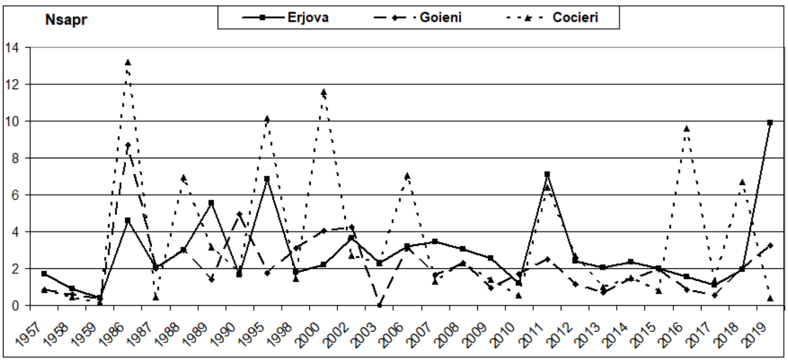

Figure 6. Interannual dynamics of saprophytic bacteria (Nsapr. × 103 CFU / ml in the Dubossary reservoir (St. Erojova - St. Cocieri) in different years

The analysis of the data presented above indicates that by now the Dubossary reservoir has passed 3 stages of formation. The first stage (1958-1959) was characterized by a low abundance (fig. 7) of saprophytic microflora (average index -0.81 ± 0.35 × 103 CFU / ml), which was most likely explained by the lack of trophic substrate for these groups of organisms.

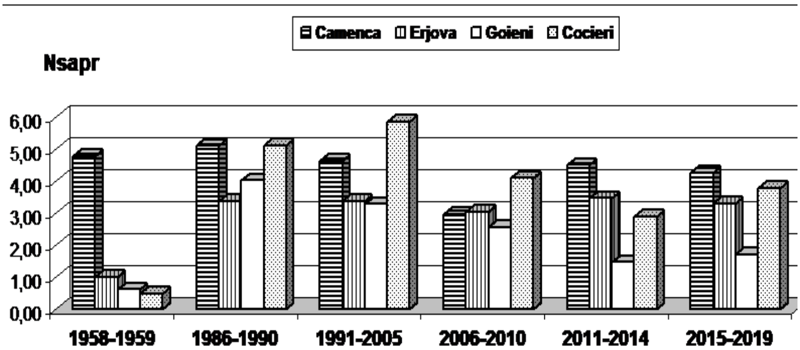

Figure 7. Dynamics of the number of saprophytic bacteria (Nsapr. × 103 CFU / ml) in the river. Dniester (St. Camenca) and Dubossary reservoir (St. Erjova- St. Cocieri) in various periods of research

The second stage (1986-2005) was accompanied by an increase in discharges of poorly treated wastewater from enterprises of various profiles that were intensively operating at that time. These wastewaters were a good nutrient medium for this group of bacteria, which also explains the increase in the number of saprophytes (average value -4.35 ± 1.66 × 103 CFU / ml). And, finally, at the last stage (2006-2019), there is (with rare exceptions) a relative stabilization of the conditions for the existence of saprophytes (the average is -2.68 ± 0.75 × 103 CFU / ml).

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APPLICATION OF VERMIFILTRATION FOR SUSTAINABLE MANAGEMENT OF SEPTAGE

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Abstract. The current article presents an innovative technical solution for sustainable management of septage, via a vermifiltration system, in the Republic of Moldova. A technical description of the main components of a demonstration model that was implemented at a household level is presented. The results from the model show an 98.85% reduction of ammonia content, 49,21 % CODMn (an indicator of degradation of easily degradable organic substances) and 85,28% of CODCr (showing degradation of hardly degradable organic substances). Considering the fact that the concentration of ammonium in the effluent was considerable reduced, while that of nitrate nitrogen remained rather low, it was assumed that the activity of earthworms was rather limited, probable the microorganisms, interacting symbiotically and synergistically with earthworms not being well developed in the vermifilter bed. The study suggests that the vermifiltration technology can provide a sustainable wastewater management solution and its application can be promoted in certain settings such as schools and kindergartens in Moldova. However, good insulation should be considered to avoid the freezing problems during winter while the odor nuisance in the septic tank can be reduced by adding lactic acid bacteria can also be applied.

Introduction

A septic tank is a watertight chamber made of concrete, fiberglass, PVC or plastic, through which blackwater and greywater flows for primary treatment. Septic tanks are simple technologies, with low operation costs, long service life and limited land requirements, as they are usually built underground. Settling and anaerobic processes reduce solids and organics, but the treatment is only moderate. In the context of climate change, with intensification of droughts, reuse of wastewaters for irrigation as an alternative to drinking water is gaining significant importance. In the Republic of Moldova, septic tanks or infiltration pits are widely used in rural areas, however, they contribute to a high pollution of the groundwater sources. As such, the introduction of new technologies for the treatment of wastewater from the septic tanks to the level that it can be reused would be a good alternative to the current existing models.

One such technology is the vermifiltration or lumbrifiltration, which is the application of earthworms in wastewater treatment [1-2]. Vermifiltration is a new technology based on aerobic treatment of wastewater by earthworms. The use of vermifiltration systems is important for rural areas of Moldova, where flush toilet systems are commonly applied. These systems are usually connected to infiltration pits, often leading to pollution of groundwater, which serves as drinking water sources for a large portion of the population. In this study, a vermifitration unit was applied in the treatment of a septic tank effluent. The potential reuse of th treated wastewater was evaluated by WiSDOM association in collaboration with Ecotox association and Laboratory of Hydrobiology and Ecotoxicology, Institute of Zoology. A vermifiltration system usually consist of a biological reactor, containing a filtration media that filters the oranic material and an earthworm layer, which contributes to the reduction of pathogens and organic matter (fig. 1).

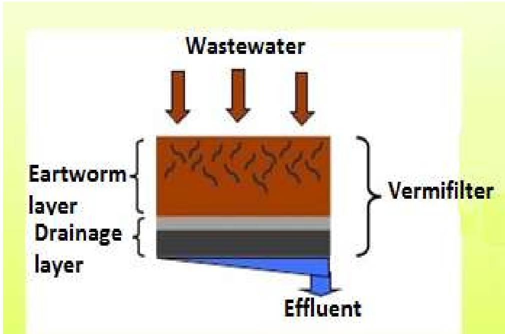

Figure 1. A schematic view of a vermifilter. Source: [3]

Materials and methods

The demonstration vermifiltration system was built with 4 main components including a septic tank with two compartments (1), an equalization tank (equalizer) (2), a distribution reservoir with associated distribution pipe system (3) and a vermifilter (4) (fig. 2). In the first chamber of the septic tank, settling of the solids as well as anaerobic digestion take place, which contribute to volume reduction. After this primary treatment, the liquid fraction of the wastewater is transported via an opening into the baffle wall to the second chamber, where additional sedimentation takes place. Excess liquid, relatively clear, is then drained through an outlet pipe into the equalization tank. This tank acts as a buffer to maintain a constant flow and prevent over peaking, which could force solids and fresh organic material to be pumped into the vermifilter. A submersible pump with a level sensor is installed in the equalizing tank. The level sensor measures the level of wastewater in the equalizing tank and triggers the pumping process, which pumps the wastewater into the distribution reservoir

The distribution reservoir is located above the vermifiler, thus the wastewater flows by gravity via a system of perforated pipes and is distributed further over the entire surface of the vermifiltre. In the vermifiltre the top layer is the substrate for earthworms (Eisenia foetida)- a layer of shredded paper, covered with a layer of soil mixed with organic waste, where the compost earthworms Eisenia foetida are placed. Beneath the earthworm substrate, a layer of biochar is placed, which absorbs pollutants that have not been decomposed by earthworms. Under the biochar layer there are also two layers of gravel of sizes 2-10 and 20-40 mm, which serve as filtration media. At the bottom of the vermifiltre there is a system of perforated pipes that directs the treated effluent to a drip irrigation system. In order to prevent freezing of the vermifilter during the cold period and to ensure that the earthworms are active, the vermifilter is covered with a polyethylene foil cover that maintained temperature at above 00C. The number of users for the treatment system is two, with a water consumption rate at around 70 L of water per person per day, the wastewater is pumped into the vermifilter every second or third day.

For the assessment of the raw and treated wastewater, samples were collected from the

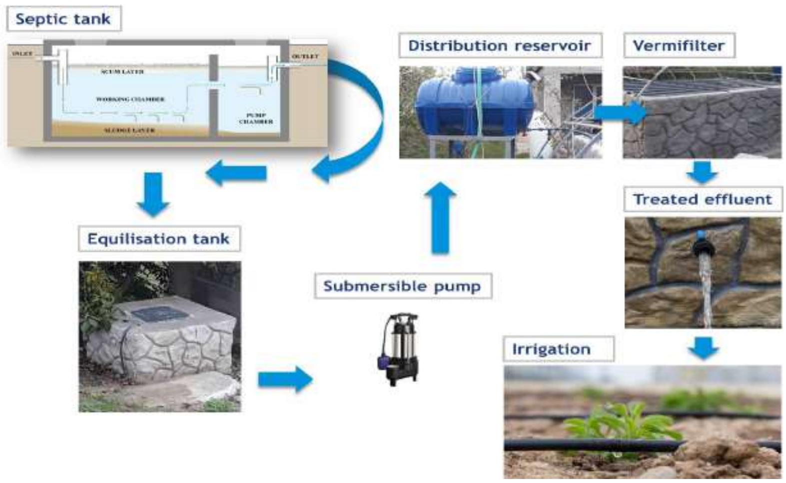

Figure 2. A schematic view of a demonstration vermifiltre built by WiSDOM association in a rural area of Moldova, at household level [3]

first chamber of the septic tank and from the effluent tap of vermifiltration unit. The values of permanganate chemical oxygen demand (CODMn) (allowing to potentially assess the level of degradation of easily degradable organic substances) and dichromate chemical oxygen demand (CODCr) (allowing to assess degradation of hardly degradable organic substances) were obtained by titrimetric methods, using automatic burettes Pellet and digital Solarus, after boiling and catalytic oxidation of the wastewater samples [5, 6]. The content of nutrients was determined by spectrophotometric methods [7-10] using the UV-VIS Specord 210 AnalyticJena spectrophotometer.

Results and discussion

As can be seen in Table 1, there was a considerable reduction of ammonia content (with 98,85 %) (see also evidence in Figure 3), a moderate reduction of CODMn (easily degradable organic substances) – with 49,21 % CODCr (hardly degradable organic substances) – with 85,28 %. Considering the fact that the concentration of nitrite nitrogen and nitrate nitrogen was very low – 0,002 mg/l, it can be assumed that the role of earthworms in ammonium cleavage was insignificant and the reduction of ammonium nitrogen was mainly due to its absorption on biochar. It can be assumed that at the time of sampling it was not sufficient time (at least few months) for the microorganisms, interacting symbiotically and synergistically with earthworms to be developed in the vermifilter bed, which would have allowed an efficient organic and ammonia transformations processes. Additional research shall be done after the community of microorganisms will be well developed. For an effective wastewater treatment, a particular attention shall also be paid to earthworm density – 15,000 specimens/m2 also, the use for bedding and filter media also have effects on the establishment of microbial biofilms and the microbial community structure within the vermifilter and the treatment performance [11].

Table 1. Content of nutrients and chemical oxygen demand in raw wastewater and treated effluent

	Untreated wastewater	Treated wastewater
NH_4^+-N, mg/l	14,60	0,17
NO_2^--N, mg/l	0,002	0,002
NO_3^--N, mg/l	0,002	0,002
PO_4^{3-}-P, mg/l		0,01
COD_{Mn}, mgO_2/l	39,00	9,29
COD_{Cr}, mgO_2/l	330,23	57,14

For an efficient wastewater recycling, the vermifilter was connected to a greenhouse, where some vegetables and decorative plants are grown. At the moment, after 10 months of use, the following issues affected the functionality of the vermifilter:

1) Freezing of the wastewater in the vermifilter and the pipes during long cold periods (26 January-04 February), when the temperature during the day and night was fluctuating between -5 and -8 ° C. The vermifilter could not be used for one week due to freezing of water in the pipes, also a fraction of the earthworms died. A good insulation is required of the greenhouse and the pipes in order to avoid such problems in the future.
2) Smell issue during the distribution of wastewater from the distribution reservoir onto the vermifilter. This was solved by adding activated lactic acid bacteria from kefir or yogurt starters, which are available in the supermarket. The cost of these starters in the Republic of Moldova is 60 lei and one box of 4 phials can be sufficient for 4-6 months, thus the costs are insignificant for the users of the vermifilter. At the moment, no complains from neighbors about the smell were received yet. However, it is necessary to further reduce the smell for the comfort of the users themselves.

Figure 3. Representative color change for ammonia content in treated and untreated wastewater

Considering the first results from the use of vermifiltration systems for treatment of grey-water (wastewater coming from the kitchen and laundry), such systems seems to be suitable to be applied at household level or even for some institutions, e.g. school or kindergarten, where the water consumption is not very high and thus the production of wastewater. The reason why vermifiltration can be implemented in rural areas is that this technology is environmentally friendly, as it allows reuse of treated wastewater in irrigation, it is relatively easy to manage and not capital-intensive [1]. This is particularly important in Moldova under the conditions of water stress during summer dry periods.

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ICHTHYOFAUNA OF THE LOWER COURSE OF THE PRUT RIVER (LARGA RIVER SECTOR (KM 120) - CONFLUENCE WITH RIVER DANUBE)

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Summary

The present written work represents the results of the research of the ichthyofauna from the lower course of the Prut River (including the Manta puddle and the Beleu lake) in terms of succession. It was established that, as a result of anthropogenic actions (drying of over 27-30 thousand ha of puddles, dam of the banks of the Prut River and its tributaries, extraction of sand and river stone from the minor river-bed, irreversible use of water in irrigation and industries, pollution, construction of the Costești-Stânca dam, destruction of wood cuts for lithifile and phytophilic species, etc.) in the ichthyofauna on the lower course of the Prut River, there were significant changes in diversity, quantitative and qualitative composition, and functional status of fish populations.

Introduction

The Prut River on the km section 120 (mouth of the river Larga (left tributary) to the confluence with the Danube River is characterized by a slow flow regime (instability during floods and showers) with major floodplain.

The flow and water level depends on the amount of atmospheric deposits that vary according to the type and seasonal changes, being unevenly distributed throughout the basin - the water level withstands large fluctuations. The lowest atmospheric deposits occur in winter and the highest in summer in the form of showers. Melting snow and spring rains as well as summer showers condition the spring and summer floods. Spring floods usually begin in late February - early March, approximately with the thawing of ice.

Raising the water level during this period, on average, is 0.3-0.6 m reaching up to 1m over 24 hours. During the summer floods (most intensive in July - August) the rise of the level also occurs quite abruptly. In the last 3 years, the spring-summer floods have shifted in May-July.

On this section, the river has many meanders, and the banks have low slopes - floodplain. Both banks are covered with tree roots, shrubs and macrophytes on which favorable conditions are created for the natural reproduction of the main fish species. For fattening, mainly Manta puddle and Beleu lake are used.

Methods and materials applied

During the years 2020 – 2021, the ichthyological material was collected by carrying out control fishing with nets and stationary and drifting with the size of the eye on the side 32-40-45-50-55-70-100 mm, with a length of 25 m and 50 m, the net for brood L - 5 - 20-50m, h - 1,5 - 2m, Ø 10x10 - 20x20 mm, ichthyoplankton mesh and fishing net race. The determination and analysis of the ichthyological material was performed using classical ecological and ichthyological methods [8, 9,10,17,19,20,21]. The data obtained were statistically processed using the program Excel - 2007. In total, 2183 fish were collected in the years 2020 – 2021.

Also, for the synthesis analysis of the results of multiannual scientific research of the Laboratory of Ichthyology and Aquaculture of the Institute of Zoology on the state of fish fauna in the Prut River (lower course) from the mouth of the river Larga (km 120) to the confluence with the Danube.

In the quantitative and qualitative description of the ichthyofauna on this segment of the Prut River were also used the results of the scientific research carried out by the Research Station in the Field of Fish Culture, the Department of Zoology of the Pedological Institute from Tiraspol, Research-Development Institute for Aquatic Ecology, Fisheries and Aquaculture Galati, as well as ichthyologic observations made by the State Inspectorate for the Protection and Reproduction of Fishery Resources and Fisheries Regulation in the Republic of Moldova.

Results obtained and discussions

Scientific research carried out (since the 50th century and until now) on the fish fauna of the lower course of the Prut River has found that until the regularization of the flow of the Prut River (Costești - Stânca dam, 1978) the ichthyofauna of the Prut River basin, after some authors, it consisted of 98 species and subspecies of fish, assigned to 23 families [3, 4, 6, 18]. After the regularization of the flow as well as the development of massive amelioration measures (drying of the puddles in the lower course - about 27-30 thousand ha and damming of the banks) essential changes have taken place in the specific diversity of the ichthyofauna. Valuable species have disappeared such as: - starlet, basratd strungeon, burbot, eel.

The population of some semi-migratory species and potamodromes have significantly decreased (ide, carp, vimba bream, sichel). Simultaneously with the introduction of valuable fish species, from the Amur River, new species have completed the ichthyofauna – silver carp, bighead, mackerel, etc. Therefore, at the end of the 70s (last century) the fish fauna of the lower course of the Prut River consisted of 54 species and subspecies of fish assigned to 19 families (aa. 1960-1974 - 16 families, a. 2017 -15 families).

The next period (from the mid-1980s to the present) is characterized by intensified negative anthropogenic influence, regularization of tributary flows, pollution, damming of many parts of banks against floods, excavation and extraction of sand, gravel and river stone, irreversible use of water for irrigation , industries and other activities related to the aquatic ecosystems.

These productive activities have caused substantial changes in the specific diversity of fish fauna and have led to a change in the ecological status of many fish species as well as to the degradation of the populations of economically valuable and endangered species.

From the composition of the ichthyofauna, burbot, Danube streber, barbel, huchen, crucian crap have practically disappeared. The population of starlet, ide, eastern mudminnow, etc. is on the verge of extinction. They passed in the category of rare species - mackerel of Danube, sichel, tench, barbel, spined loach [3, 4]. The populations of economically valuable species (previously numerous) have been considerably reduced – asp, pike-perch, carp, catfish, freshwater bream, vimba bream, pike. On the other hand, some species have adapted to the new environmental conditions, expanded their range, and their populations are progressing. The mentioned ones refer first to the species without economic value (Stone moroko, gudgeon, bleak, rudd, european perch, silver crucian carp (forms with low growth rate).

In connection with the border regime, industrial / commercial fishing in the Prut River was not practiced, this being the reason why no obvious statistics were taken. Industrial / commercial fishing was practiced only in the puddles located downstream of Ţiganca. In the period immediately after the Second World War, starting with 1946, industrial / commercial fishing was organized and, annually, until 1967, 495 tons were fished.

Until 1959, carp predominated in catches - 150 tons (30% of catches), followed by pike 90 tons (18% of catches), catfish - 16 tons and freshwater bream- 7 tons [7].

The drying of over 27000 ha of ponds during the years 1950 - 1960, essentially modified the aquatic ecosystems in this area, and from the famous puddles only the puddles from the village of Manta (2200 ha) and Lake Beleu from the area of Slobozia Mare (900 ha) remained.

With the drying of the ponds, important areas for the reproduction of many valuable species, the development of juveniles fish, the growth and fattening of fishing species (carp, freshwater bream, pike-perch, pike, tench, catfish, etc.) were liquidated.

Catches of industrial / commercial fishing, during the years 1968-1970, decreased to 227 tons, and towards the end of the 70s of the last century up to 100 tons dominant in catches becoming the silver crucian carp - 28 tons and bream -20 tons [7] (Tab. 1).

Tab. 1 The dynamics of industrial fishing in Manta puddle and Lake Beleu in the Prut River basin (tons).

Years	Carp	Freshwater Bream	Pike-Perch	Asp	Catfish	Crucian Carp	Pike	Tench	Rudd	European Perch	Other species *	Total
1	2	3	4	5	6	7	8	9	10	11	12	13
1946	178,4	24,1	32,4	0	41,0	0	27,0	0	35,0	16,0	141,9	495,8
1947	21,9	4,8	1,1	-	6,1	-	13,7	-	-	-	84,7	132,3
1948	211,4	5,1	5,7	-	2,4	-	2,4	-	-	-	530,0	757,0
1949	349,7	23,8	4,6	-	19,7	-	107,9	-	-	-	352,1	857,8
1950	374,2	27,6	1,7	6,0	11,6	-	89,4	-	-	-	266,8	777,3
1951	102,3	14,7	-	1,2	6,1	-	43,1	-	-	-	237,4	404,8
1952	96,1	10,1	0,1	1,2	5,3	6,4	60,0	3,6	21,3	10,7	206,2	421,0
1953	120,6	12,8	0,4	-	6,6	2,8	43,9	2,0	23,8	18,5	176,1	407,5
1954	74,0	11,9	-	-	4,2	8,3	41,6	5,9	8,5	2,3	126,4	283,1
1955	112,1	6,5	-	0,2	20,6	-	98,7	5,6	5,0	13,0	143,6	405,3
1956	128,6	22,5	0,7	1,0	36,9	32,8	355,0	38,3	51,4	48,6	207,5	923,3
1957	159,0	5,6	0,3	0,5	28,0	8,7	162,4	13,6	10,3	9,6	236,0	634,0
1958	77,0	4,9	-	-	18,2	10,0	94,4	25,0	17,2	7,0	191,4	445,1
1959	80,0	3,6	-	-	18,8	9,2	103,9	20,0	15,0	5,0	187,8	443,3

1960	22,7	0,4	-	-	16,8	-	101,2	20,0	16,0	5,0	227,5	409,6
1961	12,4	1,4	-	0,5	10,7	8,4	67,7	40,2	-	13,4	232,0	386,7
1962	17,3	2,1	-	-	3,1	106,0	63,6	23,7	19,9	34,4	126,8	396,9
1963	11,0	1,7	-	-	7,1	91,5	117,8	-	31,6	46,2	171,5	478,4
1964	13,9	1,6	-	-	3,9	45,6	44,7	-	9,1	23,3	102,4	244,5
1965	4,8	4,5	-	0,9	16,0	1,0	71,9	-	15,6	29,9	188,2	332,8
1966	6,4	31,4	1,5	0,3	39,5	43,8	68,5	-	10,8	25,0	187,6	414,8
1967	15,4	9,0	0,7	0,2	18,2	78,4	51,3	0,4	10,0	27,9	199,5	411,0
1968	1,4	4,4	0,2	-	8,2	3,7	35,6	0,1	11,0	4,8	157,7	227,1
1969	2,0	4,8	-	-	11,0	34,5	43,6	6,7	82,7	19,0	67,7	272,0
1970	6,1	3,6	0,1	-	8,1	4,4	44,8	11,2	43,7	4,4	96,8	223,2
1971	4,0	3,5	1,4	-	6,2	18,4	22,4	5,4	33,3	13,2	45,9	153,7
1972	5,1	3,8	3,2	-	12,0	22,6	13,7	1,2	20,4	5,2	90,6	177,8
1973	5,0	14,0	6,0	-	-	13,0	8,0	-	18,0	6,0	42,0	112,0
1974	3,0	17,7	4,4	-	19,1	52,4	12,9	-	-	-	58,7	168,2
1975	18,8	34,7	9,3	-	14,6	43,7	6,8	-	10,1	15,1	35,1	188,2
1976	38,0	11,9	22,5	-	3,3	24,2	3,7	-	24,2	14,0	13,2	155,0
1977	20,6	2,4	3,4	-	9,4	9,5	-	-	30,2	30,0	19,1	124,6
1978	25,4	5,3	1,9	-	11,6	45,3	-	-	-	-	22,8	112,3
1979	24,0	17,9	2,8	-	7,0	41,1	1,8	-	17,0	21,0	7,4	140,0
1980	13,6	17,3	5,7	3,2	9,0	35,9	3,2	-	5,9	6,0	2,2	102,0
1981	7,8	30,1	6,8	1,4	5,6	30,8	2,2	-	1,5	11,0	13,0	110,2
1982	12,4	29,9	7,0	0,6	3,3	39,9	-	-	1,5	-	5,0	99,6
1983	12,5	48,6	6,9	-	2,5	24,8	-	-	-	-	3,6	98,9
1984	9,4	48,8	7,7	-	4,8	29,2	-	-	-	-	8,4	108,3
1985	32,0	28,8	2,1	1,7	1,9	38,4	1,0	-	2,0	2,2	9,4	119,5
1986	5,0	11,0	1,2	1,3	-	19,0	0,8	-	-	3,7	3,1	45,1
1987	12,8	5,3	0,1	1,3	0,2	10,6	1,0	-	-	-	5,0	36,3
1988	26,7	10,9	4,6	-	3,1	11,3	-	-	-	-	22,1	78,7
1989	29,2	12,8	1,3	-	2,2	14,4	-	-	-	-	24,9	84,8
1990	10,3	8,6	0,5	-	0,1	5,0	-	-	-	-	7,6	32,1
1991**	-	-	-	-	-	-	-	-	-	-	-	-
1992	2,2	4,8	-	-	-	5,0	-	-	-	-	12,2	24,2
1993	0,7	1,2	1,0	-	-	10,1	-	-	-	-	17,0	30,0
1994	-	0,8	-	-	-	0,2	-	-	-	-	2,4	3,4
1995	-	-	-	-	-	1,1	-	-	-	-	1,7	2,8
1996	0,2	1,1	-	-	-	-	-	-	-	-	-	1,3
1997	-	-	-	-	-	-	-	-	-	-	-	-
1998	-	-	-	-	-	-	-	-	-	-	-	-
1999	-	-	-	-	-	-	-	-	-	-	-	-
2000	-	-	-	-	-	-	-	-	-	-	0,6	0,6

2001	-	-	-	-	-	-	-	-	-	-	0,2	0,2
2002	-	-	-	-	-	0,14	-	-	-	-	0,05	0,19
2003	0,26	0,15	0,1	-	-	1,0	-	-	0,11	0,1	0,18	1,9
2004	-	-	-	-	-	-	-	-	-	-	-	-
2005	0,4	0,2	0,2	-	-	0,4	0,2	-	-	-	-	1,4

* Other species included: white bream, white-eye-bream, roach, ruffe, common nase, fitofagus, round goby, bleak, sichel .
**In connection with the inclusion of Lake Beleu in the component of the nature reserve "Prutul de Jos", since 1991, industrial fishing took place only in the Manta puddles where, since 2006, it is no longer practiced.

Control and scientific fisheries conducted during the years 1981-1991 as well as the analysis of catches from industrial / commercial fishing found that ichthyofauna was represented by 36 species of which 12 species from the fishing group, but only 9 species of all were of economic importance: roach, asp, freshwater bream, vimba bream, white-eye-bream, silver crucian carp, carp, catfish, pike-perch[11, 22] (Tab. 2, 3).

In the catches, the following species were completely missing: starlet, ide, barbel, sichel, white bream etc., which in previous years were found very common. Of the rare species, in June 1991, one eel (length 1.05 m, weight 1.2 kg) was caught in the Manta puddles and another eel of 58 cm in length was fished in May 2003, as well as starlet (from fishermen's reports have not been reported in this area since 1984).

Tab. 2 The qualitative and quantitative composition of the fish fauna from the Prut river in the area of Văleni village a. 1981

Fish species	***No. of fish examined***	**Species ratio %**		**Body length, *cm***			**Ichthyomas, *g***		
			Values			***Values***			
		abundance	**Ichthyomas**	***average***	***min***	***max***	***average***	***min***	***max***
Freshwater bream	*38*	*33,33*	*14,0*	*19,5*	*12,0*	*35,0*	*205*	*45*	*650*
European Perch	*16*	*14,07*	*6,0*	*17,5*	*16,0*	*24,3*	*200*	*70*	*330*
Asp	*15*	*13,15*	*30,3*	*40,6*	*30,3*	*62,0*	*1220*	*500*	*3270*
Pike	*11*	*9,64*	*7,8*	*31,7*	*26,8*	*40,0*	*409*	*170*	*725*
Pike-perch	*10*	*8,77*	*8,3*	*32,1*	*27,9*	*34,8*	*471*	*290*	*595*
Catfish	*9*	*8,89*	*12,3*	*41,9*	*29,8*	*78,5*	*795*	*250*	*3470*
Carp	*6*	*5,26*	*11,0*	*36,0*	*24,0*	*51,0*	*450*	*300*	*600*
Silver crucian carp	*7*	*6,14*	*4,6*	*2,5*	*21,3*	*28,0*	*368*	*325*	*440*
Barbel	*2*	*1,75*	*5,5*	*50,0*	*46*	*54,0*	*1510*	*1120*	*1900*
In total	***114***								

Tab. 3 The qualitative and quantitative composition of the fish fauna from the Prut river on the segment of Brînza village - Câşliţa village in the aa period. 1990-1991

Fish species	*No.* *of fish examined*	Species ratio %		Body length, *cm*			Ichthyomas, *g*		
			Values			*Values*			
		abundance	Ichthyomas	*average*	*mini*	*max*	*average*	*min*	*max*
Eel	*1*	-	-	-	-	-	-	-	-
Roach	*78*	*12,5*	*5,2*	*15,7*	*13,7*	*18,8*	*165,6*	*120*	*190,5*
Asp	*54*	*8,6*	*5,7*	*31,7*	*22,2*	*58,1*	*260,1*	*205*	*305,5*
Freshwater bream	*85*	*13,5*	*8,8*	*31,0*	*16,4*	*35,5*	*256,8*	*205*	*305,5*
White-eye-bream	*66*	*10,6*	*3,0*	*16,6*	*15,2*	*24,2*	*12,7*	*100*	*210,0*
Vimba bream	*4*	*0,6*	*1,4*	*16,2*	*10,5*	*24,2*	*103,2*	*34*	*150*
Silver crucian carp	*202*	*32,5*	*25,2*	*14,1*	*8,2*	*25,8*	*197,4*	*20*	*365*
Carp	*100*	*16,0*	*43,2*	*33,6*	*8,4*	*41,3*	*1060,1*	*850*	*2332,0*
Catfish	*18*	*2,9*	*3,9*	*36,9*	*25,2*	*62,5*	*525,0*	*275*	*3350,0*
Pike-perch	*17*	*2,8*	*3,5*	*33,4*	*25,5*	*40,2*	*499,3*	*280*	*7905*
In total	***624***	***100***							

Annually, in the pre-reproductive period, there are massive migrations of carp, freshwater bream, roach, crucian carp, asp, catfish and other species from the Danube River to the breeding sites. From May to the beginning of July, the juveniles and the young fish are taken by the current of the river water downstream to fl. Danube. In years 1990-1991 according to the numerical abundance, roach, freswater bream, crucian carp and carp predominated and after ichthyomas (specific weight): carp, catfish, crucian carp, freshwater bream, asp. The significant decrease in ichthyofauna was due to low water levels, intensive pollution, lack of natural floods and showers, low water levels in the Danube River, which disturbed the natural reproduction, development and growth of fish in various ontogenetic stages.

Another negative factor that contributed to the reduction of fishery resources on the lower course of the Prut, Manta puddles and in Lake Beleu was the regularization of water flows by building the Costeşti-Stânca dam (1979), changing the hydrological regime of the river.

The current state of the fishery resources in the Prut puddles entirely depends on the degree of water supply and the penetration of fish from the Danube-Prut river system. As a result of systematic ichthyological research and observations, it was found that the intensive entry of fish for reproduction and fattening in the Manta puddle and Beleu lake occurred during the periods with the average annual flow of the Prut River of 80m3/s and the increase of water level in river Danube.

With the regularization of the Prut flow, the average annual water flow is 50m3 / s with variations from 31 m3 / s to 77m3 / s. In such conditions, fish no longer enter the Prut puddles, and industrial fishing, from the years 90 (last century), are no longer practiced and have lost their importance for reproduction where the areas of cattle breeders were about 750-900 ha in Manta puddle and 300-500 ha in Lake Beleu [16].

A negative impact on the aquatic biological resources in the Prut River basin was also caused by various economic activities: extraction of sand, gravel and river stone (only the Republic of Moldova, since the sixth decade, last century, annually excavated from the minor riverbed 360 - 380 thousand m3 of sand and gravel); irreversible use of water for various purposes (on the left bank there were 35 pumping stations - most were operated without fish protection facilities); wastewater pollution; irrational exploitation of fishery resources (lack of a common agreement with Romania on joint research on the state of fishery resources, regulation and record of fishing, improvement of fisheries measures, prevention of illegal fishing) [7].

Significant changes in the water level and flow regime occurred, especially in the lower course after the regularization of the Prut River flows and the commissioning of the Costești-Stânca reservoir. Over the years, with more water, during the period of increasing level, to a greater or lesser extent, the puddles and lakes in the Prut meadow are connected to the riverbed by ravines and canals which are often barred (clogged) and cannot ensure the normal water supply of lakes with average depths of 1-2m. In some years, for example in 1990, Lake Beleu has dried up completely and the entire surface was covered with macro-vegetation, losing its fishing importance. During the great floods of spring and summer and the flooding of the lakes, as it was in 1991, due to the abundant atmospheric deposits, favorable conditions were created for the reproduction of the fish and the fattening of the juveniles fish. Downstream of the town. Leova riverbed, until 1967, was bordered by lakes and puddles. After their drying (approximately 27000 - 30000 ha) only the Manta puddle (2100 ha) and the Beleu lake (900 ha) were preserved, which are fed with water, predominantly from the Prut River, and a large part of the lake surfaces they are covered with macrophytes constituting the main places for the natural reproduction of many species of fish, the development and fattening of the juveniles fish.

The influence of water flow on the effectiveness of natural reproduction is demonstrated by the years 1989-1990, that are completely different due to the water flow in the spring-summer period. In 1990, only 10 species of fish were identified compared to 1989 (19 species), and the share of species with economic value decreased 3 times from 8.7 million. (a. 1989) up to 2.9 milion. (year 1990), (Tab. 4). It should be mentioned that in all ichthyological samples it was found the presence of species that live permanently in small waters (coastal areas, bays) where the water heats up faster, rich in feed organisms and has a low flow. In these areas, the following species predominate: - bleak, gudgeon, bitterling, round goby, stone moroko, whose share is - 81.4%.

Tab. 4 The diversity and abundance of juvenile fish during the passive migration in the lower course of the Prut river (the segment of the village Brânza - the village Câşliţa), (thousands of individuals

Fish species	Years							
	1989	1990	1991	2000	2002	2005	2009	2012
Fam. Clupeidae								
Mackerel of Danube	–	24,0	-	–	–	–	-	-
Black sea shad	–	–	-	155,6	45,1	158,6	106,0	14,7
Fam. Cyprinidae								
Roach	1272,6	288,3	2716,0	757,6	45,7	1110,2	1082,0	175,1
Chub	205,2	–	-	9829,1	248,0	1823,8	1035,5	24,0
Asp	246,0	–	-	20165,6	78,9	634,4	752,0	35,0
Bleak	1252,1	841,0	7663,7	1509,2	1590,5	2379,0	2933,0	220,2
White bream	184,7	–	2182,7	323,4	320,1	555,1	526,0	51,3
Freshwater bream	492,6	48,8	3880,5	982,9	2797,6	475,8	403,0	77,6
White-eye-bream	–	–	-	196,6	327,1	396,4	342,0	61,7
Vimba bream	143,7	–	-	196,6	–	79,3	323,5	28,9
Carp	718,4	–	1067,1	1249,2	1545,4	1030,8	653,0	54,5
Silver Crucian Carp	533,7	96,1	2619,2	3360,9	879,8	951,6	858,5	88,4
Barbel	164,2	–	-	–	248,1	79,3	61,5	-
Common nase	–	–	-	2029,2	45,5	237,9	221,5	-
Stone moroko	–	48,1	242,5	196,6	248,1	79,3	53,5	8,2
Gudgeon	985,3	192,2	970,0	1572,0	169,2	1506,7	1288	144
Ide	–	–	-	66,2	–	–	-	-
Tench	–	–	-	63,8	–	–	-	-
Rudd	821,0	–	145,5	–	124,0	–	76,0	5,7
Belica	410,5	–	194,0	–	–	–	-	-
Bitterling	20,5	1129,3	970,0	–	–	–	-	-
Fam. Percidae								
Pike-perch	102,6	–	533,3	11287,7	710,6	1665,3	1327,5	97,7
European perch	–	–	97,0	589,7	124,2	634,4	522,0	82,6
Ruffe	82,1	–	-	–	–	1903,2	1366,5	175,8
Fam. Siluridae								
Catfish	41,0	–	145,5	1179,5	169,2	475,8	426,0	49,6
Fam. Odontobutidae								
Round goby	780,0	216,2	485,0	5834,1	1083,0	3568,5	2515,5	329,7
Fam. Cobitidae								
Burbot	287,4	72,1	242,5	60,2	925,0	555,1	490,0	51,2
Fam. Centrarchidae								
Danube streber	–	–	-	63,1	–	–	-	-
Sun Perch	-	-	48,5	-	-	-	-	-
Total	8743,2	2956,1	23330,7	61668,8	11725,1	20299,6	17362,5	1775,9

Note : years 1989, 1990, 1991 - Scientific Research Station in the field of Fisheries, years: 2000, 2002 – Service of Fisheries, years: 2005, 2009, 2012 - Institute of Zoology

The onset of mass brood migration usually occurs in the second decade of May. The migration of juvenile fish, with some decreases, in significant quantities continues until the third decade of July, which is probably related to the gradual exit of juvenile fish, as they grow, from Manta and Beleu lakes.

In 1990, the Manta puddles were supplied with water at the minimum level and their area decreased by 420 ha. In 1991, after long rains, the water level in the Danube River and in the Costești-Stânca accumulation lake and starting with April 30, the water level in the Prut River rose suddenly. Lake Beleu, which dried up completely in 1990, at the end of May was filled with water and the migration of fish to the breeding sites was found. But the unstable hydrological regime and the low water level in the Manta puddles and Beleu lake require the fattening of the migratory juvenile fish only in the Danube River [11, 13].

Therefore, it can be concluded that the lower course of the Prut River is generally used for breeding, and its fishery resources are directly dependent on the water flows during the breeding period and the state of the fishery resources on the lower course of the Danube River.

Research conducted in 2014 on the Chircani – Cahul segment showed that among the economically valuable species, resulting from the migration of juvenile fish, predominated the populations of freshwater bream (73.3 thousand ex.) and pike-perch (68.1 thousand ex.) followed by the populations of carp (20.9 thousand ex.) and catfish (10.5 thousand ex.) (Tab. 5). Among the species with lower economic value, the populations of roach (120.4 thousand ex.) and silver crucian carp (78.5 thousand ex.) had the highest productivity, followed by those of white bream (60.3 thousand ex.) and perch (26.2 thousand ex.). The highest productivity had the economically inalienable species: bleak (268.6 thousand ex.), round-goby (387.0 thousand ex.), ruffe (206.8 thousand ex.) and gudgeon (163.8 thousand ex.).

Tab. 5 Abundance of sapling in the Prut River on the section between Chircani village - Câșlița village during the migration period of 2014 (thousands of individuals)

Fish species	segment S. Chircani - or. Cahul		segment s. Brînza - s. Câșlița	
	Abundance	**Percentage ratio%**	*Abundance*	**Percentage ratio%**
Black sea shad	17,3	1,1	16,6	0,9
Roach	120,4	7,7	99,1	5,3
Chub	-	-	16,5	0,9
Asp	-	-	35,6	1,9
Freshwater bream	73,3	4,7	71,6	3,9
White-eye-bream	-	-	60,6	3,3
Silver crucian carp	78,5	5,0	82,6	4,4
Vimba bream	-	-	5,5	0,3
Rudd	6,0	0,4	5,7	0,3
White bream	60,3	3,8	57,8	3,1

Bleak	258,6	16,7	247,5	13,5
Carp	20,9	1,3	88,1	4,7
Gudgeon	163,8	10,4	157,0	8,5
Stone maroko	8,6	0,5	8,2	0,4
SCatfish	10,5	0,7	49,6	2,7
Freshwater bream	26,2	1,7	82,6	4,4
Pike-perch	68,1	4,3	143,2	7,7
Ruffe	206,8	13,2	198,2	10,7
Burbot	60,3	3,8	57,8	3,1
Round goby	387,8	24,7	371,7	20,0
Total	**1567,4**		**1855,5**	

In the lower course of the Prut River small species with low economic value predominate (55.5%) and are represented by silver crucian carp - 15.3% with 7 age groups in which 2 years old groups predominate (24.5%), as well as 3 years (27.5%) and 4 years (29.5%).

Compared to the period 2010-2015, the silver crucian carp populations decreased by 19.4% when they had a share of 34.7%. This is explained by the disturbance of natural reproduction (low water levels in recent years during the breeding period, so that the bushes in Manta and Lake Beleu puddles were not covered with water on their entire surface, the small number of carp breeders and freshwater bream, etc.).

The crucian carp is followed by european perch 15.3% (during the years 2008-2014 - 5.0%). The European Perch population is represented by 6 dominant age groups, the groups of 2 years (14.6%), 3 years (34.8%) and 4 years (33.8%). White-eye-bream with flattened muzzle constitutes 11.2% (during the years 2008-2014 - 23.3%) and is represented by 6 dominant age groups being the groups of 2 years (11.1%), 3 years (34.8%) and 4 years (33.8%).

Large species constitute 36.3%, being represented by freshwater bream 11.9% (2.3 times higher than in the year 2008-2014) and by 6 dominant age groups being the 2-year groups (33.3%), 3 years (22.7%) and 4 years (16.7%). Carp follows- 8.5% represented by 7 dominant age groups being the groups of 2 years (16%), 3 years (30.5%) and 5 years (19.6%).

The pike-perch constitutes 6.8%, but the population is found in small numbers. A slight increase was reported to catfish (3.0%). Species without economic value constitute 8.2% [7].

Conclusions

The ichthyofauna of the lower course of the Prut River is mostly influenced by anthropogenic actions (drying of ponds - about 30 thousand ha only on the territory of the Republic of Moldova, construction of Costesti-Stânca dam, tributary regularization, sand excavation, pebbles and river stone). from the minor riverbed, pollution with untreated wastewater and domestic water, etc.) is in continuous quantitative and qualitative decline, especially of species with economic value (starlet , basratd strungeon, burbot, ide , eel).

Many species currently introduced in the Red Book (3rd ed.) Of the Republic of Moldova have become extinct or are on the verge of extinction.

There are no, on both sides, systematic studies on the state of fishery resources, structural-functional changes of fish populations in current and future ecological conditions.

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SECTION 2. INVERTEBRATES

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NEW CONTRIBUTIONS TO THE KNOWLEDGE OF HONEY BEE (APIS MELLIFERA) PESTS

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Abstract. The paper aimed to identify the pest complex facing Apis mellifera, in autumn and winter, based on an apiary in Tomai, Leova district. In the presence of pests, 60 bee families were checked during the sanitation and feeding work of the bees, between April and May. A total of 3 species of pests have been identified, that cause damage to bees and honeycombs. Each identified species also contains information on the origin of the species, diet, range, some biological features, methods of detection and protection against these pests. Strict monitoring of pests can help to avoid negative effects on apiaries without losing bee families and their products.

Introduction

Bees are among the most important pollinating insects of plants cultivated in the world They pollinate over 150 species of cultivated entomophilous plants. Therefore, the plants that are the essential source of life support depend on pollinators. The amount of fruit and seeds obtained per hectare depends directly on the degree of pollination of flowers by insects, including bees [4]. Bees contribute annually to the production of agricultural crops of sunflower, rapeseed, mustard, vegetables, fruits and berries by approx. 9%, which represents about 153 billion euros in the global economy [1 Bees also offer many bee products such as honey, pollen, propolis, royal jelly, venom and wax [2]. Due to climate change and globalization, bee diseases and pests have become more widespread. The disappearance of bees from nature would have serious consequences for the environment, biodiversity, economy and human life. In addition to pests, bees are also directly affected by environmental factors (cyclonic rains, temperature fluctuations, strong winds).Given the enormous role that bees play in human life, it is absolutely necessary to know and combat their pests. In both the warm and cold periods of the year, bees are affected by various pests.The aim of the research was to highlight the pest complex of honey bees in autumn and winter period.

Materials and methods

The faunistic material discussed in the paper was collected from the apiary in Tomai, Leova district, in April and May of 2021. The apiary was placed in the household of the beekeeper from the above-mentioned locality. The available literature in this regard was used to identify the species [5]. For the analyzed species, they were also described some aspects from the specialized literature [5; 6; 7].

Results and discussions

During the spring work, along with the cleaning of the hives, some bee pests were also collected. The honey bee pests (*Apis mellifera*) in the researched apiary are represented by 3 species of pests, which belong to 2 classes (Insecta and Arachnida), 3 orders (Lepidoptera, Hymenoptera and Parasitiformes) and 3 families (Varroidae, Sphingidae and Vespidae). Bee pests can be classified depending on the attack they produce such as parasites (arachnida), predators (wasps) and wax pests (butterflies). In order to facilitate the analysis of pests, their presentation was chosen according to the classes they belong. The following is a list of pests identified in the apiary investigated in the cold season of the year:

Class Arachnida

Order Parasitiformes

Family Varroidae

Varroa destroyer Anderson & Trueman, 2000. Originally from Southeast Asia (Java, Indonesia), it was first described in 1904 by A. C. Oudemans, as V. jacobsoni. In the Republic of Moldova, Varroa destructor has been present since 1964 in all bee families from all apiaries (previously identified as V. jacobsoni) [7]. Adults feed on the hemolymph of adult bees and larvae. Females live throughout the year, males between 15-20 days. From a single female in the hive, a population grows to 30 thousand individuals [5]. The parasite has a strong economic impact on the beekeeping industry.

There are numerous methods of collecting the parasite, the most harmless methods were used without destroying the bees: the powdered sugar method and the sticky surfaces method. Interpretation of results. If there are 10 varroa parasites per 100 bees analyzed, the infection is considered weak, up to 20 parasites - medium, and more than 20 parasites denote a strong infection. In the absence of immediate treatment, the entire bee colony can be destroyed [7]. Following the study of the apiary, more than 10 mites per 100 bees were found. The main methods of treating varroatosis are: chemical treatment, zootechnical measures, heat treatment and their combinations. In order to prevent the spread of the parasite, epizootic monitoring of mite invasion and preventive treatment of bee colonies are constantly performed.

Class Insect

Order Lepidoptera

Family Sphingidae

Acherontia atropos Linnaeus, 1758 (African death's-head hawkmoth). Originally from North Africa, now widespread in Europe, the European part of Russia, the Middle East, Africa. It is a migratory species. In years with mild climates, some specimens can survive and multiply. It is a polyphagous butterfly, the adults feed on the sap of the trees and the juice of the ripe fruits, but they also enter the hives of the bees and consume 5-10 grams of honey, until they are detected by the bees and killed. Death's-head butterfly is a large butterfly 50 mm long, with a pair of brown wings, and the other pair - yellow with two black stripes. The basic feature is the presence of the skull shell design on the thorax. The male is smaller than the female. After mating, females lay eggs on the leaves of different plant species. It can be found in various agricultural crops (potato - Solanum tuberosum), as well as in shrub plantations (oleander - Nerium oleander, raspberry - Rubus idaeus, jasmine - Jasminum officinale, euonymus - Euonymus europaeus, etc.). There are two generations per year (May-July; September-October) [5]. Only one specimen was found. It is a rare species. Does not cause damage to apiaries.

Order Hymenoptera

Family Vespidae

Vespula germanica (Fabricius, 1793), originating from Europe, North Africa and temperate Asia. They are polyphagous predators that feed on various arthropods, fruits, honey, meat with body dimensions reaching 12-15 mm in length and are black and yellow. They build their nests from vegetation chewed with saliva, most often in the soil, sometimes on the surface of the soil, and other times in attics. Prefers forested areas. In the spring, the queen lays the eggs, from which appear working individuals. At the end of summer, appear males and females which will become queens. The latter are wintering. A colony can exceed 10,000 individuals, but their number depends on climatic conditions. 7 individuals were detected in the analyzed apiary. The specialized literature confirms the entry of the pest into the hives to steal honey [3].

The study was carried out within the project 20.80009.7007.12:

Conclusions

During the spring work, along with the cleaning of the hives, three species of bee pests were collected and identified (*Varroa destructor, Vespula germanica, Acherontia atropos*). According to observations, the biggest danger to the apiary is represented by Varroa destructor mites, which negatively affected the health of bees, which will influence the production of honey. This negatively affects not only the quality and quantity of bee products, but also involves serious problems in agriculture, because bees are the main pollinators of agricultural crops. Our observations can be used by beekeepers in the fight against pests in apiaries.

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NEW RECORD OF COLLEMBOLA (HEXAPODA) FROM THE PRUTUL DE JOS SCIENTIFIC RESERVE

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Abstract. The paper presents new data about the species diversity of the order Collembola from the Prutul de Jos Scientific Reserve. A total of 13 species of Collembola from 11 genera and 5 families were identified for the first time in the floodplain forest of the Prutul de Jos Scientific Reserve. Among the identified collembolan species one - Endonura lusatica Dunger, 1966 is new for the fauna of the Republic of Moldova.

Introduction

Prutul de Jos Scientific Reserve was created on April 23, 1991 in order to protect the flora and fauna of Lake Beleu and the floodplains around it. The protected area is located in the lower reaches of the Prut River in the south west of the Republic of Moldova. It includes Lake Beleu and its surroundings, a network of ponds that, as a whole, form a unique ecosystem. The surface of the reserve is 1691 ha, about ⅓ of its surface is occupied by the waters of lake, the rest of the territory is presented by marshy vegetation and floodplain [5]. On 2018, the reserve was registered in the UNESCO World Network of Biosphere Reserves, becoming the first biosphere reserve established in the Republic of Moldova.

Material and methods

The samples were collected in the spring 2021 in a floodplain forest located on the shores of Lake Beleu formed 27-30 years ago (fig. 1), in which white willow (Salix alba) predominates [5]. The willow forest is floodable, in which water enters during floods rising up to 1 m. Many trees have grown too tall, others have dried up and break very easily from strong winds.

Figure 1. Floodplain forest in the Prutul de Jos Scientific Reserve

The specimens of Collembola were extracted from decomposed wood using modified flotation method [1] then were treated with lactic acid, discolored in KOH and mounted in permanent slides.

The taxonomic identification of invertebrate species were performed using MBS-10, Meiji Techno binoculars, microscope Leica and determination keys [1, 6].

Results and discussion

As a result of investigation 13 species of Collembola from 11 genera and 5 families were identified for the first time in the wood decompose covered with mosses and lichens from the Prutul de Jos Scientific Reserve. Among the identified species one *Endonura lusatica* Dunger, 1966 from the family Neanuridae is new for the fauna of the Republic of Moldova.

Table 1. List of species identified in the Prutul de Jos Scientific Reserve

N.	Taxon	Data of collection	Nr. of ind.	Ecology
	Neanuridae			
1	**Endonura lusatica* Dunger, 1966	23.03.21	14	Silvicolous and hydrophilous species
	Hypogastruridae			
2	*Choreutinula inermis* (Tullberg, 1871)	23.03.21	1	Litter species
3	*Schoettella ununguiculata* (Tullberg, 1869)	23.03.21	44	Euribiont
4	*Xenylla brevicauda* Tullberg, 1869	23.03.21	7	Silvicolous, briophilous species
	Isotomidae			
5	*Isotoma riparia* (Nicolet, 1842)	23.03.21	6	Hygrophilous species
6	*Isotoma viridis* Bourlet, 1839	23.03.21	1	Euribiont
7	*Folsomia quadrioculata* (Tullberg, 1871)	23.03.21	12	Euribiont
8	*Proisotoma minuta* (Tullberg, 1871)	23.03.21	4	Ruderal and thermophilous species
	Entomobryidae			
9	*Entomobrya marginata* (Tullberg, 1871)	23.03.21	8	Euribiont
10	*Lepidocyrus paradoxus* Uzel, 1890	23.03.21	1	Mesophilous species with preference for wet habitat
11	*Lepidocyrtus violaceus* Lubbock, 1873	23.03.21	13	Silvicolous species with preference for wet habitat
12	*Willowsia nigromaculata* (Lubbock, 1873)	23.03.21	1	Corticicolous and silvicolous species
	Katiannidae			
13	*Sminthurus* sp.	23.03.21	1	

The analysis of the wood decompose covered by moss collected along the banks of Prut River using collembolan communities' structure emphasized several peculiarities of collembolan species diversity and distribution in riparian habitat. Thus, if we compare the list of species identified in the Prutul de Jos Scientific Reserve with other habitats located along the Prut River no euedaphic species were found [3].

The revealed species presented different types of the hygropreference, the largest part of them are mesophilous and silvicolous species (Tab. 1).

New revealed species *Endonura lusatica* Dunger, 1966 is the second species from genus *Endonura* identified in the Republic of Moldova. According to research conducted by Smolis it is one of the most hygrophilous species of the genus [6].

The riparian habitats remain rather attractive places for the soil invertebrates due to the carry-over and accumulation on their surface of the biogenic elements. Thereby, Collembola inhabited all the studied localities from the banks of Prut and Dniester Rivers and their species diversity is varying [1, 2, 3, 4]

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NEW RECORD OF LEUCORRHINIA PECTORALIS (CHARPENTIER, 1825) (INSECTA: LIBELLULIDAE) IN THE REPUBLIC OF MOLDOVA

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Abstract: The paper includes new record of rare and protected species of Odonata - Leucorrhinia pectoralis in the Republic of Moldova. The species was cited firstly in 2009 from the Cioburciu village. In June, 2021 one adult male was identified on the palustral vegetation in the Plaiul Fagului Reserve. This is the second record of this species in the Republic of Moldova.

Introduction

The first papers dedicated to the diversity of dragonfly species in the Republic of Moldova were published at the beginning of the 20th century [2, 3]. The dragonflies are relatively large, intensely colored insects and attract people's eyes. Several species of Odonata are protected by law both locally and internationally. One of such species is **Leucorrhinia pectoralis** (Charpentier, 1825), a dragonfly species from the family Libellulidae, extremely rare on the territory of the Republic of Moldova. Until now, the species has been cited from the southern region of the Republic of Moldova along the bank of Dniester River [5, 8, 9]. Some Odonata species identified in the Plaiul Fagului Reserve were cited previously [1], but does not include the newly identified species.

Material and methods

The Plaiul Fagului Reserve is situated in the North – West of the Central Moldavian plateau, 70 km away from Chisinau, at approximately 47°17'28"N 28°3'16"E in the Central Region of the Republic of Moldova. This reserve is an important part of the Moldavian protected areas with a total surface of 5,558.7 km2. Many rare species of plants, vertebrate and invertebrate animals are mentioned here, including some Carpathian elements. On the territory of the reserve there are several lakes with an area of 24 ha and 7 ha of swamps (fig.1) [7].

In summer of 2021, several field trips were made to collect entomological materials. In order to identify the species of dragonflies from the Plaiul Fagului Reserve, the specimens of Odonata were collected around the lakes and the photos of the large species were captured.

Results and discussions

As a result of investigation, new data about the species diversity of the order Odonata from the Plaiul Fagului Reserve were obtained.

Among the species identified in the Plaiul Fagului Reserve one species - *Leucorrhinia pectoralis* is the most interesting. The male of this species was photographed on 24 June 2021 on the palustral vegetation on the water of lake, at about 5 meters from the water edge (fig. 1b). For the first time on the territory of the Republic of Moldova Leucorrhinia pectoralis was found near the border of the forest and swampy meander, downstream from the Cioburciu village (Stefan-Vodă District) on 24 Mai 2009 [5] and then cited from Lower Dniester Region in ”Grădina Turcească” and Olănești-Crocmaz Valley without any data of collection [8, 9].

Figure 1. a – Lake in the Plaiul Fagului Reserve **b – male of Leucorrhinia pectoralis**

According to the Red Book of the Republic of Moldova Leucorrhinia pectoralis is a critically endangered species, which lives in the southern part of Moldova, especially in the Lower Dniester Region [4]. The species prefers mesotrophic freshwater basin, isolated ponds in the forest and swamps.

In the summer of 2014, after fifty years, *Leucorrhinia pectorali*s was rediscovered in Romania. Two males of this species were registered in the “Tinovul Mare Poiana Stampei”, Succeava country [6].In Ukraine the species is regularly observed in the Volynska, Zhytomyrska, Kharkivska, Dnipropetrovska, Kyivska and other regions [10].At international level the species *Leucorrhinia pectoralis* is protected and included in the Bern Convention (Appendix II), the Habitats Directive (Council Directive 92/43/EEC) (Annexes II and IV), the IUCN European Red List of Dragonflies, the IUCN Red List of Mediterranean Dragonflies and in the Red List of Dragonflies of the Carpathians [6].

Conclusions

In spite of investigation carried out in the Plaiul Fagului Reserve during long time, new insect species can be still identified. The presence of the rare and protected at European and local level species, such as Leucorrhinia pectoralis, indicate the importance and conservation value of a wide range of habitats that exist in the Plaiul Fagului Reserve, including the lakes.

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NEW RECORD OF INVERTEBRATES ASSOCIATED WITH DECOMPOSED WOOD FROM THE PLAIUL FAGULUI RESERVE

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Abstract. The paper presents new data about the diversity of invertebrates associated with decomposed wood from the Plaiul Fagului Reserve. A total of 60 invertebrate species belonging to 45 genera, 20 families and two classes Collembola and Insecta (Coleoptera and Hymenoptera) were identified. A rare species - Carabus intricatus and C. ullrichi included in the third edition of the Red Book were highlighted. Four new species of Coleoptera are recorded for the first time for the reserve.

Introduction

The Plaiul Fagului Reserve is situated in the North – West of the Central Moldavian plateau, 70 km away from Chisinau – the capital of the country, at approximately 47°17'28"N 28°3'16"E in the Central Region of the Republic of Moldova. This reserve is an important part of the Moldavian protected areas with a total surface of 5,558.7 km2. Many rare species of plants and vertebrate animals are mentioned here, including some Carpathian elements [14].

The invertebrate fauna of the Plaiul Fagului Reserve was studied and the data are published in several papers and books [3, 4, 6, 14]. Notwithstanding the studies presented above, a wide range of insects species have not been studied yet, and some large groups have not even been mentioned before this research. The diversity of invertebrates associated with decomposed wood from the reserve has not been studied separately.

The aim of this paper was to reveal the invertebrate species associated with dead woods and to increase the knowledge about insects involved in wood decomposition from the Plaiul Fagului Reserve.

Materials and methods

The vegetation of the reserve consists of several types of temperate mixed forests typical for the European region. The arboreal vegetation is dominated by the oak, lime, ash and hornbeam. Of the total forested area, 5% is occupied by beech forest, being the largest territory covered by monodominant beech in the whole country [14]. Several old fallen trees were kept on the territory of the reserve in order to protect rare invertebrate species the life cycle of which is linked to the decomposed wood (fig. 1).

The invertebrates were collected from the decaying wood from the Plaiul Fagului Reserve in October 2020 (sample I) and in March (sample II), April (sample III) and Mai (samples IV and V) 2021. The list of species and number of specimens are included in the Table 1.

Saproxylic beetles and ants were collected manually under the bark or from the dry trees fallen to the ground and from trunks in an advanced degree of decomposition. Supplementary soil and decomposed wood samples were taken for the extraction of small invertebrates (beetles, ants and collembolans).

Collembola, small beetles (Staphylinidae) and some ant specimens were collected from decomposed wood by exhauster.

The taxonomic identification of invertebrate species was performed using MBS-10, Meiji Techno binoculars and microscope Leica. The modern determination keys specific to each group were used [1, 5, 7, 10, 15].

Figure 1. Decomposing trees in Plaiul Fagului Reserve - host of invertebrates

Figure 2. Carabus ullrichii – species included in the Red Book

Results and discussions

As result of the investigation, new data about the invertebrates associated with decomposed wood from the Plaiul Fagului Reserve were obtained. A total of 60 invertebrate species from two classes (Collembola and Insecta) belonging to 45 genera and 20 families were recorded in the microhabitat formed by decomposed wood in the forest ecosystem (Table 1). For each species a brief information regarding the number of specimens, some data about the ecology is presented.

Four coleopteran species – *Litargus connexus, Bolitophagus reticulatus, Pyrochroa coccinea and Thanasimus formicarius* were identified for the first time in the reserve. Two identified species *Carabus intricatus* and *C. ullrichii* (fig. 2) are rare and included in Red Book of the Republic of Moldova [13].

Table 1. Invertebrates associated with decomposed wood from the Plaiul Fagului Reserve

Family	Genus	Species	Sample				
			I	II	III	IV	V
Odontellidae	*Superodontella*	*S. montemaceli* Arbea& Wiener, 1992	-	2	-	1	-
Neanuridae	*Thaumanura*	*T. carolii* (Stach, 1920)	-	-	7	21	-
Isotomidae	*Isotoma*	*I. viridis* Bourlet, 1839				1	-
Entomobryidae	Orchesella	*O. pseudobifasciata Stach, 1960*	-	-	-	1	-
		O. pannonica Stach, 1960	-	-	-	1	-
	Lepidocyrtus	*L. lignorum* (Fabricius, 1775)	-	-	-	1	-
		L. violaceus Lubbock, 1873				1	-

Tomoceridae	*Pogonognathellus*	*P. flavescens* (Tullberg, 1871)	-	3	-	5	-
	Tomocerus	*T. minor* (Lubbock, 1862)	-	-	-	1	-
		T. vulgaris (Tullberg, 1871)	-	2	-	4	-
Carabidae	*Abax*	*A. parallelepipedus* (Dejean, 1828)	-	-	-	-	1
	Amara	*A. aenea* (De Geer, 1774)	1	-	-	-	-
	Agonum	*A. assimile* (Paykull, 1790)	11	-		-	-
	Carabus	*C. cancellatus* Illiger, 1798	1	4	-	-	-
		C. convexus Fabricius,1775	-	-	1	-	-
		C. coriaceus Linnaeus, 1758	-	-	-	1	-
		C. granulatus Linnaeus, 1758	-	-	-	1	-
		C. *excellens* Fabricius, 1798	-	-	-	-	4
		C. intricatus Linaeus, 1761	6	3	-	-	-
		C. ullrihi Germar, 1824	-	-	-	1	-
	Panagaeus	*P. cruxmajor* (Linnaeus, 1758)	-	-	1	-	-
	Molops	*M. piceus* (Panzer, 1793)	-	-	-	1	2
	Nebria	*N. rufescens* (Stroem, 1768)	-	-	-	-	4
	Notiophilus	*N. laticollis* Chaudoir, 1850	-	-	-	-	1
	Platynus	*P. krynickii* (Sperk, 1835)	-	-	-	1	-
	Pterostichus	*P. oblongopunctatus* (Fabricius, 1787)	-	-	-	1	-
Coccinellidae	*Coccinella*	*C. septempunctata* (Linnaeus, 1758)	1	-	1	-	-
	Propylea	*P. quatuordecimpunctata* (Linnaeus, 1758)	1	-	-	-	-
	Harmonia	*H. axyridis* (Pallas, 1773)	1	-	-	-	-
Elateridae	*Ampedus*	*A.pomonae* Stephens,1830	2	-	-	-	-
		A.sanguineus (Linnaeus, 1758)	-	3	-	-	-
		A. sanguinolentus (Schrank, 1776)	-	-	-	-	1
	Megapenthes	*M. lugens* (Redtenbacher, 1842)	1	-	-	-	-
Geotrupidae	*Geotrupes*	*G. stercorosus* Hartmann in Scriba, 1791	-	-	-	-	1
		G. vernalis (Linnaeus, 1758)	-	-	-	1	2
Erotylidae	*Triplax*	*T. aenea* (Schaller, 1783)	-	2	-	-	-
Mycetophagidae	*Litargus*	**L. connexus* (Geoffroy, 1785)	-	2	-	-	-
Silvanidae	*Uleiota*	*U. planata* (Linnaeus, 1761)	5	2	-	2	3
Scarabaeidae	*Cetonia*	*C. aurata* (Linnaeus, 1758)	-	1	-	-	-

Staphylinidae	*Aleochara*	*A. curtula* (Goeze, 1777)	-	-	-	-	1
	Atrecus	*A. affinis* (Paykull, 1789)	-	2	-	-	-
	Scaphidium	*S. quadrimaculatum* Olivier, 1790	2	-	-	2	2
	Sepedophilus	*S. marshami* (Stephens, 1832)	1	2	-	-	-
	Paederus	*P. littoralis* Gravenhorst, 1802	1	-	1	-	-
	Ocypus	*O. nitens* (Schrank, 1781)	1	-	-	-	-
	Tachyporus	*T. hypnorum* (Fabricius, 1775)	-	1	-	-	-
Tenebrionidae	*Bolitophagus*	**B. reticulates* (Linnaeus, 1767)	1	-	-	-	-
	Scaphidema	*S. metallicum* (Fabricius, 1792	3	-	-	-	-
	Stenomax	*S. aeneus* (Scopoli, 1763)	-	-	-	2	-
	Uloma	*U. culinaris* (Linnaeus, 1758)	3	-	-	-	4
Zopheridae	*Bitoma*	*B. crenata* (Fabricius, 1775)	-	2	-	-	-
Pyrochroidae	*Pyrochroa*	**P. coccinea* (Linnaeus, 1761)	-	-	-	1	-
Lucanidae	*Dorcus*	*D. parallelipipedus* (Linnaeus, 1758)	-	-	-	-	1
	Lucanus	*L. cervus* (Linnaeus, 1758)	-	-	-	1	
Cleridae	*Thanasimus*	**T. formicarius* (Linnaeus, 1758)	-	-	-	-	1
Formicidae	*Aphaenogaster*	*A. subterranea* (Latreille, 1798)	-	-	39	-	-
	Lasius	*L. fuliginosus* (Latreille, 1798)	8	-	-	-	-
		L. emarginatus (Olivier, 1792)	-	-	36	7	-
		L. neglectus Van Loon, Boomsma & Andrasfalvy, 1990	-	39	-	-	-
		L. niger (Linnaeus, 1758)	-	-	49	10	-

* - new species for the reserve

The climatic conditions with high humidity during the winter - spring 2020-2021 created optimal conditions that favored the development of a wide range of invertebrates associated with decomposed wood. During material collection, the decomposed wood was soaked with water. While the wood was broken, a large number of invertebrates were observed. Only a few specimens of each species were collected so as not to damage natural decomposition processes.Collembolan species included in the present study are extracted only from wood in an advanced state of decomposition. A total of 10 collembolan species belonging to 7 genera and 5 families were revealed. All these species are considered silvicolous and saproxylophagous, active participants in wood decomposition processes. The increased preference of the species from the families Neanuridae and Odontellidae (genus *Superodontella*) to the secular trees oaks residues were observed, in which the unique microhabitats invaded by a rich microflora were formed. The species of two genera *Thaumanura and Superodontella* can be observed only in natural forest habitats, in which the dominant trees are oak. The species *Thaumanura carolii* is an indicator of natural deciduous forests of Central European type detected in litter and decomposed wood [4].

The order Coleoptera was represented by 14 families, 36 genera and 45 species. The most numerous families were Carabidae with 10 and Staphylinidae with 7 species. From the family Carabidae the genus *Carabus* was represented by 7 species, the other 3 genera were represented by a single species, while the family Staphylinidae was represented by 8 genera with only one species each. They were followed by the families Tenebrionidae with 4 and Coccinellidae with 3 genera and one species of each genus, Elateridae with 2 genera, of which the genus Ampedus was represented by 3 species, and *Megapenthes* by one species. Family Lucanidae was represented by 2 genera with one species each, while family Geotrupidae was represented by one genus and two species. Other 7 families were represented by one species each (Table 1). Two species *Carabus intricatus* and *Carabus ullrichii* are rare, protected by law and included in the 3rd edition of the Red Book [13].Recent research has highlighted several species of ants associated with decomposed wood. Between them the species *Aphaenogaster* subterranea is a widely distributed Mediterranean ant species, which mainly inhabits moderately wet and warm deciduous forests, nesting in the ground, under stones, in rotten wood, rarely in litter. They are active at night, but also extremely active during the day, exhibiting aggressive behavior towards common forest species [11].*Lasius fuliginosus* has a wide distribution in Europe and Asia, is a zoophagous-xylophagous species. Nests of this ant are most commonly found in the stems of old trees, especially oak, sometimes under rocks or even in open ground. The ant queen of L. fuliginosus can behave as a temporary social parasite, taking the place of the queen of other Lasius sp. developing in their own colony, by eliminating the hosts over a period of time [8]. *Lasius niger* is one of the commonest European species, which occurs in deciduous, coniferous and mixed forests, in gardens and in open meadows. In forests it tends to nest in the trunks of rotten trees. It has a varied diet: insects, nectar from aphids or seeds. The species is active both day and night [12].*Lasius emarginatus* is a very common species occurring in central and southern Europe, their nests most often is located under rocks in dry places, but also in dry wood. It avoid wet places [12]. The species *Aphaenogaster subterranea, Lasius fuliginosus, L. emarginatus and L. niger* were cited as being present in the Codri Tigheci in 1980 [9]. The ant *Lasius neglectus* is an invasive species, which appeared in Europe in the late of twentieth century. The species is native to Asia from where it expanded its range, affecting and substituting other species of ants. The nest can be placed under stones or construction waste and under the bark of trees and in the ground [2].In order to protect useful insect species is important to keep decomposed wood in natural forest ecosystems such as the Reserve Plaiul Fagului. Fallen or dead trees have great importance especially for protection of invertebrate fauna. Not all insects found in dead wood are xylophagous, some of them find shelter during the cold period of the year, most of them being zoophagous and contribute to the regulation of phytophagous species in the ecosystem.

Conclusions

Notwithstanding the long-term research carried out in the Plaiul Fagului Reserve, some new invertebrate species from classes Collembola and Insecta can be still identified and such high invertebrate abundance was observed for the first time. The climatic conditions with high humidity during the winter - spring 2020-2021 created optimal conditions that favoured the development of a wide range of invertebrates associated with decomposed wood. The maintenance of intact patches of natural oak-based forests, with enough quantity of decomposed wood covered by moss could improve forest biodiversity. Especially it is important the presence of carabides and ants that consume a large number of forest pests and contribute to the regulation of phytophagous species activity being active zoophages.The presence of the rare species as well as newly identified species, indicate the importance and conservation value of studied microhabitats in the Plaiul Fagului Reserve.The study was performed within the project 20.80009.7007.02.

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DONACIINAE (COLEOPTERA: CHRYSOMELIDAE) OF THE REPUBLIC OF MOLDOVA FAUNA

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Abstract. The Donaciinae of the Republic of Moldova (including Transnistria) fauna are reviewed based on collected material, museum collections and available literature data. In total, 14 species of Donaciinae are known from Republic of Moldova.

Introduction

The leaf beetles (Coleoptera, Chrysomelidae) is one of the largest beetle families and according to some bibliographical sources, from 35 to 50 thousand species are known in the world. Donaciinae is a subfamily of Chrysomelidae and sometimes are called longhorned leaf beetles because of the long antennas comparatively than other chrysomelids. Both the adult and larval stages feed on the same plants and occur in lotic and lentic habitats.

Materials and methods

The Donaciinae subfamily material presented in the paper includes collections (fig. 1) from the 1924-1939 years stored in the National Museum of Ethnography and Natural History [1] and the recent period (2004-2021) from the Institute of Zoology and available literature data [4-8]. Collecting was performed using standard methods: manual collecting and sweeping with a sweep net. The taxonomic identities of specimens were determined using the key [2].

Figure 1. Example of beetles from the National Museum of Ethnography and Natural History collection

Results and discussion

In the Republic of Moldova, the following species were identified:

Genus Donacia **Fabricius, 1775**

Donacia clavipes Fabricius, 1793

General distribution: Europe, Siberia

Distribution in R.Moldova: Chişinău [1], Chişinău [4], "Iagorlîc" Scientific Reserve [5, 6, 7]

Biological data: host plants are Phragmites Adans., Eleocharis R.Br.

Donacia cinerea **Herbst, 1784**

General distribution: Europe, Siberia.

Distribution in R.Moldova: Chişinău [4], "Iagorlîc" Scientific Reserve [5, 6, 7]

Biological data: host plants are Typha L., Carex.

Donacia dentata **Hoppe, 1795**

General distribution: Europe, Middle Asia.

Distribution in R.Moldova: Chişinău [1], Merenesti [7]

Biological data: host plants are Sagittaria sagittifolia and Alisma plantagoaquatica

Donacia brevicornis **Ahrens, 1810**

General distribution: Europe

Distribution in R.Moldova: Ploti [7], Badragii vechi, 14.07.2016

Biological data: host plants are Scirpus lacustris and Carex L.

Donacia simplex **Fabricius, 1775**

General distribution: Paleartic region

Distribution in R.Moldova: Goian (Transnistria) [1], Tighina (Bender) [4], "Iagorlîc" Scientific Reserve [7]

Biological data: host plants are Sparganium L., Glyceria L., Carex L.

Donacia polita **Kunze, 1818**

General distribution: Northern Africa to South and Eastern Europe.

Distribution in R.Moldova: Chişinău, [4]

Biological data: host plants are Sparganium L., Glyceria L.

Donacia antiqua **Kunze, 1818**

General distribution: Europe

Distribution in R.Moldova: Chişinău, [1]

Biological data: Carex L.

Donacia bicolora **Zschach, 1788**

General distribution: Europe.

Distribution in R.Moldova: Chişinău, [4]

Biological data: host plants are Sparganium L., Carex L.

Donacia marginata **Hoppe, 1795**

General distribution: Northern Africa, Europe, Middle Asia

Distribution in R.Moldova: Chişinău [1]

Biological data: host plants are Sparganium L. and Carex L.
Donacia semicuprea **Panzer, 1796**
General distribution: Northern and Middle Europe
Distribution in R.Moldova: Goian (Transnistria), Chişinău [1]
Biological data: host plants Glyceria aquatica L.
Donacia versicolorea **(Brahm, 1790)**
General distribution: Europe
Distribution in R.Moldova: ubiquitous [4]
Biological data: host plants are Typha L., Potamogeton L.
Genus Plateumaris **Thomson, 1859**
Plateumaris affinis (Kunze, 1818)
General distribution: Europe
Distribution in R.Moldova: r.Orhei, s.Vatici, 20.05.1978 (fig. 2a)
Biological data: host plants are Sparganium L., Carex L.
Plateumaris bracata **(Scopoli, 1772)**
General distribution: Spain to Central Asia
Distribution in R.Moldova: Chişinău [4]
Biological data: host plants are Phragmites australis, Eriophorum
Plateumaris sericea **(Linnaeus, 1758)**
General distribution: Paleartic region
Distribution in R.Moldova: Goian (Transnistria) [8]; r.Orhei, s.Ivancea, 12.05.1968; (fig. 2b) Rădenii vechi, 19.05.2006; Țaul, 20.06.2004
Biological data: host plants are Carex L., Iris L., Sparganium L.

a **b**

Figure 2. Plateumaris affinis (a) and Plateumaris sericea (b)

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IMPORTANCE OF EXORISTA LARVARUM (LINNAEUS, 1758) (DIPTERA: TACHINIDAE) SPECIES IN THE BIOLOGICAL REGULATION OF LEPIDOPTERA SPECIES

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Abstract. Exorista larvarum is a dipteran polyphagous larval endoparasitoid particularly known as antagonist of Lepidoptera. Exorista larvarum is a good biocontrol candidate against forest lepidopterous defoliators. This parasitoid has positive features, among which, it can be efficiently reared in vivo and in vitro. In the laboratory, box tree moth larvae were accepted by E. larvarum females. A lower number of eggs were laid on C. perspectalis than on G. mellonella, but the difference between the two moth species was not significant, although a quite long 3 hours exposure time was necessary for oviposition. We can speculate that, although not the preferred host, C. perspectalis may be accepted by E. larvarum also in nature. The overall results suggest that the mortality of C. perspectalis and G. mellonella larvae due to the partial development of E. larvarum may be useful to regulate the populations of this invasive pest in a context of conservative biological control.

Introduction

Exorista larvarum (Linnaeus, 1758) is a dipteran endoparasite known especially as an antagonist of Lepidoptera species. This species is part of the Tachinidae family, which includes about 8,500 species of parasitoids present worldwide [17]. This Palaearctic tachinid fly is distributed from Europe, to northern Africa, and certain Asian regions. It has also become established in North America during the 20th century, when it was introduced there to control *Lymantria dispar* (Linnaeus, 1758), which is a lepidopterous defoliator species causing serious damages in the forests [12].

The species develops in the caterpillars of about 45 species of host butterflies [13, 14]. It is well known as an enemy of *Lymantria dispar* and other defoliating butterflies: *Malacosoma neustria* (Linnaeus 1758), *Tortrix viridana* (Linnaeus, 1758), *Hyphantria cunea* (Drury, 1773) *Dendrolimus pini* (Linnaeus, 1758), *Peridroma saucia* (Hübner, l808), *Spodoptera littoralis* (Boisduval, 1833), *Pseudaletia unipuncta* (Haworth 1809) [5, 9, 18, 28]. This tachinid is also an antagonist of some agricultural noctuid pests as *Xestia c-nigrum* (Linnaeus, 1758), *Agrotis segetum* (Denis & Schiffermüller, 1775), *Prodenia litura* (Fabricius, 1775), *Mamestra brassicae* (Linnaeus,1758), *Pieris brassicae (Linnaeus*, 1758), *Autographa gamma* (Linnaeus, 1758), *Lacanobia oleracea* (Linnaeus, 1758) [6, 7, 12 ,15, 19].

Moreover, other lepidopterous species, not attacked in nature, may be suitable for the development of *E. larvarum* in vitro, one of these is the wax moth *Galleria mellonella* (Linnaeus, 1758). Due to the potential to reduce and regulate the number of phytophagous insects, Tachinidae species can be used in classical biological control programs [10].

The purpose of this study is to investigate the potential of E. larvarum as a biological control agent of lepidopteran pests by testing the effectiveness of fly on the larvae of artificial hosts *G. mellonella* and *Cydalima perspectalis*.

Materials and methods

Rearing of *Exorista larvarum:* A laboratory colony of *E. larvarum* was maintained on the factitious host *Galleria mellonella* [14]. The artificial medium used during the study consisted of skim milk (30 ml), egg yolk (5.5 ml), yeast extract (2.7 g), sucrose (0.8 g), gentamicin (10-1mg solution), 0.01 ml/ml [2, 15].

Plastic plates with 24 holes were used as growth containers. Each hole had a diameter of 17 mm, a height of 18 mm and a capacity of 3.3 ml. *E. larvarum* eggs were collected from infected larvae of *G. mellonella* and were placed by the method an egg on an orifice [2].

After laying the parasitoid eggs, the plates were sealed with paraffin paper and kept in the dark at 26 ± 1°C and 70% humidity throughout the development period, except for daily inspections. The food was prepared according to the method of Farneti et al. [6]. The diet of adult flies consisted of sugar cubes mixed with pollen, yeast extract and cotton balls soaked with a prepared solution of 20% honey and 80% water. The change of these cotton balls was done three times a week to avoid drying and contamination with mold [8].

For the standard colony maintenance, up to 2-5 parasitization procedures were performed weekly, by exposing last instar larvae of *G. mellonella* to flies however younger larvae can also be suitable for them [1]. According to the number of flies 40-70 host larvae were inserted into the adults cage. Exposure occurred after about 5 days from the beginning of fly emergence: the females were, therefore, mated, since in *E. larvarum* mating 18 occurs soon after emergence [1].

Prior to exposure, cotton balls, pollen, yeast and sugar, as well as the remaining pupae were removed from the cage. Alternative hosts were removed after infestation with 3-4 parasite eggs clearly visible on their cuticle, this being the optimal number for each host, in order to avoid excessive over parasitization [14, 15].

The dolls collected from the previous procedures were placed in a new box. Sometimes, the hatched flies earlier were gathered together with the hatched ones later to keep about 50-70 individuals in the box [16].

Rearing of *Galleria mellonella:* The colony of *Galleria mellonella* species was grown in the laboratory under controlled conditions [3].

In a sideboard at a fixed temperature of 30-32 ± 1°C, humidity of 65 ± 5% in complete darkness were kept a number of 10 boxes (24 x 13 x 8 cm) of different *Galleria mellonella* larvae continuous ages. Three times a week, the larvae were fed the ground diet. The requirements for the amount of food were different depending on the stages of development, the young larvae consumed more food. Adult moths lay eggs on filter papers, which were collected weekly. Unused eggs were stored in a refrigerator at 4°C.

Artificial diet preparation was made monthly. The diet contained corn flour, white flour, and wholemeal flour, milk powder, lyophilised brewer's yeast; the ingredients were mixed together in a big pot; solid wax and liquid glycerin were put in two other pots, and two bottles of honey were prepared in advance. All of the ingredients were placed in a special oven set at 100°C for 3-4 hours. This is a method to disinfest the materials and to melt the solid ingredients. In a special mixing machine all components were mixed until they got mash texture; before the whole dollop became rigid it was mounted to a plastic tray and cut into smaller pieces. When the diet cubes were cold they became solid and some of the cubes were grinded in a shredding machine. The grinded diet was placed for 24 h into a freezer (-24°C), and the following day it was unfrozen and was used for larval feeding. The remaining diet was stored in a fridge set at 4-5°C until further use [3].

Infection of *G. mellonella* larvae with eggs of *E. larvarum*. For the experiment, a total of 34 instare larvae of *G. mellonella* and *C. perspectalis* were exposed separately to *E. larvarum*. The exhibition took place in plastic cages of 20 x 20 x 20 cm. After exposure, the parasitoid females were removed and placed in another cage, then the eggs of the parasitoids on the larvae were counted (fig.1).The infected larvae were placed separately in plastic boxes with a diameter of 9 cm and were kept in the climate chamber and monitored daily until the visible effects of parasitization or non-parasitism by the fly. The larvae were considered infected when at least one parasitoid egg was found on their integument [2].

Figure 1. Infection of G. mellonella larvae with E. larvarum eggs in the growth box

Statistical analysis. For statistical analysis, the software STATISTICA 10.0 (StatSoft, 2010) was used. The data were analyzed by one-way ANOVA or, in case of variance heterogeneity, by Kruskal-Wallis nonparametric test. The percentages of moth adults were analyzed separately for the larvae exposed to *E. larvarum* females and for those which were not. Prior to analysis, the percentage values were transformed using an arcsine transformation [21].

Results and discussions

In the laboratory, box tree moth larvae were accepted by *E. larvarum* females. A lower number of eggs were laid on *C. perspectalis* than on *G. mellonella*, but the difference between the two moth species was not significant, although a quite long 3 hours exposure time was necessary for oviposition. We can speculate that, although not the preferred host, *C. perspectalis* may be accepted by *E. larvarum* also in nature.

As a result of the study, as expected, no significant difference was obtained between *G. mellonella* larvae that were fully accepted and between those of *C. perspectalis* accepted on average by 86%. No significant difference was also found for the parasitoid eggs/accepted larva, but higher oviposition was obtained on *G. mellonella*. No puparia formed in any accepted C. perspectalis larva. Conversely, puparia were obtained from *G. mellonella* larvae and all puparia emerged as adults (Table 1). The percentage of moths obtained from the larvae exposed to *E. larvarum* was low for both *C. perspectalis* (4.2 ± 2.6) and G. mellonella (7.3 ± 1.8) [2].

Importance of *E. larvarum* as an applied biological control agent. Effective production of biological control agents requires efficient mass-rearing techniques at competitive costs [2]. The role of tachinid parasitoids in applied biological control programs has been sometimes underestimated; they receive less attention than hymenopteran parasitoids, on which more research has been carried out [9].

Table 1. Acceptance and suitability of C. perspectalis and G. mellonella for the parasitoid E. larvarum: accepted larvae (%) based on moth larvae exposed to parasitoids, E. larvarum eggs/accepted larva, E. larvarum puparia (%) based on parasitoid eggs laid on larvae, adults (%) based on parasitoid puparia

Host species	Accepted larvae (%)	*E. larvarum* eggs/ accepted larva (n.)	*E. larvarum* puparia (%)	*E.larvarum* adults (%)
C. perspectalis	86.1 ± 11.1	4.2 ± 2.6	0	0
G. mellonella	100	7.3 ± 1.8	15.5 ± 5.4	100

The members of Tachinidae can be applied in classical and augmentative biological control programs. Yet, the potential of these beneficial enemies as antagonist of target insect pests is still underestimated and misunderstood. Difficulties may arise from the scarcity of knowledge about the biology, behavior and ecology of both the tachinids to be used as biological control agents and the target insect pests. Also, the rearing techniques and shipping methods of tachinids flies are relatively poorly known in comparison with hymenopteran parasitoids [9].

Exorista larvarum is a good candidate for biocontrol against forest Lepidoptera defoliators. One of the most important target pest is L. dispar (native in Eurasia), impairing deciduous and evergreen species. This defoliator damages more than 300 species of trees. The pest was introduced from Europe into the USA in the end of the 19th century [17]. To date, however, E. larvarum has been used as a biological control agent against L. dispar only in inoculative releases in the northern United States, where it has become established [18]. The potential of *E. larvarum* as an antagonist of forest and agricultural insect pests deserves to be better exploited. This parasitoid has positive features, among which, it can be efficiently reared in vivo and in vitro (on artificial media, without the host) too [9].The results of laboratory studies are encouraging regarding the possible augmentative releases of *E. larvarum* against populations of agricultural interest, such as *Spodoptera littoralis or Mithymna unipuncta*, and also such species as *Agrotis segetum, Prodenia litura, Mamestra brassicae, G. mellolnella, C. perspectalis* [6, 7, 11, 19].

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PARASITE FAUNA DIVERSITY IN RED FOX (Vulpes vulpes) FROM NATURAL AND ANTHROPIZED ECOSYSTEMS OF THE REPUBLIC OF MOLDOVA

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Abstract: The study of the diversity of the parasite fauna in the investigated foxes showed a high level of infestation (100%). The taxonomy of parasitofauna in foxes includes 12 parasitic invasions (Isospora canis – 14,3%, Alaria alata – 51,0%, Mesocestoides lineatus – 21,7%, Taeniidae spp – 27,0%, Syphacia obvelata – 17,0%, Strongyloides stercoralis – 13,3%, Toxocara canis – 59,0%, Toxascaris leonina – 65,5%, Ancylostoma caninum – 8,7%, Trichuris vulpis – 26,1%, Trichuris muris – 4,4%, Capilaria hepatica – 35,0%), which belong to 5 classes, 10 families, 11 genera and about 12 species. The share of species from the Sporozoa class is 8.3%, from the Trematoda class - 8.3%, from the Cestoda class - 16.7%, from the Secernentea class - 41.7% and from the Adenophorea class - 25.0%. Analyzing the parasitic species on epidemiological criterion, it was found that 10 species (83.3%) with large spread have zoonotic impact (A. alata, M. lineatus, Taenia spp., S. obvelata, S. ratti, T. canis, T. leonina, A. caninum, C. hepatica, T. vulpis) with a major risk to public health, and the identified invasions (100%) can parasitize domestic animals, as well as game fauna.

Introduction

Canids play a key role in the stability of the development cycles of a large number of parasitic species, including those with zoonotic impact. These animals pollute the environment with parasitic forms, which is why they pose a major danger to humans and the environment [19; 20; 1]. The presence of these animals in close contact with humans is a potential risk of increased infection, through contact with soil, vegetables, grapes and berries contaminated with parasitic forms [4; 7].

The close human relationship with canids and the common environment can increase the risk of human infection with vector-borne zoonotic pathogens, while wild mammals in their vicinity may act as reservoirs for such pathogens [9]. The evaluation of the population of Vulpes vulpes in the Republic of Moldova according to the number of reproductive burrows in the spring period, highlighted an increased density of foxes by 7-8 times [13].

Helminthfauna in *V. vulpes* in the Republic of Moldova previously (1958-1984) was studied and described by O. Anreyko. The author describes in this host several species of parasites as follows: class Trematoda *Alaria alata*; class Cestoda *Dipilidium caninum*, *Mesocestoides lineatus, Taenia cressiceps*; class Nematoda *Capillaria plica, Thominx aerophilus, Trichocephalus vulpis, Trichinella spiralis, Uncinaria stenocephala, Gnatostoma spingerum, Toxascaris leonina, Toxocara canis* [17].

The prevalence of parasites identified in V. vulpes in Romania was for: *Eimeria spp. 20.4%, Alaria alata 6.9%, Mesocestoides lineatus 31.5 %, Taenia pisiformis 24.5%, Dypilidium can-*

inum 6.0%, Toxocara canis 39.4%, Ancylostoma caninum 14.81%, Uncinaria stenocephala 10.2%, Pterigodermatites affinis 6.5%, Trichocephalus vulpis 21.8% [5].

Similar studies performed in Ukraine has revealed in V. vulpes several parasitic species: *Pearsonema plica 13.2%, Aonchotheca putorii 0.6%, Eucoleus aerophilus 12.0%, Trichuris vulpis 18.8%, Trichinella spiralis 0.6%, Ancylostoma caninum 0.6%, Uncinaria stenocephala 27.1%, Crenosoma vulpis 6.2%, Molineus patens 3.6%, Toxascaris leonina 39.2%, Toxocara canis 22.9%, Spirocerca arctica 0.6%, Pterygodermatites affinis 5.4%, Heligmosomum costellatum 0.6%, Syphacia agraria 0.6%* [15].

Nematoda helminths in V. vulpes in Polonia were identified as follows: *Toxocara canis 30.2%, Toxascaris leonine 26.0%, Uncinaria stenocephala 34.0%, Trichuris vulpis 11.9%* [14].

Parasite fauna monitoring in foxes and specifying their epidemiological role is of major importance for preventing the transmission of pathogens to animals and humans, which are involved in the evolutionary cycles of parasites. The bibliographic data described above denote the purpose of this paper, which provides the bioecological study on the role of canids (Vulpes vulpes) in the transmission of parasitic species in the zoonotic and epizootic chain of natural and anthropogenic ecosystems.

Materials and methods

Parasitological investigations, according to the methods Popova, Baermann, Fuileborn, Darling, of the successive washing [18], were performed in the laboratory of Parasitology and Helminthology of the Institute of Zoology, on biological samples collected from foxes from natural and anthropized biotopes from different areas of the Republic of Moldova. In the ovo-coproscopic diagnosis, the *Teneidae oncospheres (T. hydatigena, T. pisiformis, M. multiceps, M. serialis E. granulosus, E. multilocularis)* are very similar to each other, which is why they are noted as *Tenea* sp. oncospheres [8].

The parasitological evaluation is based on the determination of the prevalence (%), intensity (specimens / animal) and abundance (specimens / plot) of the parasitic species in the investigated canids.

Results and discussions

In recent years, many vector-borne diseases have (re) emerged and spread, due to global and / or local changes that have led to the invasion of new arthropod-vector species, improved their vector capacity, or the introduction and establishment of territory of new species of pathogens [12].

Research on the role of wild canids in the spread of parasitic species in the zoonotic and epizootic chain of natural and anthropogenic ecosystems began with the evaluation of the fox population by the number of breeding population in spring. The fox is quite numerous in the southern (10 ind./1000 ha) and in the central part (9 ind./1000 ha), having low density in the northern part (6.2 ind./1000 ha). At the spring evaluations, the fox was observed with densities of 5.5 ind./1000 ha in the agricultural funds. Thus, in the agricultural ecosystems in the spring were evaluated about 15000 foxes, over 6000 foxes have been registered in localities and 3700 foxes were counted in the forest ecosystems, total number of about 25000 individuals. Toward the end of 2020, in the autumn period, the fox population number was of 35000 individuals.

The fox population decreased in the last years, while in 2014-2017 its density was of 14 - 16 ind. / 1000 ha. The concentration of foxes in forested ecosystems and in localities is an ecological-ethological adaptation caused by trophic resources availability and the stability factor particular to these types of ecosystems [13].

The study of the parasite communities diversity in fox highlighted several species of parasites (tab.1). The species *Isospora canis* from the Sporozoa class was identified with a prevalence of 14.3% and an intensity of 2-8 oocysts. The Trematoda class was represented by *Alaria alata* with a prevalence of 51.0% and an intensity of 1-2 eggs. From the class Cestoda there was identified *Mesocestoides lineatus* with a prevalence of 21.7% and an intensity of 3-15 eggs and Taeniidae spp with 27.0%, respectively (3-4 oncospheres). The species from the class Secernentea are represented by *Syphacia obvelata* with a prevalence of 17.0% and an intensity of 3-4 eggs in the microscopic field, respectively, *Strongyloides stercoralis* - 13.3%, (50-100 larvae), *Toxocara canis* - 59.0% , (2-5 eggs), *Toxascaris leonina* - 65.5%, (12 eggs), *Ancylostoma caninum* - 8.7%, (1 egg). The parasitic invasions from the class Adenophorea were represented by Trichuris vulpis with a prevalence of 26.1% and an intensity of 2 eggs in the microscopic field, respectively *Trichuris muris* - 4.4%, (2-3 eggs), *Capillaria hepatica* - 35.0%, (3-4 transit eggs). An important fact is that 100% of the total number of parasitologically investigated foxes were infested.

Table 1. Parasite fauna in Vulpes vulpes

Class	Family	Species	Prevalence (%)	Intensity (sp.)
Sporozoa	Eimeriidae	*Isospora canis* (Levine, 1977)	14,3	2-8 ouă
Trematoda	Diplostomidae	*Alaria alata* (Goeze, 1792)	51,0	1-2 ouă
Cestoda	Taeniidae	*Taenia spp*	27,0	3-4 ouă
	Mesocestoididae	*Mesocestoides lineatus* (Goeze, 1782)	21,7	3-15 ouă
Secernentea	Oxyuridae	*Syphacia obvelata* (Rudolphi, 1802)	17,0	3-4 ouă
	Strongyloididae	*Strongyloides stercoralis* (Bavay, 1876)	13,3	50-100 larve
	Ascarididae	*Toxocara canis* (Werner, 1782)	59,0	2-5 ouă
		Toxascaris leonina (Linstow, 1902)	65,5	12 ouă
	Ancylostomatidae	*Ancylostoma caninum* (Ercolani, 1859)	8,7	1 ou
Adenophorea	Trichuridae	*Trichuris vulpis* (Froelich, 1789)	26,1	2 ouă
		Trichuris muris (Scrank, 1788)	4,4	2-3 ouă
	Capilariidae	*Capilaria hepatica* (Bancroft, 1893)	35,0	3-4 ouă

Similar research conducted previously (1958-1984) in the Republic of Moldova revealed several species of parasites as follows: *Alaria alata – 28,9%, Dipilidium caninum– 3,7%, Mesocestoides lineatus– 59,3%, Taenia cressiceps – 3,71%,Capillaria plica– 11,2%, Thom-*

inx aerophilus– 40,8%, Trichocephalus vulpis – 3,7%, Trichinella spiralis – 7,4%, Uncinaria stenocephala– 29,7%, Crenosuma vuples – 3,7%, Toxascaris leonina– 81,5%, Toxocara canis – 40,8% [17]. Comparing the previous data with the recent ones, insignificant differences were found, but with the maintenance of a high level of infestation.

From taxonomic point of view the parasitic species identified in the fox fall into 5 classes, 10 families, 11 genera and about 12 species, including 1 parasitic species of the class Sporozoa, 1 species of the class Trematoda, 2 species of the class Cestoda, and 4 species of the class Secernentea and 3 species of the class Adenophorea.

The helminthofauna hierarchy was evaluated according to the prevalence level,being identifying 3 eudominant species *(Toxascaris leonina, Toxocara canis, Alaria alata)* with 65,5 – 51,0%, 3 dominant species *(Capilaria hepatica, Trichuris vulpis, Taenia spp, Mesocestoides lineatus)* with 35,0 – 21,7%, 3 subdominant species *(Syphacia obvelata, Isospora canis, Strongyloides stercoralis)* with 35,0 – 21,7% and 2 rare encountered species (*Ancylostoma caninum, Trichuris muris)* with8,7 – 4,4%.

The development feature denotes 9 species that develop according to the monoxene model *(Syphacia obvelata, Capilaria hepatica, Trichuris muris, Strongyloides stercoralis)*, species of Taenia have dixen evolution cycle *(Taenia spp),* one specie with trixen development *(Mesocestoides lineatus)* and one species with tetraxen evolution *(Alaria alata)*.

The epidemiological feature highlights 2 categories of parasitosis, zoonotic and canine specific. Zoonotic parasitosis are caused by 10 parasitic species, including 1 species of the class Trematoda *(A. alata)*, 1 genus and several species of the class Cestoda *(M. lineatus, Taenia spp.)*, 5 species of the class Secernentea *(S. obvelata, S. ratti, T. canis, T. leonina, A. caninum)* and 2 species of the class Adenophorea *(C. hepatica, T. vulpis)*, while 2 species induce diseases particular to canids, including 1 species of the class Sporozoa *(Isospora canis)* and 1 species of the class Adenophorea *(T. muris)*.

Table 2. Epidemiologic characteristics of parasite fauna in fox

Impact	**Class**					
	Sporozoa	**Trematoda**	**Cestoda**	**Secernentea**	**Adenophorea**	**Total**
Zoonotic	-	*A. alata*	*Taenia spp* *M. lineatus*	*S. obvelata* *S. stercoralis* *T. leonina* *T. canis* *A. caninum*	*C. hepatica* *T. vulpis*	**10 (83,3%)**
Fox	*I. canis*	-	-	-	*T. muris*	**2 (16,7%)**

Analyzing the parasitic invasions on epidemiological criteria, we find that 10 species (83.3%) with increased spread have zoonotic impact *(A. alata, M. lineatus, Taenia spp., S. obvelata, S. ratti, T. canis, T. leonina, A. caninum, C. hepatica, T. vulpis)* with major risk for public health, andtwo of them are particular for canids (16,7%).

Zoonotic parasitosis are of major epidemiological importance because they have a direct impact on human health, and carnivores are definitive hosts or reservoirs of more than 60 parasitic species with zoonotic impact [7; 6].

Several gastrointestinal parasites of canids, especially *Toxocara spp., Ancylostoma spp., Echinococcus spp., Dipylidium spp., Taenia spp.* are considered species with increased zoonotic potential [3; 2]. According to some authors, zoonotic parasites can be divided into 4 groups [16; 3; 10; 11].

From the research results we find zoonotic parasites that directly infect humans through animals (*Toxascaris leonina, Toxocara canis)*; saprozoonotic parasites that are transmitted through soil or water contaminated with parasitic forms (*Ancylostoma caninum, Strongyloides stercoralis*); metazoonotic parasites that infect humans through intermediate invertebrate hosts (*Alaria alata, Dipylidium caninum*); cyclozoonotic parasites that infect humans through vertebrate intermediate hosts (*Echinococcus granulosus, Taenia spp*).

At the same time, it is important that all identified parasitic species (100%) have an epizootic impact, which indicates that they can also infest domestic animals, as well as wild animals, which is why they pose an eminent danger to the gamespecies.

These parasitosis cause economic damage to domestic and wild animals, causes considerable damage to their number, including the integrity of the spectrum of main and complementary species that ensure the hunting fund.

Monitoring the parasitofauna in canids, highlighting the epidemiological role, is of major importance for preventing the transmission of pathogens to animals and humans, which are involved in the evolutionary cycles of parasites.

The results obtained denote a rich diversity of parasitic species with increased prevalence and intensity. This is due to the considerable increase in the number of foxes (7-8 times), as well as the ecological plasticity of this species with a tendency to synanthropy.

These are the key factors in the emergence and maintenance of outbreaks of parasitosis in natural and anthropogenic ecosystems.

The results obtained from parasitological investigations represent the premise of developing new procedures to control / reduce parasitosis in wild canids in order to strengthen bioecological and epidemiological security in natural and anthropogenic ecosystems.

The studies were performed within the State Program projects 20.80009.7007.12 and 20.80009.7007.02.

Conclusions

The evaluation of the population of Vulpes vulpes according to the number of reproductive burrows during the spring, showed an increased density of foxes, this being about 9individuals / 1000 ha, compared to the normal density of 1- 2 individuals / 1000 ha, which indicates a considerable increase of 4–5 times.

The study of the diversity of parasite communities in the investigated foxes, denotes an increased level of infestation with parasitic species – of 100%.

The taxonomy of parasitofauna in investigated foxes includes 12 parasitic invasions (*Isos-*

pora canis, Alaria alata, Mesocestoides lineatus, Taeniidae spp, Syphacia obvelata, Strongyloides stercoralis, Toxocara canis, Toxascaris leonina, Ancylostoma caninum, Trichuris hepis) the parasitic species identified in the fox fall into 5 classes, 10 families, 11 genera and about 12 species. The share of species in the Sporozoa class is 8.3%, in the Trematoda class - 8.3%, in the Cestoda class - 16.7%, in the Secernentea class - 41.7% and in the Adenophorea class - 25.0% .

As epidemiological criteria, 10 species (83.3%) with increased prevalence have zoonotic impact (*A. alata, M. lineatus, Taenia spp., S. obvelata, S. ratti, T. canis, T leonine, A. caninum, C. hepatica, T. vulpis*) with a major risk to public health, and all identified invasions (100%) can parasitize domestic animals, as well as game fauna.

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SOME RARE HETEROPTERA SPECIES (HEMIPTERA) FROM THE „COBÎLENI" NATURAL RESERVE, REPUBLIC OF MOLDOVA

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Abstract. A list of the some rare heteroptera species from the „Cobîleni" Natural Reserve is published. The list contains 9 species from 5 families: Corixidae (3 species), Anthocoridae (1), Miridae (3), Lygaeidae (1) and Pentatomidae – 1 species. Data on bio-ecology and host plants are given. It is noted that the „Cobîleni" Reserve are the guarantor of the preservation of both typical biotopes and rare and endangered species of true bugs.

Introduction

The suborder Heteroptera (Hemiptera) is one of the most studied groups on the territory of the Republic of Moldova [2, 3, 4, 5, 6, 7, 8, 9, 10]. At the same time, many species of bugs from the local fauna are rare. Due to the changing habitat, some species of true bugs are on the verge of extinction. The reserves are the only guarantor of the preservation of both typical biotopes (of a particular zone of the Republic of Moldova) and some rare and endangered species of insects.

Material and methods

The researches were carried out during the vegetation period of 2016-2018 in the „Cobîleni" Nature Reserve (47°30´51´´N, 29°01´20´´E) located near Lopatna village (Orhei District) on the right bank of the Dniester river. The „Cobîleni" reservation is a natural forest area, with an area of 33.5 ha, it belongs to the Susleni Forest District (fig. 1).

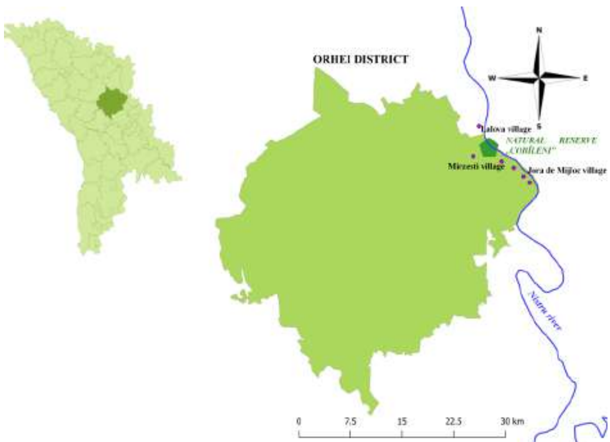

Figure 1. The location schematic map of „Cobîleni" Natural Reserve, Orhei District, Republic of Moldova [19]

The territory of the „Cobîleni" Reserve is located on the right bank of the Dniester and its terraces. In the Reserve there are biotopes with floodplain vegetation, forest and stony steppe sectors. The rock forest, specific to the steep slopes of the Dniester valley is formed by Quercus robur and *Fraxinus excelsior*. The steep sectors in the center of the Reserve, facing east, contain *Stipa pulcherrima, Amygdalus nana, Rhamnus cathartica, Thalictrum minus, Silene fabaria* and others. Numerous species of ferns, mosses, lichens (*Cladonia pyxidata, C. fimbriata,* etc.) and some angiosperms grow on the limestone rocks of the Dniester valley, which together form a mosaic similar to that of mountainous areas [19].

In the present investigations there were used field collectings from different biotopes. The true bugs were collected using usual entomological methods. Also in our studies, light traps with white and ultraviolet lamps were used.

Results and discussions

As a result of research, 9 rare species of true bugs were registered on the territory of the „Cobîleni" Reserve. These species belong taxonomically to 5 families: Corixidae – *Corixa affinis* Leach, *Hesperocorixa sahlbergi* Fieb., *Sigara iactans* Janss.; Anthocoridae – *Amphiareus obscuriceps* Popp.; Miridae – *Hallodapus suturalis* H.-S., *Pilophorus cinnamopterus* Kirschbm., *Psallopsis neglecta* Konst.; Lygaeidae – *Peritrechus meridionalis* Put. and Pentatomidae – *Clorochroa pinicola* Mls. & Rey.

In the list of heteroptera species shown below, are presented the date of collection, number of caught specimens, geographical spread and bio-ecological peculiarities.

CORIXIDAE Family

***Corixa affinis* Leach, 1817**

Material: Two males captured July 5, 2016 on the ultraviolet light trap near the Dniester river.

Bioecology: Brackish ditches and pools, mostly near the coasts of central and southern England and Wales [1]. There is also evidence that the species prefers stagnant water [14]. Probably, a general increase in temperature provokes an increase in the concentration of salts in water, which contributes to the spread of hallophilic species.

Distribution: Holomediterranean species [16]. Is present in the countries of Central and Southern Europe, Northern Africa, Turkey, Iran, Iraq, Caucasus, south of european part of Russia, Kazakhstan, Turkmenistan, Uzbekistan and Tajikistan (to the Amu Darya river), north of India.

***Hesperocorixa sahlbergi* (Fieber, 1848)**

Material: One male was caught July 19, 2017 in a light trap with an ultraviolet lamp in the „Cobîleni" Reserve.

Bioecology: In England this very common species occurs in densely vegetated ponds and lakes, which have a bed o f dead leaves or mud. In the meres it is confined to pools and ditches in the fringing Alder woods [12].

The *Hesperocorixa sahlbergi* found in ponds, ditches, lake margins and neglected aquatic habitats especially with dead vegetation. The overwinters adults mate in early spring when the water is still quite cold and the resulting nymphs take at least two months to develop into adults.

Distribution: This species is widespread in Europe, Caucasus, Eastern and Western Siberia.

Sigara iactans Jansson, 1983

Material: From the „Cobîleni“ Reserve total in the period June, 13 – September 19, 2016-2018 was collected 64 specimens.

Bioecology: The species seems to seek permanent water bodies, mesotrophic to eutrophic, which have little submerged aquatic vegetation with a pH greater than or equal to 7. If it can withstand weakly flowing waters, however it prefers lentic waters soft and brackish dunes. In its stations, it is most often found in company of Sigara falleni (Fieber, 1848), very rarely alone [11].

Distribution: Central and south-eastern part of Europe. On the territory of the Republic of Moldova, this species, until recently, was mixed with Sigara falleni Fieb. morphologically similar to it; therefore, all previous records should be reviewed. Our data indicate a more northern confinement of this species in the Republic of Moldova [8].

ANTHOCORIDAE Family

Amphiareus obscuriceps (Poppius, 1909)

Material: Total in the period June, 4 – September 25, 2016-2018 in the „Cobîleni“ Reserve was collected almost 300 specimens of this species.

Bioecology: As habitat for A. obscuriceps can by different open areas and woodlands, associated with dead-leaf habitats in the trees or on the ground, also brush piles and old tent of caterpillar nests. The minute pirate bugs feed on small arthropods, probably including aphids, thrips, etc.

Distribution: Is considered native to Asia, recently introduced to North America (has spread across the eastern half of the US & Canada) and Europe [13].

MIRIDAE Family

Hallodapus suturalis (Herrich-Schäffer, 1837)

Material: July 13, September 17, 2016, 2 specs. (to the ultraviolet light).

Bioecology: Apparently, he lives in rocky areas of the Reserve, under grassy vegetation. It feeds on sap on the roots of various steppe plants.

Distribution: Southern part of Europe, North Africa, Turkey, Caucasus, Central Asia.

Pilophorus cinnamopterus (Kirschbaum, 1856)

Material: Total in the period June, 17 – September 27, 2016-2017 was collected on the white light 14 specs. and 21 specs. – on the ultraviolet light.

Bioecology: In the Reserve was noted on a pine tree (Pinus nigra), among a colony of aphids from the genus Cinara.

Distribution: Widespread in all Europe, Caucasus (Azerbaijan), Asian part of Turkey, Eastern Siberia.

Psallopsis neglecta Konstantinov, 1998

Material: August 17, 23, September 3, 2016, 4 specs.

Bioecology: According to F. Konstantinov [15] the host plants not clarified, but certainly annual Chenopodiaceae. Some specimens from collections are labelled as collected from annual Suaeda, Halogeton and Petrosimonia.

Distribution: Ukraine, south of European part of Russia, Kazakhstan, Uzbekistan, West Siberia and Mongolia.

LYGAEIDAE Family

Peritrechus meridionalis Puton, 1877

Material: August 5,7, 2016, 2 specs. (to the white light).

Bioecology: Most often noted under plants from genera Suaeda, Salicornia and Puccinellia [17].

Distribution: South and south-east Europe, Caucasus, South-West and Central Asia, North Africa [4].

PENTATOMIDAE Family

Clorochroa pinicola (Mulsant & Rey, 1852)

Material: August 7, 2016, 1 spec., on Pinus nigra.

Bioecology: This species lives and feeds on pine trees (Pinus spp.), but can also be found on Abies alba and Juniperus communis. Hibernates in the adult stage. Has one generation per year. [18].

Distribution: Europe (except United Kingdom Island), Caucasus, Kazakhstan, West Siberia.

The research was carried in the project 20.80009.7007.02. from the State program at the Institute of Zoology.

Conclusions

Thus, in various biotopes of the „Cobîleni" Natural Reserve, 9 rare species of true bugs from 5 families were registered: Corixidae – Corixa affinis Leach, Hesperocorixa sahlbergi Fieb., Sigara iactans Janss.; Anthocoridae – Amphiareus obscuriceps Popp.; Miridae – Hallodapus suturalis H.-S., Pilophorus cinnamopterus Kirschbm., Psallopsis neglecta Konst.; Lygaeidae – Peritrechus meridionalis Put. and Pentatomidae – Clorochroa pinicola Mls. & Rey. For each species data on bio-ecology and host plants are given.

Our research has shown that the „Cobîleni" Reserve are the guarantor of the preservation of both typical biotopes and some rare and endangered species of true bugs.

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ENVIRONMENTAL POLLUTION - PARASITIC POLLUTION

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Abstract. The paper presents the danger caused by environmental pollution (water, soil, vegetables, fruits, animal and plant products) with parasitic elements eliminated and spread by humans and animals, as well as the importance of conducting research in this field. An important role is also played by the high level of infestation, with various parasitic agents, of animals in the Republic of Moldova. Mention is made of the huge volume of invasive forms eliminated by infested animals.

Introduction

The ecological aspects of zooparasitology began to develop intensively in the first half of the twentieth century [44, 50, 51, 54]. The term "ecological parasitology" was introduced and gradually this direction became dominant [5, 26, 38, 46]. Parasitology is an integral part of ecology, as the science of the relationships of organisms with each other and the environment. Some ecologists have long underestimated the importance of the concepts and data of parasitology. A turning point occurred at the end of the twentieth century, when, together with the greening of parasitology, the parasitologizing of ecology is observed [41].

The subject of ecological parasitology are parasites, their hosts and the environment with the full diversity of their interactions. These components form parasitic systems with varying degrees of complexity, outside of which the existence of parasites is impossible. The most significant contribution to the development of the concept of parasitic systems was made by Beklemishev [39, 40].

He wrote that "the parasitic system consists of the population of parasites, along with all the populations of the host, which directly support its existence." By the parasitic system, he meant the population of various parasitic agents, along with all the species populations of its hosts, which directly support its existence.

The problems of ecological parasitology are widely and efficiently addressed in many countries. Parasitism, as one of the life forms, is a general biological phenomenon and is characteristic of many taxa of living organisms, from viruses to multicellular animals and plants, although the understanding of the essence of this phenomenon is not yet unambiguous. To date, there is no clarity about the object of parasitology as a science and the validity of its division into independent disciplines through research objects and methods [2, 3, 23, 25, 26, 27] (Fig. 1).

Pollution of the environment - of water, soil, air, vegetables, fruit, products of animal and plant origin and others - with parasites removed and disseminated by humans and animals, constitute a permanent, mass danger of exposure to infestation and thereby willingly entering the biological cycles of the huge number of parasite species.

It is enough to emphasize the danger of pollution with enormous amounts of invasive elements, in children's playgrounds by cats and, especially, by stray dogs, highly parasitized, or inside cattle farms, a huge number of invasive elements eliminated by caring people, infested with Taenia saginata and others. [25, 30].

The World Health Organization (WHO) indicates that 4.3 billion people are infected with various parasitic agents, over 16 million deaths per year, or about a third of all cases, are caused by infectious and parasitic diseases (helminthic): nematode, trematode, cestode [37].

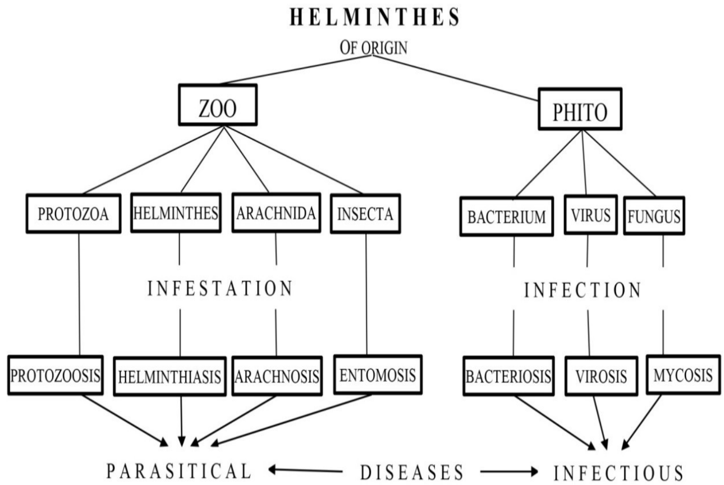

Fig. 1. Diversity of parasites according to origin and diseases caused by them (after [25])

Of great importance is the pollution of vegetables, etc. by infesting them with parasite eggs, it reached the lands in question by fertilizing it with manure from parasitized animals. To this is added the massive pollution with parasitic elements from dogs, cats, people, etc.

Data on water infestation with Cryptosporidium and Giardia cysts pose a danger to humans. The percentage of positive soil samples for the presence of cryptosporidae oocysts is different: in the grazing areas it is 22.3 ± 1.2%, in the bodies on the water surface it is 18.8 ± 1.1%, and in the wildlife habitats - 13.7 ± 1.1% [1, 4, 28, 43, 45].

Materials and methods

Investigations, regarding the determination of parasite species from various animal species, or conducted in the laboratory of Parasitology and Helminthology of the Institute of Zoology. Biological samples were collected from animals from various natural and anthropogenic biocenoses of the Republic of Moldova.

Coproovoscopic (*Fulleborn, Darling*), coprolarvoscopic (*Popov, Baermann*) methods, special examination in sarcocystosis according to the Lubyanetsky [48] method, partial parasitological investigations [after 53] and successive washing were used to achieve the proposed objectives.

Results and discussion

According to scientists, environmental protection is a priority of socio-economic development. In the late 1960s, all industrialized countries adopted laws and administrative measures to protect the ecosystem. Therefore, environmental protection issues become state work programs, along with economic and social aspects.

In response to the suggestions of the international commissions, on 31.07.87, during the session of the Supreme Soviet of the MSSR, a complex long-term program on the issue of environmental protection and rational use of the resources of the Moldovan Soviet Socialist Republic was approved, lasting until 2005. It is well known that the issue of the environment and resources is international in nature.

In recent years, new data have accumulated on the results of research in the field of parasitology. Some of them determine the trend of the research perspective in this field. A synthesis of these data and theoretical hypotheses can be made on a modern basis. This is the ecological basis of the requirements, taking into account the economic feasibility of the practical application of the technological methods developed.

Despite the fact that the definitions of a phenomenon such as "environmental pollution" given by different scientists may vary, almost all agree on the main point: "Pollution" is understood to mean any adverse change in the environment for living organisms caused by human destructive action on it. Environmental pollution is caused by various factors. This is largely due to a variety of industrial, agricultural and household waste. This includes types of pollution: Chemical, organic, physical, radionuclide and other types of pollution.

However, for many years, the main attention has been paid to technogenic pollution factors and to a much lesser extent to natural factors, ie biological pollution, which consists in changing the properties of the environment due to the increasing number of various types of microorganisms, plants and animals, initiated by anthropopressure.

In this context, it is quite clear that one of the forms of biological pollution (its basic component) is "parasitic pollution". It is not necessary to demonstrate the enormous role that parasitic organisms play in the biosphere. In nature, there are practically no organisms that are not infected with parasites. Humans, animals and plants are populated with a diversity of parasites, which possess various ecological and informational connections with the environment, forming complex parasitic systems [14].

As mentioned by academician Konstantin Skriabin [52], these systems formed during the evolution of the biosphere, uniting various components of biocenoses into independent complexes. Such complexes occur either in the "systemic inorganic" stage (according to Обухов's terminology, 1989) [49], when rigid and predominantly unilateral connections are observed between components fi is "systemically organic", when fi each of the components is not able to exist independently (outside the system).

One of the most difficult examples of complexes is parasitic systems. Parasitic systems are self-regulating structures, characterized by certain vectors and their rate of change, depending on environmental factors. Often, the changes observed in the number of members of the parasitic systems, caused by the actions of the self-regulating mechanisms, do not lead to the destruction of the systems themselves. Any parasitologist may give examples where the annual dynamics of host infestations with one or other parasite agent fluctuate strongly and yet the multi-annual results show that the state is fairly constant. This is how parasitic systems behave, which are not subjected (or are less subject to) to the influence of anthropogenic pressure.

Environmental pollution with parasitic elements is permanent, throughout the time when humans and infested animals eliminate in nature eggs, larvae, proglottids of parasites or even adult parasites. The dimensions of this phenomenon are almost incomprehensible in a short period of time. The issue of the phenomenon is part of the coordinates of ecological parasitology.

Most parasitologists, who have studied the etiological agents of various parasitosis, have also addressed issues related to their relationship with the environment. In the years 1963-1964, the participants in the works of Complex Parasitological Expeditions (CPE) in Romania paid special attention to the study of the phenomenon of polyparasitic pollution of the environment. For this purpose, 10 complex parasitological expeditions were made. The results obtained showed that the environment is strongly polyparasitic in all its components. It is worth mentioning the intense polyparasitic pollution of manure, livestock farms, feed depots populated by dogs, cats, mice, rats, etc., animal shelters, grazing areas, places for watering animals, means of transport etc. [24, 25].

A similar situation existed in the Republic of Moldova. Until the 90s of the last century in the republic there were 19 pig breeding complexes of 54 thousand heads each, 38 complexes - of 12-24 thousand each, 12 complexes - of 9-11 thousand each, 22 complexes pigs of 7-9 thousand heads each. In the republic there were 3 milk-cargo complexes (with over 1000 milking cows), 26 reed breeding complexes, 31 bull fattening complexes, in 837 households in the republic there were milk-cargo farms. In 1990 alone, there were about 3.430.000 pigs in the republic, about 477.630 head of cattle and about 1.243.000 sheep/goats [47].

The results of our research showed that cattle in households with various maintenance technologies, ages and geographical areas of the Republic of Moldova are infested with echinococci in 13.4-83.3% of cases, fascioles - 16.9-59.5%, dicroceles - 23.5-63.5%, strongyloids - 20.0-47.5%, eimers - 54.4-94.4% (2-6 months), sarcocysts - 81.2-97.6% and cryptosporids - in 20.0-34.5% (calves up to 2 months) of cases [14].

Rusu Stefan et al. [30] mention that canine youth in urban and rural areas in Chisinau were infested with *Echinococcus granulolosus* - in 3.3-6.3% of cases, *Dipilidium caninum* - 5.5-18.5%, *Toxocara canis* - 9 , 6-12.6%, *Toxascara leonine* - 40.9-64.9%, *Ancylostoma caninum* - 5.4-9.4%, *Trichocehpalus vulpis* - 5.5-6.2% and *Eimeria canis* in 30.9 -35.9 cases, and adult stray dogs were infested with *Echinococcus granulolosus* in 14.3-42.7% of cases, *Dipilidium caninum* - 25.5-47.3%, *Toxocara canis* - 39.6-52.8 %, *Toxascara leonina* - 1.9-12.4%, *Ancylostoma caninum* - 7.4-16.2%, *Trichocehpalus vulpis* - 10.5-24.6% and with *Eimeria canis* - in 2.9-4.2 % of cases.

The grazing of animals of various species and ages and limited lands is the most used system in the Republic of Moldova. Based on the concrete epizootological data, it is possible to establish the level of environmental contamination, as well as the impact of deforestation on it. Therefore, overpollution of pastures with animals of different ages and species, as well as their uncoordinated deforestation, is an important factor that can contribute to increasing the extent and intensity of animals with various parasitic agents.

It has been established that each species of *Eimeria* is genetically programmed for a number of merogonic generations. It is estimated that around 92,000,000 merozoids can result from a single oocyst. The dynamics of the elimination of oocysts express an increasing intensity in calves up to 6 months and then decreases to higher ages [10]. In lambs and kids suffering from eimeriosis, the level of co-elimination reaches from 1000-2000 to over 100,000 eggs per gram (OPG) [12, 31].

Coprological eliminationsof oocysts in piglets with symptoms of eimeriosis reach over 50.000 OPGs [18].

In apparently healthy rabbits of eimeriosis, oocyst co-elimination is 0.1x1.9x106 OPG. Oocysts survive up to 150 days in bedding and shady areas. In foals, the co-elimination period of eimeria oocysts is 30-35 days, and the amount reaches from 25 to about 1,100 fecal OPG, and the amount of pollution with eimeria oocysts in cats exceeds 106 OPG [32, 33].

Krull (1941) demonstrated that from a single miracle of Fasciola hepatica, which enters a snail - *Lymnaea truncatula* (intermediate host), about 4000 searches can occur, which turn into metacercises - the invasive form. Dicrocoelium eggs remain viable after 6 months in the faecal masses of pastures and from an egg ingested by terrestrial gastropods, the first intermediate host, then the search finds the second intermediate host - ants, where up to 400.000 metacercariae can appear - form infesting [11, 32].

It has been established that the expanding Moniezia expansa, at its maturity, can eliminate up to 100 eggs full of eggs and up to 1.000.000 eggs per day, respectively. It is known that *Moniezia expansa* lives in the intestine of the host up to 11 weeks, so the infested animal can spread on the pasture a huge number of parasitic elements (oncospheres) that can take the intermediate host (pasture mites - *Oribatidae, Galumnidae*) [10]. *Taenia* cestodes lay 2-3 ovary proglottids containing 100.000 eggs/proglott daily [16, 29], and *Echinococcus granulosus* oviplot proglottids contain about 1500 eggs [35].

Egg proglottids, after elimination into the outdoor environment, may leave the feces and move more than 25 cm (*Echinococcus granulosus*) or up to 90 cm (*Taenia hydatigene*), which contributes to the dispersion of eggs in the outdoor environment. The eggs of teniid may be spread over a distance of at least 175 meters by infected dogs and it is estimated that a single dog can polite eggs of at least 30.000 hectares [21].

Insects, birds, and wildlife contribute to the dispersal of eggs over very large areas, sometimes more than 60 km [21, 36].

Dogs experimentally infected with *Echinococcus granulosus* lay eggs up to 22 months [36]. Difilobotriosis is caused by the cestode *Diphyllobothrium latum* which measures up to 25 m in length and can contain 4000 proglottids. Infected humans and/or animals can lay between 2 and 40 million eggs/day [22].

Taenia saginata has a length of 4-10 meters, with 1000-2000 proglottids. The proglott contains from 97.000 to 124.000 eggs, and the annual production of an adult basket is 594.000.000 eggs. The proglottids are evacuated intermittently, up to 12 per day.

Taenia solium produces about 50,000 eggs in each proglott. Cestoda *Taenia solium* is 3 to 7 m long. The strobila contains 700-1000 proglottids, and the ovigere ones contain about 50.000 eggs. The parasite grows at a rate of 7 cm per day, reaching maturity in two months. Adult proglottids, full of eggs, are eliminated with the feces in groups of 3-5 specimens each [10].

Animals infested with nematodes from the family Trichostrongylidae can lay a big number of eggs daily (about 10.000). Biothermically sterilized and spread on pasture manure is an important source of contamination [8].

Carnivores infected with the nematode Ancylostoma caninum pose a danger to the external environment. A female lays 10.000-20.000 eggs/day [7].

A horse infected with strongyls can remove 40x106 eggs/day [34]. Egg removal takes place throughout the year, but there is a seasonal fluctuation - in winter the smallest number of eggs is eliminated [13].

Ascaris suum larvae lay 200.000-1.000.000 eggs daily, leading to massive environmental contamination. In addition, a role in the spread of eggs can be played by paratenic hosts, first of all earthworms [6].

Females of *Oxiuris* equvi lay up to 60.000 eggs [17]. The females of Parascaris equorum have a very high progeny, being able to eliminate, when they parasitize the foals, up to 1.000.000 eggs/day. The eggs are very hardy, surviving over a year in the outdoor environment. The female *Toxocara canis*, the dog's nematode, can lay up to 200.000 eggs/day. Due to the thick shell, *Toxocara canis* embryonated eggs are very resistant to unfavorable factors. Under natural conditions, they remain viable for years [15].

It is known that the main source of parasite for humans is the soil, however more recently it is established that a potential source can fi and dog hair [19].

A female of *Ascaridia galli* lays up to 170.000 eggs. The source of environmental contamination with parasites are sick and carrier birds. Wild birds can be a reservoir of infestation for domestic birds [33].Prof. Olteanu Gh. [25] asks the question if someone regularly controls whether or not there are parasitic elements on the sands of the beaches on the coast, in the swimming pools and in general in the external environment in Romania? The same situation is in the Republic of Moldova. If the results of such periodic inspections were made known, we would be horrified. Beaches, playgrounds for children, land near animal husbandry, etc. they are "honored" by periodic wanderings of packs of stray and semi-stray dogs, which seem to perpetuate their presence and tendency to grow in number and aggression in all respects. The parasitic load of each of the components of the dog packs is enormous and regenerates in the permanent circuit with the parasitic elements from the environment strongly aggressed by the polyparasitic pollution that it ensures and maintains aggravating it. Polyparasitic pollution of the environment by wild animals, stray dog packs, as well as by other categories of dogs, including those belonging to the canine elite, have a standard of living well above the level of many ordinary people, through access to living conditions in apartments, beds, kitchens, living rooms, balconies, hallways, luxury cars, etc., is a danger of exceptional gravity for the human population.The problem of vectors (insects, ticks, rodents and other animals) of pathogens of diseases caused by protozoa, helminths, bacteria, viruses is far from fi solved, as well as the interruption of the transmission chain of pathogens, taking into account the cycles and characteristics of development itself of vectors. The vector-host-pathogen system acts constantly with the species for different species of parasites and conditions. The ability to efficiently suppress the vector population, for a long period of time, is considered decisive in choosing the optimal strategy for combating vector-borne diseases [20].

The difficulty in solving the problem of parasitosis consists in the diversity of parasite species, the high resistance of pathogens to environmental factors, as well as in the existence of the large range of intermediate, complementary hosts in a constantly changing environment.

The problem of parasitic zoonoses, and especially zooanthroponoses, is relevant to the whole world. The transmission of pathogens through the elements of the natural environment and mainly through animal metabolites is dominant in epidemic situations. In this case, the effects of infecting people with parasitic agents from animals are often tragic.

Some theoretical and applied aspects of the parasitosis problem are the following:

► first, in the strategy to control parasites, a constant negative point is observed - the "treatment" of the consequences, without eliminating the cause;

► with the poor sanitation of pathogens and the insufficient use of drugs, it is necessary to constantly take into account the emergence of new generations of resistant parasites and their spread in the environment.

In recent years, there is a trend in parasitology, in terms of the ecological nature of processes and technologies. Of particular importance is the ability of parasites, from the stages of the biological cycle, to adapt to complex environmental conditions. It is observed that they can survive in extreme conditions during adaptation, expansion of the host spectrum and even in the shortening of biological cycles [20].

The high degree of adaptation of helminths and protozoa and counteracting the body's protective forces is explained by the fact that parasites in the molecular structure of their proteins, reproduce the structure of a number of host immunoregulatory proteins and suppress their immunity. At the same time, they survive and develop successfully.

Ecological prevention of parasitic diseases is based on the results of basic research in different directions. These directions in parasitology are important in general biology, veterinary medicine, human medicine and agronomy. In contemporary veterinary parasitology, the ecological direction of research is becoming increasingly important in the case of restructuring animal husbandry technologies. Ecological aspects refer, first of all, to pathogenic parasitic agents, whose biological development cycles are closely related to the environment. For this reason, it can fi formulate the following scientific direction fi that research - the bioecology of pathogens, *the basic elements and biotechnological principles of environmental protection from pathogens*.

This problem is multifactorial. In this context, ongoing epizootic monitoring and complex research are needed. Based on the results obtained, it is necessary to develop modern regulatory documents and standards, respecting the rules of ecological safety of objects in the veterinary complex.

In the strategy to combat parasitosis, this direction, in terms of science and application, requires independent development. Global environmental pollution, associated with anthropogenic pressure, requires the development of integrated control methods. We emphasize in particular the fact that, in the last years, there is an obvious orientation of the promotion of ecological parasitology both in general, theoretical, and in applicative, medical plan.

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ESTABLISHING THE ROLE OF AMPHIBIANS (ANURA) IN THE PROPHYLAXIS OF HELMINTHS SPECIFIC TO DOMESTIC, WILD AND PET ANIMALS

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Abstract. This work is based on helminthological data of amphibians, collected since 2013 until 2020, in the Republic of Moldova. The investigations on anura amphibians were conducted in the laboratory of Parazitology and Helminthology of the Institute of Zoology. One of the most common parasitic diseases in ruminants is fasciolosis, caused by the trematode Fasciola hepatica species. The results of parasitological research showed that adult cattle were infected with fascioles in 66.4% of cases, and young cattle - in 46.1% of cases. This is largely due to the grazing of animals of different species and ages in limited areas.The presence of the trematode species Haplometra cylindracea was established in 78% of cases in the amphibians in the Ranidae and Bufonidae families (Rana ridibunda, Rana lessonae, Rana temporaria, Bufo viridis). The results of laboratory helminthological investigations have shown that the relationships between the Fasciola hepatica miracidium and the Haplometra cylindracea miracidium are antagonistic. Amphibians of the Ranidae and Bufonidae families (Rana ridibunda, Rana lessonae, Rana temporaria, Bufo viridis) infested with Haplometra cylindracea tremateda may play an important role in the prophylaxis of fasciolosis.

Introduction

The most common helminths in domestic, wild and domestic animals are ecto-endoparasitosis, which causes them major economic damage [2]. The main biotic and abiotic factors (biological properties of parasite species, specific diversity and numerical efficiencies of hosts, thermal conditions, humidity, etc.) determine the existence and functioning of the main groups of endo- and ectoparasites in agrocenoses and in the natural biotopes.

Helminthological research performed on adult cattle, depending on the maintenance technology and the geographical area, has shown that they are infested with *Fasciola hepatica* in 34.8 - 48.2% of cases. In the body of cattle infested with *Fasciola hepatica* there are considerable changes in the liver and muscle tissue of the content of vitamins (A, C, B1, B6, E), micro- and macroelements (Ca, Mn, Na, K, Fe, P), leading to a considerable decrease in the quality of these products [2, 3].

Animal infestation occurs through the consumption of *Fasciola hepatica* adolescarium (infesting form), either with grazing or drinking water from biotopes favorable to fasciolosis.

The presence and circuit of the parasitic agent in the body of ruminants, causes essential changes in metabolism, digestive system, decreases the ability to assimilate food, which leads to weakening the body, decreased productivity, daily weight gain, and sometimes their death [5].

Ruminants or productive animals are the definitive hosts of the *Fasciola hepatica* species, but in the development cycle of this species also participate some mollusks species of the genus Lynmaea, which are an important source of food for amphibians.

The ecaudata amphibians can be definitive optional hosts in the development cycle of various helminth species (*Haplometra cylindracea, Alaria alata, Spirometra erinacei-europaei,* etc.), dangerous for the animals and humans [1, 6, 7].

Materials and methods

In order to identify the role of amphibians in the biological prophylaxis of helminths in animals, laboratory helminthological investigations of biological samples of amphibians were performed, on the presence of helminths or helminthic elements (eggs, larvae), which contribute to the formation and maintenance of common parasitic foci domestic, wild and pet animals.

Observation, collecting and obtaining data on the complex of anurans from *Ranidae (Rana ridibunda, R. lessonae, R. esculenta, R. temporaria, R. dalmatina) and Bufonidae (Bufo bufo, B. viridis*) families was performed in the center and south of Moldova.

The helminthological analysis of biological samples was performed according to the standard method proposed by K.I. Skrjabin, which involves the examination of all the internal organs of the animal [9].

Helminthological research of the parenchymal organs was performed with the help of compressors, and the digestive tract - by successive washes.

The collection, fixing, determination and processing of the helminthological material was carried after the methods proposed by various authors [6, 7, 8].

The diagnosis of the helminthosis in ruminants was established on the basis of complex parasitological research, with evidence of clinical manifestations of the disease, epizootiological, helminthocoproscopic, pathological and anatomical data, dissection of corpses, dissection of carcasses of slaughtered animals at meat slaughterhouses and in households. For the diagnosis of the fasciolosis in living condition were used the method of successive washing of biological samples, the methods described by Demidov, Vişncauscas, Darling and others [5].The miracidium of *Fasciola hepatica* and *Haplometra cylindracea* were obtained in laboratory conditions, in the Laboratory of Parasitology and Helminthology of the Institute of Zoology.

Fasciola hepatica eggs were placed in a thermostat at the temperature of 24-26°C during 10 days, after which, *Fasciola hepatica* miracidium were obtained.

Haplometra cylindracea miracidium were obtained by examining all the internal organs of the amphibians (*Rana ridibunda Rana lessonae, Rana temporaria, Bufo viridis*), by applying methods specific to the field of research. The *Haplometra cylindracea* specimens were kept alive in the thermostat, at a constant temperature of 24-26 ° C, in physiological solution.

In order to quantify the characteristics of helminthes contamination, the intensity indexes (II, specimens) was calculated - the minimum and maximum number of parasites of a species and the extent of invasion (EI, %) - the percentage of host contamination by a parasite species.

Results and discussions

Previous parasitological research has shown that adult cattle were infected by fascioles in 66.4% of cases and young cattle in 46.1% of cases [2]. This is largely due to the grazing of animals of different species and ages in limited areas.

From the body of ruminants infested with *Fasciola hepatica* (cattle), in the environment (pasture) eggs are eliminated, which reach the body of the species of mollusks *Lymnaea truncatula* - freshwater snail, which at the optimum temperature (18-25 ° C), inhabits in swampy areas. If the egg hit into a wet area, within 9-14 days the larva emerges - miracidium. The vitality of the larva is several hours, maximum 2-3 days, resembles a ciliate, has cilia, which move very quickly and have a special tactics for snails. This is a mandatory stage for the evolution of the helminth, apart from which the larvae cannot become contagious to cattle.

In the snail, the miracidium transforms into sporocyst 1. It divides into sporocyst 2, then into redia, redia-daughter, migrates into the hepatopancreas of the snail, where it transforms into a cercariae. The cercariae form the digestive tract and have a tail.

From a snail, in general, several hundred cercariae are eliminated. After coming out of the snail, the cercariae are fixed on the grass, with the help of cystogenic glands, which secrete a shell and turn into a larva trapped under the name of adolescaria. Their cycle lasts 2-2.25 months. Adolescaria are very resistant to the environment (they have a cystic form), they resist on the grass until autumn (the disease has a higher frequency in autumn).

Cattle become infected with *Fasciola hepatica* either when grazing grass with adolescarias in wet habitats, or by eating already infested hay. Once in the liver, it passes through the Glisson's capsule, which is sifted through the places where it crosses the young bundles due to its mechanical (cell breakage) and toxic action. The host organism (cattle), after 2-3 months, begins to remove eggs. The biological cycle of the trematode species Fasciola hepatica lasts about 5-6 months [3].

At the sanitary-veterinary expertise of the carcasses and organs, depending on the intensity of the fascioles infestation, it is recommended to establish three levels of infestation: small, medium and high.

The low level of infestation is detected in case of liver damage with a single specimen of fasciola (10-15%). At this level, as a rule, no visible changes are established on the surface and in the section of the liver parenchyma; when the bile ducts are suppressed, unique fascioles are removed from them. The low level of infestation is found in young people aged 2-3 years (25-28%).

The average level of infestation is detected when visually the damage is found not more than 2/3 of the organ. The section shows live fascioles and partial thickening of the bile duct walls with catarrhal inflammation of the affected part of the liver. Usually, this level is observed in animals aged 3-5 years (22-26%).

High level of infestation - the whole organ is affected. The liver swells and is hyperimulated, when touched the walls of the bile ducts are strongly thickened; their inner surface is rough due to the excessive growth of connective tissue; affected sections with dark red spots, coagulated blood and fascioles of different sizes (up to 1000 and more specimens) are detected in the section. The high level of infestation is characteristic for adult cattle aged 6-7 years and over (14-18%). Thus, in animals slaughtered more frequently, the medium and low intensity of infestation was detected.

The results obtained show that in cattle with low intensity of fasciole infestation, the moisture content in meat increases by 2.45%, average - by 3.53%, in those with increased infestation intensity - by 5.84%, and in the liver, respectively - by 2.44%, 5.59% and 9.46%.

In cattle with low infestation intensity, the dry matter content in meat decreased by 6.82%, average - by 9.85%, increased - by 16.67%, and in liver, respectively - by 5.63%, 12.91 % and 21.85%.The amount of protein in meat, in cattle with low infestation intensity, decreased by 3.23%, average - by 5.53% and in those with increased infestation intensity - by 8.3%, and in the liver, respectively - by 6, 01%, 9.87% and 16.31%. In cattle with low infestation intensity, the fat content in meat decreased by 25.74%, average - by 28.57% and increased - by 65.74%, and in the liver, respectively - by 2.27%, 29.55% and by 56.82%.

In cattle with low infestation intensity, the amount of mineral substances in meat decreased by 16.67%, average - by 16.67% and increased - by 25%, and in liver, respectively - by 7.14%, 14.29 % and by 28.57%.

In order to determine the nutritional value of muscle tissue and liver in cattle infested with fascioles, the content of vitamins (A E, B1, B2, C), micro- and macroelements (Ca, Mg, Na, K, Fe, P) was determined. It was established that in cattle infested with fascioles, the content of vitamin A in the liver decreased by 1.4 times, E - 3.18, B1 and B2 - 1.08 and 1.23, respectively, and C - 2.08 - 3.01 times, phosphorus - 4.17, calcium - 1.56, Mg - 1.04, and the Na and K content increased by 1.61 and 1.25 times, respectively, compared to uninfested animals.

In meat, the content of vitamin A was 1.83 times lower, E - 1.67, B1 -1.21, B2 - 1.11, C - 3.73, calcium - 1.60, phosphorus - 1 , 46, and magnesium, sodium, potassium and iron - respectively 1.34, 1.82, 1.30 and 3.14 times higher than in healthy animals. In the treatment of this disease are recommended various remedies of chemical origin (Albendazole, Closantel, Clorsulon, Triclabendazole, Oxyclozanide, etc.), to which over time the fascioles become resistant.

The role of ecaudata amphibians (*Rana ridibunda, Rana lessonae, Rana temporaria, Bufo viridis*) in the biological control of ruminant fasciolosis is explained by the fact that they are definitive hosts of the trematode species *Haplometra cylindracea* which in the cercariae stage parasitizes in the snail species *Lymnaea truncatula* - intermediate hosts for the species *Fasciola hepatica.*

Both parasites, meeting in the same host (*Lymnaea truncatula*), the species of trematode *Haplometra cylindracea* is antagonistic to the species *Fasciola hepatica*, causing the death of the larval stages of the fasciola. Snails of the genus Lymnaea, as intermediate hosts, have an aquatic and semi-aquatic way of life, so that they can be found in stagnant waters or humid environment (swamps), a favorable living environment for amphibians as well.

The amphibian species *Rana ridibunda, Rana lessonae, Rana temporaria* and *Bufo viridis* inhabit the most diverse biotopes, including aquatic basins, where the biological cycle of *Fasciola hepatica* takes place.

According to helminthological investigations performed on amphibians, their infestation was established in 78% of cases with the trematode species *Haplometra cylindracea* Zeder, 1800 from the Plagiorchiidae family.

Thus, the antagonistic relations between these two species of parasitic agents end with the interruption of the developmental cycle, of the larval stages (sporocyst, redia, redia-daughter, cercarium), of the *Fasciola hepatica* trematode in the intermediate host *Lymnaea truncatula* and later the interruption of its vehicular chain in biotopes.

The studies were carried out within the research project 20.80009.7007.12 and in the scholarship program offered by the World Federation of Scientists, Geneva 2020-2021

Conclusion

1. The results of parasitological research showed that adult cattle were infected with fascioles in 66.4% of cases, and young cattle - in 46.1% of cases. This is largely due to the grazing of animals of different species and ages in limited areas.
2. The presence of the trematode species *Haplometra cylindracea* was established in 78% of cases in the amphibians in the Ranidae and Bufonidae families (*Rana ridibunda, Rana lessonae, Rana temporaria, Bufo viridis*).
3. The results of laboratory helminthological investigations have shown that the relationships between the *Fasciola hepatica* miracidium and the *Haplometra cylindracea* miracidium are antagonistic.
4. Amphibians of the Ranidae and Bufonidae families (*Rana ridibunda, Rana lessonae, Rana temporaria, Bufo viridis*) infested with *Haplometra cylindracea* tremateda may play an important role in the prophylaxis of fasciolosis.

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THE HELMINTH FAUNA OF PONTIC SHAD (Alosa immacculata Bennet, 1835) FROM LOWER DNIESTER

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Abstract. In this study 60 specimens of Pontic were caught from lower Dniester, Stefan Voda district, Olănesti. As a result of the parasitological study of Pontic shad, three species of helminths were detected: Pronoprymna ventricosa (Rudolphi, 1819) Poche, 1926, Lecithasther confusus Odhner, 1905), Hysterothylacium aduncum (Rudolphi, 1802). The degree of infestation with these species was different. The most abundant species was Hysterothylacium aduncum with an extensivity of invasion of 85% and intensivity of invasion 1 – 80 parasites per fish, followed by Pronoprymna ventricosa (EI-20%, II-2-18 ex.) and Lecythaster confusus (EI-7,8%, II-1-12 ex.).

Introduction

Fishes like many other organisms, are hosts for a wide group of parasites that include monogeneans, cestodes, trematodes, nematodes, etc., and all these parasites can invade both freshwater and marine fishes. Parasitic diseases of fish are dangerous and can be a possible source of infestation for humans. The diseases produced by parasites can cause fish exhaustion, the deterioration of nutritional quality, commercial aspect, and other properties of fish. Sometimes, the determination of the degree of fish infestation with helminths only by visual inspection (size and nature of lezions) can be decisive. However, more often, the most dangerous parasites left on or inside the fish are invisible to the naked eye. The infected fish with larvae of parasites and undercoocking of fish can lead to severe parasitic diseases [14].Pontic shad (*Alosa immaculata* Bennet, 1835) is a migratory species, widespread in the northwestern sector of the Black Sea, the Sea of Azov and their rivers – Danube, Dniester, Dnieper, Bug, Don. During the period of february-march large stocks of Pontic shad concentrates near Dniester estuary and, being attracted by the river current, enters the Dniester for reproduction. Currently, Pontic shad has become vulnerable, and it is in a continuous decline due to overfishing, water pollution with wastes, and the damage caused to aquatic habitats [11].

In the 1950s of the last century, the Pontic shad was reported from Hotin to the Dniester estuary. After the construction of the Dubasari hydroelectric power plant, the migration of Pontic shad was limited by the Dubasari dam, and its reproduction took place more intensively in the Dubasari-Varnita sector, as well as in the Turunciuc river. In recent years, the most important places for reproduction are located in the Dubasari-Speia sector. The reproduction of Pontic shad takes place in March at a water temperature of about 5 °C, reaching its peak in late April-early May at 10-17 °C, and ends in late June at 22-24 ° C [1].

Helminth fauna of Pontic shad from Black Sea, the Sea of Azov, Danube, and Dnieper river have been studied [2,3,5,6,7,8,9,10], but there are no data on the helminth fauna of Pontic shad from Dniester river. This paper presents the results of the parasitological examination of Pontic shad captured from the lower Dniester.

Materials and methods

The ichthyological material necessary for this study was collected from the Dniester river, near Olănești, Ștefan Vodă district, during the second half of May, 2021. For this study 60 specimens of Pontic shad were parasitologically examined. The parasitological research was performed in the laboratory of Parasitology and Helminthology of the Institute of Zoology, according to the standard method proposed by Skryabin K.I. (the examination of all internal organs of the animal) and the method proposed by Dogel and modified by Bykhovskaia – Pavlovskaia [13,15]. The determination of the helminths was done acording to Bauer [12]. The microscopy of the detected helminths was performed using the stereomicroscope MBS, as well the examination at the optical microscope Novex Holland B, as fresh preparation slide-cover-glass, with the objective 4x, 10x, 20x and ocular WF10X DIN/20MM. The detected nematodes were stored in Barbagallo solution (3% formaldehyde and 0,9% sodium chloride), and the trematodes were stored in 70% ethanol and stained. For the parasitological evaluation, extensivity (%) and intensivity of invasion were used.

Results and discussions

As a result of the parasitological examination of 60 specimens of Pontic shad caught from the lower Dniester, 3 species of metazoans were detected: two species of trematodes (*Pronoprymna ventricosa* (Rudolphi, 1819) Poche, 1926 (Fig. 1), *Lecithasther confusus* Odhner, 1905) (Fig. 2), and one species of nematodes (*Hysterothylacium aduncum* (Rudolphi, 1802) (Fig. 3)).

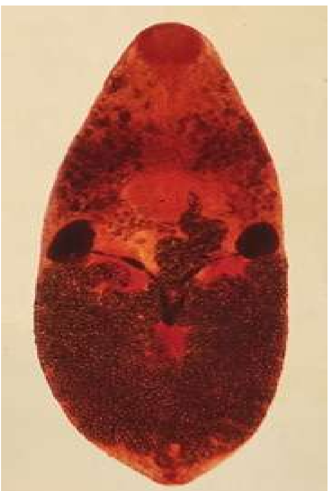

Figure 1. *Pronoprymna ventricosa* (Rudolphi, 1819) Poche, 1926 found in the intestine and pyloric caeca of Pontic shad (original photo) Republic of Moldova [19]

Figure 2. *Lecithaster confusus* Odhner, 1905 found in the intestine of Pontic shad (original photo)

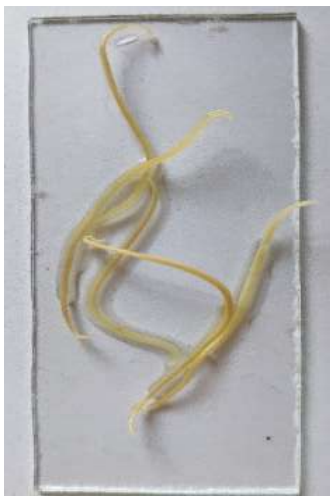

Figure 3. *Hysterothylacium aduncum* (Rudolphi, 1802) found in the stomach of Pontic shad (original photo)

Pronoprymna ventricosa is a trematode that parasitize in the digestive tract of many clupeids (Pontic shad, Caspian shad, Azov shad). This trematode has an elongated, small (0.40 mm up to 1.8 mm in length; 0.1 mm up to 0.7 mm in width) and fragile body, and highly developed dermal glandular cells in the anterior part. The oral sucker (0.07 mm up to 0.1 mm in length; 0.09 mm up to 0.20 mm in width) is subterminal, the pharynx is small, the vetral sucker lies in the anterior third of the body, and is slightly larger than the oral one (0.09 mm up to 0.2 mm in length; 0.12 mm up to 0.20 mm in width). The testes, horizontally, lie in the middle third of the body, behind the abdominal sucker. The ovary lies medianly, behind the testes. The vitelline glands are kidney-shaped and lie in front of the testes. The uterus lies in the back half of the body. Eggs are small and numerous.

During the parasitological examination specimens of *Pronoprymna ventricosa* were found in the intestine and pyloric caeca of Pontic shad. The prevalence was 20% and the intensivity of invasion 2 – 18 parasites per fish.

The parasite *Lecythaster confusus*, widespread in marine fishes (clupeids and gobiids), is a small trematode (1.2 mm up to 2.0 mm in length; 0.3 mm up to 0.6 mm in width). The diameter of oral sucker is 0.13 mm up to 0.16 mm. The ventral sucker (0.25 mm up to 0.27 mm in diameter) lies in the anterior half of the body.

During the parasitological examination specimens of *Lecithaster confusus* were found in the intestine of Pontic shad. The prevalence was 7,8% and the intensivity of invasion 1 – 12 parasites per fish.

The most abundant species found in Pontic shad was *Hysterothylacium aduncum*. The adult worms parasitize the stomach, piloric caeca, and intestine (some adult specimens have been detected migrating through viscera after the fish evisceration (Fig. 4)). The larval stages parasitize on the serous membranes of the abdominal cavity and internal organs, rarely in the muscle tissue. The parasite predominantly invades marine fishes. It invades salmonids, sturgeons, less often freshwater fishes that migrates into the seas (Black Sea, the Sea of Azov etc.).

Figure 4. Specimen of *Hysterothylacium aduncum* migrating through piloric caeca (original photo)

The life cycle of Hysterothylacium aduncum takes place with the presence of the first intermediate host represented by different species of copepodes, gastropodes, polychaetes. The first intermediate host is consumed by the second intermediate host, or reservoir host, represented by fishes of different taxonomic groups, crabs and shrimps. The definitive hosts – predatory fishes (marine and freshwater fishes) consume the second intermediate hosts, and in their digestive tract the nematodes molt twice and reach the sexual maturity [12].

As a result of the parasitological examination, creamy white specimens of *Hysterothylacium aduncum* were detetcted. During the parasitological examination many specimens of *Hysterothylacium aduncum* were found in the stomach of Pontic shad (Fig. 5). The length and width of the detected specimens varied (males – 18.0 – 20.5 mm in length, 0.43 - 0.51 mm in width; females – 24 –36 mm in length, 0.78 – 0.88 mm in width).

Pontic shad revealed to be highly parasitized with Hysterothylacium aduncum in terms of both larval and adult helminths (Fig. 6). The extensivity of invasion of Pontic shad with *Hysterothylacium aduncum* was 85% and the intensivity of invasion 1 – 80 parasites per fish.

Figure 5. High infestation of Pontic shad with *Hysterothylacium aduncum* (Rudolphi, 1802) (original photo)

Figure 6. Larvae of *Hysterothylacium aduncum* in the stomach of Pontic shad (original photo).

Conclusions

As a result of the parasitological study of Pontic shad captured from the lower Dniester, three species of helminths were detected: *Pronoprymna ventricosa* (Rudolphi, 1819) Poche, 1926, *Lecithasther confusus* Odhner, 1905), *Hysterothylacium aduncum* (Rudolphi, 1802). The degree of infestation with these species was different. The most abundant species was *Hysterothylacium aduncum* with an extensivity of invasion of 85%, followed by *Pronoprymna ventricosa* (EI-20%, II-2-18 ex.) and *Lecythaster confusus* (EI-7,8%, II-1-12 ex.).

The studies were carried out within the research project no. 20.80009.7007.12.

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RANISSUS SCYTHA (OSHANIN, 1913) (HEMIPTERA, FULGOROMORPHA, DICTYOPHARIDAE) IN THE FAUNA OF THE REPUBLIC OF MOLDOVA

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Abstract. The article contains new data on the cicada Ranissus scytha Oshanin, 1913 (Hemiptera, Dictyopharidae) from the Republic of Moldova. The first evidence of this species on the territory of the country can be found in 1966. This cicada species was rediscovered in 2015 in "Flamînda" Reserve (Pelinei, Cahul), and in 2016 in the steppe area (Vrănești, Singerei). No information about the presence of this species on the territory of the Republic of Moldova was included in the site Fauna Europaea

Introduction

Cicadas (order *Hemiptera*) represent an important component of fauna and play a significant role in ecosystems. At present, much more than 30 thousand species are described in the world fauna, from which more than 2000 species are in Europe. According to the Fauna Europaea (FE) 252 species from 13 families: Aphrophoridae, Cercopidae, Cicadidae, Tibicinidae, Cicadellidae, Membracidae, Ulopidae, Caliscelidae, Cixiidae, Delphacidae, Dictyopharidae, Issidae and Tettigometridae are recorded in the Republic of Moldova. The number of species is greater in the countries bordering with the Republic of Moldova: in Ukraine - 609 and in Romania - 430 species [3]. One family - Dictyopharidae belongs to infraorder Fulgoromorha, which species are more pronouncedly different from other groups of cicadas. Cicadas of this families are medium-sized, well-jumping insects. The head is often extended forward in the form of a cephalic outgrowth. The adults and larvae walk, raising the front part of the body. According to the FE in the Republic of Moldova there are two species of cicadas from this family *Dictyophara europaea and Dictyophara multireticulata, no information about the species Ranissus scytha in our country* [6].

Materials and methods

The article is based on the materials of our own collections in various points of the Republic of Moldova. During the research were used generally accepted methods in entomology - sampling (mowing with an entomological net over the vegetation) and collected material was determined in laboratory conditions with the help of determinants and with the involvement of an electronic resource. Also, the collection of cicadas of the Laboratory of Entomology and Biocenology of the Institute of Genetics, Physiology and Plant Protection was studied (the collection also contains this type of cicada found and identified by various entomologists in the 60s of the last century). The collected cicada specimens were confirmed by PhD Emeljanov (Russian Academy of Sciences).

Results and discussion

Geographical spread. According to FE the species *Ranissus scytha* (Oshanin, 1913) occurs in Ukraine, Bulgaria, Monaco and South European Russia. Emeljanov made the main contribution to the study of the subfamily Orgeriinae. In his works the information about phylogeny, evolution, new taxa and new data on distribution of the subfamily Orgeriinae in the Mediterranean are included [1, 2, 4].Systematic framing. The species *Ranissus. scytha* belongs to subphylum Hexapoda, class Insecta, order Hemiptera, infraorder Fulgoromorpha, family Dictyopharidae, genus *Ranissus*.Synonymous. The synonyms given during the study for *Ranissus scytha* treated in foreign sources are Orgerius scytha Oshanin, 1912 and *Schizorgerius scytha* Oshanin, 1913 [2].Morphology. The structure of the representatives of family Dictyopharidae is typical for Fulgoroidea, the body is longitudinally elongated, moderately flattened dorsoventrally, more convex below, legs most developed among Fulgoroidea. The front legs are slightly longer than the middle ones, which causes the front part of the body to be raised. Leaping hind legs are strong and proportionally longer than those of other Fulgoroidea. Species of subfamily Orgerıınae are more compact flightless monomorphically short-winged with thicker and more rigid covers.The most variable is the length and shape of the head. Ranissus has a short and broad head. Also, in its representatives, the middle keel is partially or completely bifurcated - this is the transition of the larval trait to the imago. Larvae of representatives *Ranissus genus* have been studied very poorly; they are the closest to the imago in appearance and way of life.

Bioecology of the species. *Ranissus scytha* prefers dry meadows, perennial deposits with shrubs, steppe areas, "gyrnets" slopes and herbs. In the southern regions of Ukraine, this species occurs in steppe areas with cereal grasses. Larvae were recorded in the same biotopes just like adult insects. It was found in Republic of Moldova earlier, specifically in Bendery, Dubossary, Chisinau, Kornesti, Hincesti, Rybnita [5]. The species was collected recently in "Flamînda" July-August 2015 (Peliney village, Cahul district) and July 2016 (Vrăneşti village, Singerei district) in steppe biotopes (fig. 1).

Figure 1. Biotope in the steppe area, near Vrănești village

Generally, the species of Dictyopharidae family are a thermophilic and sun-loving group, associated with undersized and therefore mainly herbaceous vegetation, with open spaces and therefore to large extent is xerophilic. The family is characterized by polyphagia and oviposition on the soil surface.

The species *Ranissus scytha* is a moderately hygrophilous species, therefore belongs to mesophilic group and often have flat life cycle. All available indirect data indicate that this group of insects has one generation per year with overwintering at the egg stage. Larvae are found in spring and first half of summer. Adults in most cases are met during all summer, gradually decreasing in number by autumn [2].

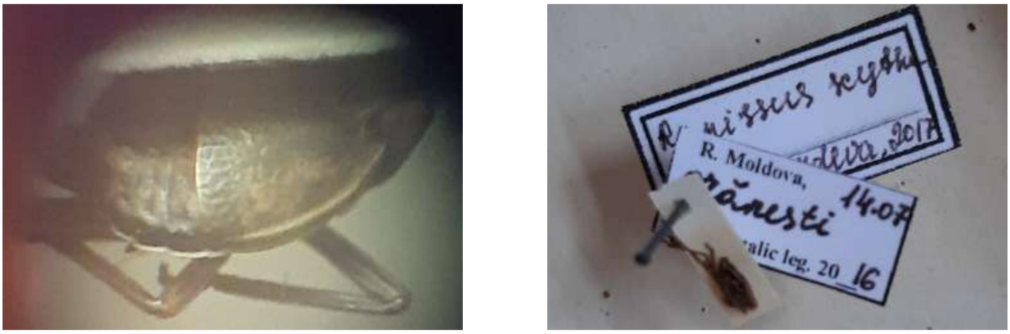

Figure 2. *Ranissus scytha*, Imago and labelling of the species

The figure above for the species *R. scytha* is the proof of identification and storage of the species in cicadas collection, which is preserved in the Museum of Entomology of the Institute of Zoology (fig. 2).

Conclusions

The faunal researches in 2015 in ”Flamînda” Reserve (Pelinei, Cahul) and in 2016 in the steppe area (Vrănești, Singerei) allowed to identified the characteristic fauna of cicadas for these areas, including the species *Ranissus scytha.* This cicada species has one generation per year with overwintering at the egg stage. Larvae are found in spring and first half of summer. Adults in most cases are met during all summer, gradually decreasing in number by autumn.

The list of cicadas collected in the Republic of Moldova in the FE needs revision.

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INVESTIGATIONS ON INVASIVE NEMATODES ASSOCIATED WITH COMPLEX INSECT PESTS FROM SOIL IN CORN IN THE ENVIRONMENTAL CONDITIONS OF THE REPUBLIC OF MOLDOVA

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Abstract: Corn is one of the major technical field crops in the Republic of Moldova, advantageous in bioecological and productive aspects, which is invaded annually by the harmful organisms with considerable parasitic impact. The phytosanitary control results, carried out annually and seasonally, comparatively in the corn plantations, notice a significant diversity of the specific diseases and of the invasive insects that seriously affect the plants from the germination phases until the harvest. In corn, were established the numerical density values (D. n.), comparative in different ecological areas, on average 15-280 (ex. /100 g soil), with an abundance by 5-25% higher in the autumn than in the spring. Phytoparasitic impact indices the frequency (F %) and intensity (I %) of the phytohelminthological disease level was estimated, being more advanced by 3-30% in the spring-summer period, observed differently depending on the area compared to the autumn period. The structure of parasitic phytonematode complexes was determined counting 20 species, included in 8 families and one order (Tylenchida), classified according to trophic specialization, with the predominance of endo-ectoparasites. It was established the diversity of the associated invasive insect species from the soil: 12 species, 8 families, 3 orders, also, with invasive ectoparasitic impact on corn plants, with the disease degree of 5-40%, detected in all phases of vegetation and sectors investigated.

Introduction

Corn cultivation in the Republic of Moldova is of universal importance for the primary and secondary finished product used in alimentation, in livestock sector and various industries. It is advantageous due to the high production capacity compared to other cereal crops; it has advanced ecological plasticity; is an efficient precursor for some field crops, absolutely mechanized cultivation techniques, with high rates of organic fertilizers use, minerals and water; various facilities for the caryopsis use and marketing of production and as seed material [5, 12, 15].

These advantages are conditioned by the bioecological adaptation qualities in impact with abiotic stress factors such as: relative tolerance to drought and heat, mechanical resistance; extended leaf area, mechanisms that ensure efficient water consumption, homeostasis regulation and induction of specific resistance. As disadvantage is the attack of a large number of harmful organisms, including communities of parasitic nematodes associated with complex pest insects from the soil, even in the first stages of growth (germination - formation of 3-5 leaves), which cause serious damage to corn annually [2, 4, 5, 12, 17, 23, 25, 26].

The phytosanitary biological control, performed periodically on corn plantations is essential for the detection of nematodes and insects invasive associations, for elaboration of prognosis and remedial application to adjust the parasitic impact on the host plant. Successful management of nematode complexes and harmful insects from soil, ensures the development of an effective program of integrated protection management, which is based on keeping the population of harmful organisms below the economic threshold of damage, to be harmless to the entire agroecosystem [1, 2, 4, 15, 23, 26]. The respective investigations were carried out in various areas and sectors of corn and seeds production, compared to the experimental investigated sectors in improvement and approval conditions of new forms and hybrids within the Phytotechnical Institute “Porumbeni”. Our purpose was to carry out phytosanitary monitoring of invasive nematodes and harmful insect complexes in maize, compared to various productive sectors, nurseries, soils of experimental approval, with the establishment of the level of parasitic impact in the technological management of integrated protection procedures, according to the economic damage threshold. Based on the mentioned purpose, we set the following research objectives:

- the diversity and structure estimation of parasitic nematode complexes associated with harmful insect species in provoked impact of agroeconomic importance to corn growing;
- carrying out phytosanitary records in establishing the degree of invasive helminthological and entomological parasitic impact on corn, comparative depending on areas, productive, experimental and demonstration sectors.

Materials and methods

The investigations were carried out by mutual agreement between the “Seminology corn”, “Technology” laboratories, the Phytotechnical Institute “Porumbeni” and the “Parasitology and Helminthology” laboratory, Institute of Zoology, 2019-2021 (figures 1-6).

In the field, the evaluation of ameliorating corn seeds and perspective hybrids was carried out, various productive and seeding sectors with an area of over 600 ha were investigated, distributed in experimental soils, homologation lands of newly created hybrids, suitable for cultivation areas. Breeding forms and productive perspective hybrids are located in three repetitions on plots with an area of 4,9 m2 and a density of 55-65 thousand plants per ha (30-35 plants on a row). There were researched more than 350 approved and perspective corn hybrids, 260 parental forms and 60 homozygous lines, used in the breeding process. Comparatively, the corn hybrids were studied, homologated and perspective from the Northern area (Pelenia v., Drochia d.), State Station for the field crops testing and homologation. Seed registration was performed according to the classification scale after the corn’s reaction to nematode complexes and pest insect species. The corn was monitored periodically, immediately after the germination of caryopsis (10-12 days until the formation of 8-10 mature leaves), with phytosanitary assessments. Over 300 soil samples were taken, at a depth of 15-30 cm, and the parasitically affected plants. The establishment of the helminthological and entomological parasitic disease was performed visually in the field with the help of a magnifying glass (optical degree, 100 MM), and in order to ascertain the criteria of extensiveness and the level of attack, indices of parasitic disease were used, using the values of number density (D. n./m2, plant), frequency

(F%), intensity (I%), reflecting the extent of the attack (GA%), reported in m2, with the analysis of 100 plants/10 samples, by finding the diseases at the level of root or plant, using the gradation of 5 balls (0 balls - no attack, 4 balls - serious diseases> 50%). Subsequently, the soil and plant samples taken, were subjected to laboratory analyzes by additional observations, using the binocular magnifier MBS - 10 and the binocular microscope, and highlighting the level of phytoparasitic, plant diseases was confirmed with the Canon EOS 1000 D camera [2, 4, 8, 9, 10].

The study of the faunal material collected and labeled preventively, was investigated according to the classical and current methods adapted in accordance with the requirements of the "Parasitology and Helminthology" laboratory, for helminthic and entomological laboratory analyzes. The nematological analyzes were performed with the methodological installation "Baermann funnel", by flotation - decantation - filtration through sets of sieves, with various perforations sizes, specific for some nematode taxa, extracts from the soil and affected organs. Then, they were listed according to the evidence, were fixed and determined by the trophic spectrum and taxonomic affiliation, using current identification keys [1, 2, 6, 13, 16, 19].

The methods of evidence on corn pests with soil and plant samples collecting were performed, depending on the sector's area, each 10 plants and soil from the rhizosphere, according to the diagonal repetition, where was noted the presence of beetles (larvae, adults), lepidoptera (larvae), dipterans, himenoptera. The density of detected pests and the affection's degree on the corn plants in the ontogenetic dynamics was highlighted. Subsequently, in laboratory conditions, some structural peculiarities were established, the taxonomic affiliation was determined, as well as the abundance and the disease level on various organs, the trophic specialization spectrum, by documenting and taking pictures. To confirm the taxonomic identity of the collected insects, the entomology publications were used [3, 7, 10, 13] as well as identification keys [18, 22 etc.].

Figures 1-3. Research and teaching activities, mutual collaboration in the laboratory and in the demonstration sector with new hybrids, Phytotechnical Institute "Porumbeni", 2019-2021

Figures 4-6. Experimental sectors phytosanitary investigated for the presence of invasive nematodes and affections caused by pests in corn, Phytotechnical Institute "Porumbeni", (Pascani v., Criuleni d.)

Results and discussions

The appearance and onset of helminthological diseases and polyphagous insects in corn, in the environmental conditions of the Republic of Moldova, are primarily determined by unstable climatic factors, quickly extensiveness and parasitic impact of pests. In the case of the onset of helminthotic diseases, with specific pathogenic and non-pathogenic effect, such as: pratylenoses, heteroderoses, tilenoses, accompanied by complexes of beetles and lepidoptera from the soil, periodically affect the plants, because the leaves absorb all nutrients from the stem to form berries, which causes the leaves to dry out, the stems to fall off, premature breaking, damage that diminishes the total yield of the corn crop. These associations of helminthic invasive agents and harmful insects on corn are in the attention of branch researchers, who frequently carry out investigations of phytosanitary records, which are included in research programs in the field of phytotechnics and breeding processes in creating forms, inbred lines, new corn hybrids, with resilience and tolerance to environmental stress factors. According to bibliographic sources, the corn crop causes damage annually, over 200 species of harmful organisms, of which 20-25 species are very dangerous, invasive, and in unstable climate conditions are manifested as significant pests of corn plants depending on the area, precocity, seed material, hybrid, environmental factors [3, 7, 11, 14, 13, 21].

Phytosanitary records on corn areas and sectors were carried out periodically, from the third decade of April - May - June - July - August. The May-June months were characterized by periodic abundant rainfall, slight increase in temperature, which determined the abundance of reproductive potential of hatching of invasive larvae, with aggressive infestation capabilities in plants, increasing the degree of parasitic helminthological impact, already in the formation phases of leaves, stems until the formation of panicle and cobs. In these months, late spring - summer the phytohelminthosis symptoms remain accentuated, more advanced and extensive by the dynamics of visual disorders of yellowing, low number of mature leaves, poorly developed, dwarfs with roots severely affected by necrosis and specific rot, caused by helminths. The frequency and intensity values of parasitic helminthic impact advance from 3% to 25%, which indicates the damage of more advanced corn plantations in the early stages (germination, 5-8 adult fungi), according to the sensitivity of plants to frequent frosts and instability of factors.

The estimated values in Table 1, characterize the comparative indices of the parasitic impact degree, by numerical density (D. n. ex./100g soil), frequency of diseases (F%), intensity of degree of attack (I %), reflecting the extent of the attack, compared to the number of soil samples analyzed and plants per m2, compared with sectors and areas investigated. The numerical density (D. n.) of the parasitic nematode complexes is by 10-25% higher in the II-III decade of June than in May, respectively and the frequency and intensity of the degree of attack is more advanced in the summer, being facilitated by favorable environmental conditions, high prolificacy and available food ration (tab. 1, fig. 7). These investigations are important for establishing a qualitative and quantitative diagnosis of the parasitic helmitological impact, for the elaboration of forecasts in the application of integrated protection measures, in the capitalization of corn with various precocity and cultivation areas.

Table1. The comparative indices estimation of helminth parasitic impact on corn cultivation, in average values by investigated areas and districts, May-August, 2020

Areas and districts investigated	May 25, 2-3 leaves			June 20, 5-10 leaves			20 July, flowering - pollination			August 25, caryopsis formation and ripening		
	D.n. (100 g sol)	F. (%)	I. (%)	D.n. (100 g sol)	F. (%)	I. (%)	D.n. (100g sol)	F. (%)	I. (%)	D.n. (100 g sol)	F. (%)	I. (%)
North, Drochia d., Pelinia v.	15-30	10-15	3-10	40-180	15-25	10-15	30-150	12-17	10-12	120-250	15-30	12-18
Center, Criuleni d.	50-80	5-10	3-7	60-180	10-17	7-12	50-180	3-10	3-7	140-280	10-20	7-12
South-East, Căușeni d., Grigoriev-ca d.	40-100	7-20	10-15	70-150	15-28	12-15	20-90	15-20	10-12	80-180	15-25	10-15
South, Ceadîr-Lun-ga, t. Svet-loe v.	60-120	5-15	7-10	130-240	12-20	8-12	40-130	10-15	7-10	40-90	10-20	8-13

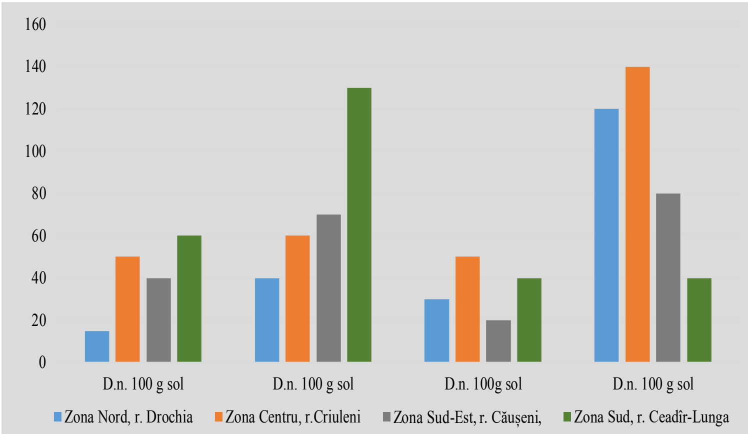

Figure 7. Estimation of the nematodes number density indices, established on corn, by areas, administrative districts, in the growth and development phases in the period 25 May – 25 August 2020

Successful management of harmful organisms is ensured by the application of a permanent phytosanitary control program and integrated protection procedures, which includes a number of components, including ways to regulate by reducing the number and parasitic impact. The approach in phytosanitary practice of the integrated management of harmful organisms is based on keeping the population of the harmful organism below the economic damage threshold, with periodic control records. Another important aspect in completing the helminthological investigations in corn is the study of establishing the frequency and structure of the parasitic nematode complexes noticed in the researched corn plantations. For the first time in the Republic of Moldova, these corn researches were performed related to the establishment of parasitic impact indices with specialized parasitic nematodes adapted to corn and the taxonomic abundance on investigated areas with their distribution according to the trophic spectrum of specialization, reflected in the table number 2. The results of taxonomic analyzes showed the presence of 21 species from 8 families with diverse trophic specialization (endo-ecto-semiendoparasite), migratory and sedentary.

It was highlighted the presence of species from the families *Hoplolaimidae, Paratylenchidae, Telotylenchidae, Criconematidae, Neotylenchidae, Tylenchidae*, present in the late spring-summer periods in various forms and biological stages extracted both from the soil and from the roots of plants (fig. 10). All species were classified according to parasitic trophic specialization in 5 groups, with the predominance of endo- and ectoparasitic forms of absorbent bristles, according to the mode of adaptation, growth phase, biotope, environmental factors [4, 8, 19, 20, 21].

Table 2. Taxonomic analysis results of parasitic phytonematode communities detected in corn, comparative on investigated areas, 2019 – 2021

Taxonomic name of the species detected	Phytoparasitic trophic specialization	North area	Center area	South – East area	South area
I. Pratylenchidae fam.: 1. *P. subpenetrans* 2. *P. nanus* 3. *P. curvatus* 4. *P. hamatus*	Migrants - endoparasites	++ ++ + +	++ ++ ++ +	++ ++ ++ +	++ ++ + +
II.. Paratylenchidae fam: 5. *P. cuvitatus* 6. *P. aciculus* 7. *P. nanus* 8. *P. tenicaudatus*	Migrants - ectoparasites	+ - ++ +	+ + ++ +	++ + + +	++ ++ + -
III. Hoplolaimidae fam.: 9. *Helicotylenchus digonicus* 10. *H. dihistera* 11. *Rotylenchus agnetis* 12. *R. incultus*	Semi-endoparasites, nutrients absorbing bristles	++ ++ ++ +	++ ++ + ++	++ + ++ ++	+ ++ ++ +
IV. Fam. Telotylenchidae: 13. *Amplimerlinius dubius* 14. *Merlinius brevidens* 15. *Bitylenchus parvus*	Ectoparasites of absorbent bristles	- + +	+ + +	+ + +	+ + -
V. Criconematidae fam.: 16. *Mezocriconema xenoplax* 17. *Xenocriconemella macrodora*	Ectoparasites of absorbent bristles	+ -	+ +	+ +	+ +
VI. Neotylenchidae fam.: 18. *Psilenchus aestuarius* 19. *P. aberans*	Ectoparasites	- +	+ +	+ +	- +
VII. Tylenchidae fam.: 20. *Tylechus filiformis* VIII. Heteroderidae fam.: 21. *H. avenae*	Endoparasitic-migratory Cyst forming	++ +	++ +	+ ++	+ ++
Total: 8 families 21 species	**5 trophic-parasitic specialization groups**	**21**	**26**	**27**	**25**

It was highlighted the presence of species from the families Hoplolaimidae, Paratylenchidae, Telotylenchidae, Criconematidae, Neotylenchidae, Tylenchidae, present in the late spring-summer periods in various forms and biological stages extracted both from the soil and from the roots of plants (fig. 10). All species were classified according to parasitic trophic specialization in 5 groups, with the predominance of endo- and ectoparasitic forms of absorbent bristles, according to the mode of adaptation, growth phase, biotope, environmental factors [4, 8, 19, 20, 21].

At the same time, the corn was monitored for the presence of insect species associated with soil nodule complexes and equal environmental conditions. Table number 3 estimates the species of harmful insects detected in corn, practically in all the researched areas, with different degree of disease and numerical density. They are visible from the first phases of vegetation, as invasive pests from the soil, which caused significant damage to young seedlings: wire larvae of the genus *Agriotes spp.* high attack estimating values of 25-30%. With the maturation of the plants, the larvae of the nocturnal owl species (*Agrotis segetum, Autographa gamma, Heliothis armigera*) are gradually associated, which also register an advanced attack of 15-25%, comparative to the sectors, then the corn borer (*Ostrinia nubilalis*) is associated, which had a high attack rate of 10-20% (figures 8, 9). Significantly, during the research period, in the sectors monitored for corn, was also reported the dangerous object of external quarantine for the Republic of Moldova - *Diabrotica virgifera virgifera* in the seed sectors, North area, Soroca district.

Table 3. Pararasitic entomofauna diversity established in corn during the vegetation period with invasive impact

Name of the species	Numerical density of pests detected on average 10 soil samples - 100 plants analysis				Affection degree	Infestation level
	May - June		July - august			
	Larvae	Adults	Larvae	Adults	%	%
Cracking beetles (true and false wire larvae from *Agriotes spp*. genus, *Elateridae* fam., Esch., 1829;*Tenebrionidae* fam., Latreille, 1802	**4**	**3**	**2**	**3**	**15-20**	**10**
Corn leaf weevil -*Tanymecus dilaticollis, Curculionidae* fam., Gyllen., 1834	**3**	**6**	**-**	**-**	**25-30**	**15**
Owl species *Agrotis segetum* Schiff., 1775; *Autographa gamma* L., 1758; *Heliothis armigera* Hüb., 1808, *Noctuidae* fam,	**3**	**7**	**12**	**17**	**20-40**	**18**
Gryllotalpa gryllotalpa, Gryllotalpidae fam., L., 1758	**3**	**6**	**2**	**2**	**5-7**	**8**
The black corn beetle -*Pentodon idiota*, *Scarabaeidae* f., Herbst, 1789	**0**	**4**	**7**	**4**	**7-10**	**5**

May beetle (white larvae)- *Melolontha melolontha, Scarabaeidae* fam., L. 1758	**5**	**3**	**-**	**-**	**5-7**	**7**
Corn borer -*Ostrinia nubilalis, Crambidae* fam., H, 1796	**0**	**12**	**33**	**14**	**10-20**	**30**
Western corn rootworm -*Diabrotica virgifera, Chrysomelidae* fam., LeConte, 1868	**-**	**-**	**15**	**7**	**5-10**	**5**
Total: 12 species; 8 families.	**3-5**	**3-12**	**2-33**	**2-17**	**5-40**	**5-30**

Figure 8. Affections caused by *Tanymecus dilaticollis* beetles

Figure 9. Corn contaminated with helminthiasis associated with wire larvae from *Agriotes spp*. genus

Figure 10. Associations of nematodes extracted from corn samples, analyzed under the microscope

Conclusions

Successful management of the pests estimated in those studies ensures the implementation of a permanent phytosanitary control program and integrated prevention and protection procedures, which includes a number of ways to regulate the number and reduce the impact of invasive parasites. The helminthological and entomological phytosanitary investigations results, carried out in 2019–2020 on corn cultivation, estimate the indices of diversity and structure of invasive nematodes communities and harmful insects from the soil, with comparative establishment of seasonal parasitic impact, vegetation phases, cultivation technologies, ecological areas.

Following the evidence surveys and helminthological analyzes performed on corn, the degree of infestation was established, by estimating the comparative indices of numerical density (D. n.), in variable values on areas of 15-280 ex./100 g soil, with the prevalence of numerical numbers more abundant by 25 -30% during May-June, comparative to July-August. Frequency indices (F %) of attack level, intensity (I %) and extensiveness of helminthological and entomological diseases in corn, estimated values of 5-40% in the critical phases of germination-maturity, which is facilitated by the plants sensitivity by accumulations of biological reserves in autumn period in the soil.

It was found the parasitic nematodes diversity and structure in corn culture in number of 32 species included in 8 families of the Tylenchida order, distributed according to the investigated areas and classified into 5 trophic specialization groups, with estimation of endo-ectoparasitic migratory species from families: *Hoplolaimidae, Paratylenchidae, Telotylenchidae, Criconematidae, Neotylenchidae, Tylenchidae Pratylenchidae* widespread abundantly in virtually all areas investigated. At the same time, the diversity and the level of associative disease of the complex insect pests in the soil were established in a number of 12 species included in 8 genera and three orders of the Insecta class, noted for severe challenges to corn roots and stems, reported in all phases and the investigated plantations, where the true and false wire larvae, *Elateridae, Tenebrionidae*, proved to be more aggressive; corn weevils, *Tenebrionidae*, owl species, *Noctuidae*; the black beetle and the corn beetle, Scarabaeidae; the larvae of the corn borer of the Crambidae family and the detection of the dangerous quarantine species of the western worm of the corn roots - *Diabrotica virgifera, Chrysomelidae,* on the northern areas.

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A COMPARATIVE GENETIC STUDY ON EXPLOITED VS. UNPERTURBED WILD POPULATIONS OF HELIX POMATIA (L., 1758): PRELIMINARY RESULTS

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Abstract. Helix pomatia is one of the most well known species of land snails across Europe and it presents economical importance due to its consumption as food. The preliminary results of the genetic comparison between two populations, one of them under the pressure of exploitation, are presented here. As expected, most indices revealed a disequilibrium in the exploited population, with the exeption of the allelic pattern which was similar among the two studied populations.

Introduction

The Roman snail *Helix pomatia* Linnaeus, 1758 is a quite well-known land snail species due to its distribution across almost the whole Europe [7]. It is also one of the biggest species, its shell reaching 4.5 cm [7]. For this reason, *Helix pomatia* is farmed for consumption, together with *H. lucorum* and *H. aspersa*, and it is highly appreciated for its taste. *Helix pomatia* is commonly consumed in the Mediterranean region and was introduced to this end in countries such as Great Britain, the Netherlands, Sweden, Denmark [14] and wild populations are also exploited. In Romania, wild *H. pomatia* populations are also exploited since the 1950' [5]. In the EU, the Roman snail is a protected species and is included in the 92/43/EEC Habitats Directive, Annex V and is mentioned in the Berna Convention, Annexe III.

The species has been extensively analysed in terms of its biology and ecology, which are more important for achieving optimal conditions necessary for successful growth and reproduction of individuals in snail farms. Land snails in general are sensitive to climate variations, and it has been shown that *H. pomatia* populations are easily affected by extreme opposite phenomena (e.g., floods, droughts) [6]. Until recently, the species in the Helix genus were barely studied from a population genetics and phylogeographic point of view. The most recent genetic studies refer to the phylogeography and phylogeny of the Helicidae family or the species within genus *Helix* [4, 8, 9, 10, 11].

The present study analyses the genetic structure of two wild populations of *H. pomatia* from Romania using DNA microsatellite markers. One of the populations is constantly exploited, while the other is left unperturbed. The population genetics analyses undertaken in this study will be able to assess the genetic state of health of the analysed populations and to highlight possible threatened populations/subpopulations of this species.

Matherials and methods

Two wild populations of *H. pomatia* from Romania were chosen for this study. One population, BT (Biertan, Sibiu County), is constantly being exploited, while the population MAD (Măda, Hunedoara County) is completely unperturbed. 30 mature individuals were collected from each population and conserved in 70% ethanol. Complete genomic DNA was extracted from foot tissue using the ISOLATE II Genomic DNA Kit (Bioline, London, UK), according to producer specifications. Nine microsatellite markers were used for genotyping both populations: HP50, HP97, HP186, HP207, HP228, HP503, HP535, HP558, HP612, with the PCR genotyping reactions and conditions were used as described in Krapal et al. (2016). The genotyping was performed on an LI-COR 4300L genetic analyzer (LI-COR Biosciences, Nebraska, USA) and the SagaGT v3.1 was used for scoring alleles.

GenAlEx v6.501 [12, 13] was used to test for Hardy-Weinberg equilibrium at each locus and to estimate the number of alleles (NA), along with observed (Ho) and expected heterozygosity (He). The fixation index (F) and the genetic differentiation between the two populations were also estimated using GenAlEx. The effective population size (Ne) for each population was estimated using the Linkage Disequilibrium method implemented in NeEstimator 2.01 [2], with a Jacknife at 0,02%. GENETIX 4.05 [1] was used for the Principal Coordinates Analysis (PCoA). Population genetic structure was assessed using a Bayesian Markov Chain Monte Carlo model (MCMC) implemented in STRUCTURE v2.3.4 [15]. Ten runs were performed for each number of populations (K) set from 1 to 4. Burn-in time and MCMC replication number were set to 150,000 and 750,000 generations, respectively, for each run. The optimal number of clusters was identified using both the Evanno method (ΔK) [3] and the highest probability for K [15] following on the CLUMPAK online platform (https://clumpak.tau.ac.il).

Results and discussion

All loci were polymorphic in all genotyped populations, with a similar allelic pattern. For the BT population, 3 out of 9 single locus tests showed a significant deviation form Hardy-Weinberg Equilibrium ($p<0.01$) after Bonferroni correction, while for MAD population only 1 out of 9 single locus tests showed a slight deviation from HWE after applying the same correction.

The inbreeding level was assessed by the inbreeding coefficient (Fis), values close to 0 indicating a random mating process. The inbreeding was higher in the BT population (Fis = 0.230, SE = 0.106) than in the MAD population (Fis = 0.009, SE = 0.071), most likely due to the smaller number of adult individuals that mate.

The genetic differentiation between the two populations was moderate to high as evidenced by the Fst values (Fst = 0.127). This result was also evidenced by the analysis of molecular variance (AMOVA) which indicated an interpopulational genetic variability of 13% as compared with the intrapopulational genetic variability of 87%.

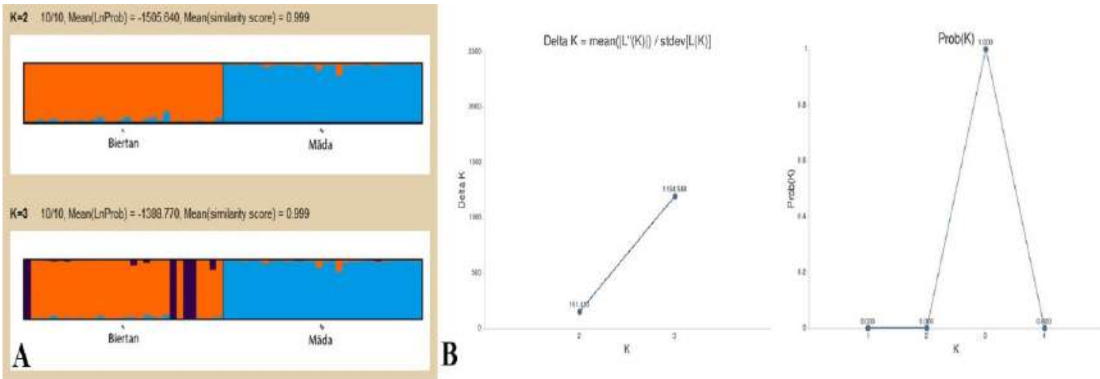

Figure 1. (A) STRUCTURE plot describing the population structure using K=3 as most probable number of genetic groups. (B) Probability by K graph using median values of Ln(Pr Data) the k for which Pr(K=k) is highest (Pritchard et al., 2000) and DeltaK graph - optimal K by Evanno (Evanno et al., 2005).

The effective population size (Ne) represents the estimated number of individuals that reproduce within a population. The MAD population seems to be healthier with Ne = 113.5 (95% CI = 41.7-infinite), while the BT population is more at risk in case of disease or other such events (Ne = 6.6, 95% CI = 4.7-8.7).

The genetic structure analysis has shown a separation of the samples in 3 clusters (K = 3), with the BT population split into 2 subpopulations (fig. 1). The same structuring was evidenced through the Principal Coordinates Analysis (PCoA), a small group of individuals from the BT population being clearly grouped in a separate cluster (fig. 2). The clustering of the BT population into 2 separate groups might signify a possible habitat fragmentation.

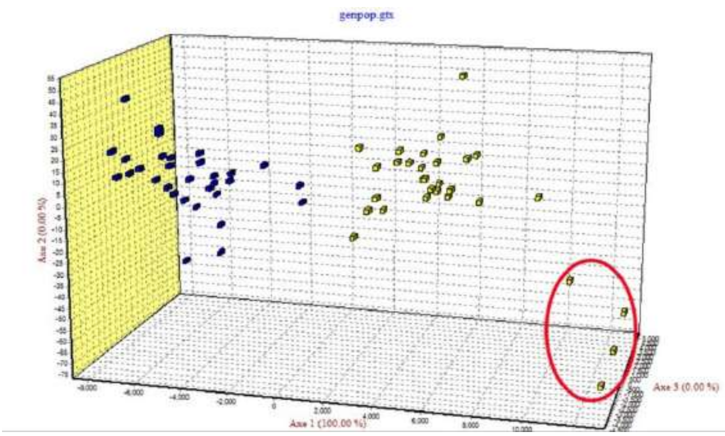

Figure 2. Principal Coordinates Analysis (PCoA) via Covariance matrix with data standardization - PCoA of genetic distances between individuals; BT in yellow, MAD in blue.

The genetic differences between the two populations shown by the HWE, the Fst values and the clear genetic structuring highlight the effects of constant pressures that the BT population is under. The constant exploitation of some populations, in the current context of climate changes (e.g., drought, sudden floods, high temperatures), represents an additional pressure for populations that may be threatened and become extinct in the absence of protective measures. The genetic parameters used in this study can be used to identify the vulnerable populations of this species in order to take measures for their protection.

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THE INFLUENCE OF THE ZOOBIOR REMEDY, USED IN AN IMPLEMENTATION STUDY ON THE HEALTH AND CLINICAL-HEMATOLOGICAL STATUS OF YOUNG HENS IN THE FIRST LAYING PHASE

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Abstract: This article presents the scientific results focused on investigating the impact of the ZooBioR remedy, obtained from Spirulina platensis, tested on young hens, in the first technological phase of laying. The research was initiated on young hens, of 24 weeks old, belonging to the "Hy-Line" hybrid, bred in identical conditions of nutrition and maintenance. The hens in the experimental group received daily food supplemented with ZooBioR, in proportion of 10 mg active substance/kg feed. During the study the birds were monitored, and periodically were determined body temperature, frequency of respiratory movements. For haematological investigations, from birds was collected blood from the axial veins and, as the case may be, from the jugular veins, in standard model test tubes. It has been established that the ZooBioR remedy is well tolerated by young hens and exhibits anti-stress and adaptive properties. In addition, the tested product has a positive evolution on the hemogram, increases the natural body resistance, inducing at the end of the study a tendency of increase of platelets in blood.

Introduction

It is a well known fact that nowadays we witness a rapid population growth on earth. This phenomenon has positive connotations, but leads to certain problems for the agri-food sector, when it comes to providing earth`s population with food, especially food of animal origin. In this regard, Chilimar S. mentions that in the high developed countries, the life standard is quite high, increasing the need of food quantity and quality [2]. According to the same source, in developing countries, as the economic level starts rising, also increases the demand in good quality food.

In this regard, poultry farming, a classic branch of modern animal husbandry, promptly intervenes in ameliorating the problem approached with double commitment: firstly, providing the consumer with meat, and secondly, namely with quality meat, with dietary and therapeutic properties, recommended for several social classes [7]. In the conditions of the Republic of Moldova, namely poultry farming is one of the most stable branches of the agro-industrial complex, which in a relatively short time has rapidly developed, reviving after a transition period the production of meat and eggs, thus contributing essential to ensuring the state food security.

Our research refers to understanding two essential moments: the prohibition of antibiotics and growth promoters in animal husbandry, and the persistent technological stress on poultry farms, etc. In recent years, according to the above-mentioned, an avalanche of research in several countries has started, highlighting the study and implementation in production of new medicinal remedies, giving priority to those of natural origin, especially of plant origin [1, 6, 7, 15].

The paper provides data on the effects of the local remedy ZooBioR, used in an implementation study, on the health and clinical-hematological status of young laying hens, in the first technological phase of laying.

Materials and methods

The research was carried out in physiological conditions of a poultry factory part of SRL - “Technological Acoustics”, from Floreni village, Republic of Moldova. The tested remedy was studied on 112 hens, belonging to the Hy-Line hybrid, divided into 2 groups of 56 heads each. The birds were analogous in terms of age, physiological condition, origin, body weight, being housed in the same shelter, with identical environmental conditions and veterinary care.

At the same time, the research objective was focused, both on the new product - ZooBioR, and on the influence of this remedy on young hens, bred for egg consumption. The tested remedy is of natural origin, containing biologically active compounds derived from Spirulina platensis, including amino acids, immunoactives ones as well, polysaccharides, phospholipids and the trace elements such as zinc and selenium.

This remedy was administered to birds in a ratio of 10 mg active substance/kg feed as the optimal dose, which was previously established, during the process of studying four different doses of ZooBioR [6, 8]. The principle of organizing this study is given in table 1.

Table 1. Scheme of ZooBioR administration to laying hens, 20 mg/ml

Specification	No of hens	Administration route	Dose, mg active substance/kg feed	Administration regimen
Control	56	-	-	
Experimental 1	56	per os with food	10,0	daily

In order to assess the health state, at the beginning of the experiment, and later on its interval, the birds were examined, and in 5 hens from each group, have been determined the body temperature and respiratory movements in one minute. To evaluate the impact of the tested product on the hematological status, blood samples were taken in three stages: at the beginning of the experiment, until the administration of the ZooBioR remedy, from 5 random hens; during the study, from 5 birds, each group - about 1 month from the beginning of the study, as well as later, at the end of this experiment, in standard test tubes. The blood samples taken from hens were read using a hematological analyzer: Mindrai BC 5150. The statistical calculation of clinical and hematological indices was made using the parametric criterion t-Student with an error less than 0.05 ($P < 0.05$).

Results and discussion

Based on the examination of birds over a period of 242 days, it has been established that the tested remedy did not cause adverse reactions in the gastrointestinal tract or in the whole body of hens. In addition, according to the research data, the tested product has anti-stress and adaptive properties in young laying hens, reflected in body temperature, which is lower by 0.22-0.48°C compared to the values of the control group. We also specify that the values of the respiration rate have the same tendency in their dynamics (with 3.6-9.6 movements/min), similar to body temperature, respectively lacking essential changes compared to the values of the control group. These data reflect the harmlessness of ZooBioR , as well as the homogeneity of the birds taken in the study. Similar results have been obtained in the case of testing this remedy on several batches of young laying hens [6], as well as in the case of testing other remedies on animals [12].

The study reveals the strong impact of the tested remedy on the evolution of hematological parameters in young chickens. The dynamics of these indicators is shown in Table 2.

Table 2. Evolution of hematological parameters in laying hens, in the first technological period of laying under the influence of ZOOBIOR product (M ± m)

Meaning	Beginning	Groups of animals	
		CG	EL
RBC, 10^{12}/l 1 sampling 2 sampling	3,16±0,09	2,92±0,19 2,28±0,15	3,01±0,10 2,41±0,06
HgB, g/l 1 sampling 2 sampling	114,40±1,75	118,4±0,98 145,8±2,46***	115,6±1,20 138,0±2,03***
HCT,% 1 sampling 2 sampling	45,04±0,72	39,40±1,91* 34,24±1,57	42,88±1,10 36,18±1,76*
MCV, fl 1 sampling 2 sampling	139,20±2,69	137,84±1,01 150,68±6,13	143,02±2,80 141,12±2,00
MCH, pg 1 sampling 2 sampling	35,70±0,69	35,52±0,63 45,66±2,60**	37,26±0,69 43,32±1,21**
MCHC, g/l 1 sampling 2 sampling	256,00±1,56	285,12±13,82 409,60±11,63	271,2±8,39 404,0±9,62

Note: * p<0,05; **p<0,01; p<0,001

The data in table 2 reveal that the parameters of the erythrocyte profile in birds from both groups fall within the physiological intervals, recorded by most bibliographic references. In addition, the number of erythrocytes in blood (RBC) in birds, at the beginning of the study was on average 3.16 ± 0.09x1012/l, a value which at the first sampling shows a decreasing tendency, the decrease being in the control group (CG) of 7.6% compared to the base values. At the end of the study, the RBC index shows a clear decreasing tendency, which in the birds from the CG is 21.9% compared to the previous research ($p<0.05$). The same decreasing tendency of erythrocytes values persists in birds in EL, but at the end of the study it is 5.7% higher than in CG. The value of hemoglobin in the blood of birds from both groups has a positive dynamics during the study, a phenomenon highlighted at the end of the research, when this index in the CG reached the highest level of 145.8 ± 2.46 g/l, the difference being significant compared to the 1st research ($p < 0.001$). According to the data of the statistical analysis the hemoglobin values increased by 19.4% in EL, compared to the 1st research ($p<0.001$). At the last stage of the research, the hematological index investigated at EL was 5.4% lower than the reference values.

According to the data in Table 2, it has been established that the mean erythrocyte volume (MCV) in hens, in the first months of laying, in birds from both groups has practically not changed, while at the end of the study, there is a clear tendency of increase in the CG, of 9.3%. In addition, at this last experimental stage, the value of the investigated parameter in EG proved to be lower by 6.3% compared to CG, a positive tendency, reported as well by other authors who administered other biologically active remedies to birds [4].

Some authors reported a low level of MCV in the blood of rabbits, a universal biological model, raised in a relatively clean region, compared to the results obtained in an intensely polluted area [13].

The value of mean erythrocyte hemoglobin (MCH) in CG hens towards the first research term practically did not change, while in birds in EG, on the contrary, it showed a weak growth tendency of 4.4% compared to the background values, and respectively of 4.9% compared to CG values. At the end of the study we can highlight the fact that MCH has a clear tendency of increase in birds, both from CG and EL, the dynamics being in both cases significant ($p<0.01$). However, at this last stage of the research the average value of MCH in birds in EL is 5.1% lower compared to the reference values.

The obtained results show that the mean value of MCHC (mean amount of erythrocyte hemoglobin) in birds to the first stage of research has a tendency of 11.4% in CG birds and of 5.9% in EG birds, respectively, compared to the reference values. At the end of the study (the 2nd research) the investigated parameter in birds from both groups has a clear upward tendency, of 1.4 times in the CG, and of 1.5 times in the EG, compared to previous values, reported at the 1st research. In addition, the parameter investigated, at the end of study in the EG, is 1.4% lower than the CG values.

This fact can be considered positive, because it argues for the correctness of the study, as well as the harmlessness of the remedy tested on hens at the molecular level. In addition, similar tendencies of MCHC decrease in ground-raised broilers, treated with BioR and Catosal remedies, towards the end of the technological process have been found by other authors [11]. The values of the hematological parameters correspond to the values indicated in the literature [5]. When investigating the birds involved in this experiment, a set of data has been obtained with reference to the values of leukocytes and leukocyte components, which are shown in tab. 3.

Table 3. Dynamics of leukocytes and basic components of the leukocyte formula in young laying hens treated with the product ZooBioR (M ± m)

Meaning	Beginning	Group of animals	
		CG	EG
WBC, 10^9/l	12,6±1,05		
1 sampling		12,00±0,38	11,90±0,68
2 sampling		12,06±0,95	14,12±1,64
Lymphocytes, %	36,4±2,23		
1 sampling		53,00±2,35***	47,80±5,07
2 sampling		32,8±3,73**	50,6±7,72
Granulocytes,%	49,8±2,56		
1 sampling		34,60±3,63**	43,60±4,93
2 sampling		55,4±3,72**	39,0±7,36
Monocytes,%	10,8±2,46		
1 sampling		12,20±1,08	8,00±0,79*
2 sampling		10,4±0,84	9,4±2,31
Eosinophils, %	0,80±0,10		
1 sampling		0,20±0,21	0,60±0,17
2 sampling		1,40±0,27	1,20±0,07
Trombocytes,10^9/l	43,6±7,73		
1 sampling		45,20±6,11	37,40±3,83
2 sampling		26,20±4,02*	30,40±0,64

Note: * p <0.05; ** p <0.01; p <0.001

According to data from table 3, the number of leukocytes in blood at the first stage of research shows a weak decreasing tendency, both in CG of 4.8% and in EG respectively of 5.6% compared to the background values, reported at the beginning of the research. Towards the end of the experiment, ZooBioR remedy induced an increase in WBC in blood compared to the CG values, an increase of 17.1%, but without statistical significance. Similar results have been reported by other authors who have administered another biologically active product to adult quails [9].

Particular attention is paid to the investigation of lymphocytes in blood. The value of this parameter in the CG, at the first research, increased significantly compared to the background values, an increase by 1.5 times (p<0.001). At the same time, there is a similar increase of lymphocytes in the EG (1.3 times), but the difference is not significant compared to the background values. At this first stage of research, the investigated parameter in birds from the EG is lower by 9.8% compared to the CG value. Towards the end of the study, the relative number of lymphocytes in the CG shows a clear tendency of decrease by 1.6 times, compared to the previous values ($p<0.01$). In addition, it has been attested a weak growth tendency at EG, by 5.9% compared to the previous values. At this stage of research, there is a significant increase of blood lymphocytes in hens in EG, which is 1.5 times higher than in CG, the difference being significant.

The value of granulocytes in blood at the beginning of the study is an average of 49.8 ± 2.56%, a parameter that considerably decreases by the first research, especially at CG by 1.4 times ($p<0.01$). At the EG this parameter`s value is with 26.0% higher than at the control group, a positive dynamics, as result of using the tested remedy. It reveals the positive dynamics of the population of heterophils/lymphocytes (fewer lymphocytes, and more heterophiles) in birds. This ratio shows an average value of 0.65 in hens in CG, while in EG this parameter is 0.91 units, being 1.4 times higher compared to the CG. Similar results have been reported in other scientific papers as well [4, 10]. The value of granulocytes in birds from CG towards the end of the study has a clear tendency of an increase by 1.6 times ($p <0.01$), repeating the late shift of this previous parameter in EL. At this stage, granulocytes value in EL, on the other hand, have a decreasing tendency of 11.8%. At the same time, the investigated parameter in EG is 29.6% lower than the CG values.

Another studied parameter, blood monocytes, shows an increase tendency in the CG of 13.0% compared to background values, while in EG, on the contrary, there is a decrease of this parameter, of 25.9% compared to background values. At the first stage of research, the value of monocytes in EG is considerably lower than in CG (with 34.4%, $p<0.05$). At the last term of research, the investigated parameter shows a decreasing tendency (-14.8%, in CG), while in EG an increase tendency of 17.5% compared to the previous values. In addition, monocytes in EG are with 9.4% lower than in CG, a dynamics that can be considered positive. It can be explained by the fact that recent scientific data mention that the decrease of monocytes in the peripheral blood may be a consequence of their migration into tissues, and their transformation and maturation into macrophages [14]. Similar results regarding the decrease of monocytes in blood when administering other bioactive remedies to broilers are also revealed by other authors [3, 7].

The analysis of the relative number of eosinophils in blood reveals a similar dynamics, without highlighting some essential divergences in the groups of birds studied.

Table 3 shows that blood platelets value in young hens at the beginning of the research was an average of 43.6±7.73 x 109/l, a parameter that, in the 1st research in CG, increased with 3.7% if compared with the background values. At the same time, in hens from EL, there is a tendency of decrease of the investigated parameter, of 14.2% compared to the background values. In addition, at this first stage, the platelet value in EG is lower than in CG, the decrease being of 17.3%. Dynamic monitoring of platelets shows that at the end of the study it had a decreasing tendency in CG, reaching the average value in the CG of 26.20 ± 4.02 x 109/ l, a decrease of 1.7 times ($p <0,05$). This decreasing tendency persists in EG birds, but of only 1.2

times compared to previous values. Still, the investigated parameter in EG is higher than in CG, the increase being of 16.0%.

Our researches have established that supplementing food with ZooBioR product increases the productive potential of hens, a topic that will be addressed in a separate study.

Conclusions

1. The research was carried out on young hens, in the first technological phase of laying eggs, raised on a poultry farm, in identical nutritional and maintenance conditions, and the ZooBioR product was well tolerated by birds and did not cause any adverse reactions.
2. In the experimental group, treated with ZooBioR, body temperature and respiratory movements decreased which proves the adaptive and anti-stress action of the tested product.
3. The haematological examination in laying hens highlights the positive dynamics of the haemogram, results that prove both the beneficial effect of ZooBioR, and its harmlessness at cellular level.
4. It has been established that the medication of hens with the remedy ZooBioR increases the natural body resistance both at general and local levels, inducing at the end of the study a tendency of blood platelets increase.
5. The product ZooBioR, a natural, autochthonus remedy determines hens` health improvement in the first technological phase of laying eggs, thus stimulating their productive potential.

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NEMATODOFAUNA OF POTATO TUBERS IN THE REPUBLIC OF MOLDOVA

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Abstract. According to the multiannual researches, it was observed that, in the conditions of the Republic of Moldova, the Nematodofauna of potato tubers (*Solanum tuberosum*) of different varieties, collected from deposits, is specific, with the dominance of saprophytes due to the spread of bacteriosis and fungal infections. In the potato tubers, sick with dithylenchosis (phases 4, 5), the presence of 30 spec8es of nematoodes was identified, belonging to 24 genera, 14 families, 5 orders, 2 classes: 1. Class Secernentea, Orders Aphelenchida, Rhabditida and Tylenchida; 2. Class Adenophorea, Orders Dorylaimida and Plectida. Most species (19 species) are saprophytes of the Class Secernentea, Order Rhabditida with the dominance of the Superfamilies Cephaloboidea and Diplogasteroidea. According to the trophic-ecological group, the species detected in the potatoes tubers are included in all 5 groups: p*lant feeding, hiphal feeding, bacterial feeding, animal predation, omnivorous.* In all researched varieties, both in frequency (100%) and density (thousands of individuals/gram of infested tissue) distinguished species *Ditylenchus destructor* Thorne 1945, Order Tylenchida, Family Anguinidae, which is also the main parasite in the culture of *Solanum tuberosum* in the Republic of Moldova, capable of causing dithylenhosis, as well as epiphytotics – mass infestation of potatoes in deposits.

Introduction

In the Republic of Moldova, one of the main crops are potatoes - Solanum tuberosum.There are currently about 4.000 varieties and hybrids on the world market. It is cultivated in almost all countries around the world on a total area of 18.8 million ha [10]. Potatoes are valued for their rich content of carbohydrates - starch (95-99% of total carbohydrates), proteins, fats,vitamins B1, B2, PP, provitamin A, significant amounts of trace elements - K, P, Na, Ca, Fe, etc., which are necessary for the existence of the human body. Among the main foods it ranks 4-th after wheat, rice, corn. It is a product not only of food importance but also fodder, industrial, ecological, etc. In the Republic of Moldova, the largest areas with potato cultivation are located in the northern districts. Favorable conditions for potato cultivation are also favorable for a large number of their pests. Among the most dangerous are phytoparasitic nematodes, which form cyst - *Globodera rostochiensis, G. pallida and migratory endoparasites - tuber nematode - Ditylenchus destructor*, included in the list of quarantine species [7]. It is a host plant of sedentary nematodes of the genus Meloidogyne - *M. hapla, M. javanica, M. hispanica, M. incognita, M. arenaria* [15; 9]. Dithylenhosis disease, caused by potato tubers and seeds by the parasitic nematode *D. destructor*, is widespread in all agro-climatic areas of the world, and the decrease in yields under certain conditions reaches 43% [14].

It is important to mention that in the tubers infested by *D.destructor*, a very important role belongs not only to the phytoparasitic species, but also to other nematode species, such as the saprophytic ones. These are active inoculators of bacterial and fungal infections, being secondary factors of dithylenosis. It is they that cause the disease of potatoes to pass into the mycosis and bacteriosis. Potatoes are also attacked by a complex of pathogenic potato viruses, including quarantine - TBRV and TRV, fungi, which cause root mold, bacteria and a. [19]. According to previous research, in the culture of food potatoes in the Republic of Moldova, the presence of a single species of phytoparasitic nematodes was detected - *D. destructor*, studied the morphological structure and indicated some measures and methods to combat it [2; 6; 12; 16]. In parallel, ecological-physiological research was performed on t2he interrelationships between the phytoparasitic nematodes *Meloidogyne incognita, Ditylenchus destructor* and *D. dipsaci* with their host plants - tomatoes, potatoes, Allium crops [3; 4; 5].

The current research, unlike the previous ones, is carried out on different varieties of potatoes, which are widely grown in the republic in recent years, in the districts of North, Center and South - Agata, Albăstriu-mov, Bellarosa, Desiree, Irga, Iagodca, Kondor, Roko, Romano, Sprinter. A total of 10 varieties, the objectives being: the evaluation of the biodiversity of the communities of parasitic and saprophytic nematodes in the potato tubers in the autumn-winter-spring period; highlighting the obligatory species of pests of potatoes and the damage caused by them.

Material and methods

The evaluation of the diversity and structure of the communities of parasitic and free phytonematodes was performed on the potato tubers, collected from: the districts of the Northern area (Briceni, Dondiuşeni, Edineţ, Soroca); of the Center (Criuleni, Telenesti, Ialoveni districts); South (Ştefan-Vodă district). Samples of potato tubers were collected, in most cases, from individual households in the autumn-winter- spring period (after harvest, or stored in warehouses), about 15-30 tubers/sample, depending on the amount of potatoes investigated. 10 varieties of potatoes origin from locals or coming from abroad were researched. Faunistic material was obtained by the classical method of extracting nematodes from plant tissue using Baermann funnels, modified by Nesterov [17]. Before being analyzed, the collected tubers were thoroughly washed with tap water, then the portions with symptoms of dithylenosis disease were shredded with a scalpel into pieces the size of -5x5 mm. For each sample, 20 grams of infested tissue were weighed, which was then introduced into household sieves. The sieves were immersed in funnels, filled with water, so that the potato particles were covered. The exposure time was 48 hours. The faunistic material obtained was fixed with hot formalin (by 60 o C) with a concentration of 4%, and further used for the assembly of permanent preparations according to the Seinhorst method, by transferring nematodes to glycerin [1].

Results and discussions

The potato tuber nematodofauna was first investigated in the Republic of Moldova. In the research process it was observed that the nematodofauna, formed in the tubers in the deposits, is specific, with the dominance of saprophytes, due to the spread of bacteriosis and mycosis infections. As a result of the analyzes, carried out for several years, in the potato tubers was detected the presence of 30 species of nematodes (tab. 1), which belong to 24 genera, 14 families, 5 orders, 2 classes: 1. Class Secernentea - orders Aphelenchida, Rhabditida, Tylenchida;

2. Class Adenophorea - orders Dorylaimida, Plectida. Most of the species detected (19 species) are of the order Rhabditida with the dominance of the superfamily Cephaloboidea - *Acrobeloides buetschlii, Heterocephalobus elongatus, Eucephalobus striatus, Panagrolaimus rigidus*; the Rhabditoidea superfamily- the *Rhabditis, Mesorhabditis* and Diplogasteroidea superfamily with the dominance of the *Pristionchus lheritieri* species; 4 species of the order Aphelenchida, with the dominance of *Aphelenchus avenae*; 3 species of the order Tylenchida, dominated by *Ditylenchus destructor*; 2 species of the order Dorylaimida - *Aporcelaimellus obtusicaudatus* and *Mesodorylaimus bastiani*; 1 species of the order Plectida - *Anaplectus granulosus*. *Aporcelaimellus obtusicaudatus* is known to be omnivorous, but according to the trophic-ecological classification [11] it is also included in the group of omnivorous and in the group of *animal predation*, which indicates that this species is attracted to potatoes diseased by dithylenosis, by the surplus of parasitic nematodes and saprophytes present in this environment. M. bastiani species is not specific for potato tubers, she enters by the paths formed by wire larvae. Of the order Plectida, only one species - *Anaplectus granulosus* - was present in potatoes suffering from dithylenosis. From the order Tylenchida, as mentioned, there were 3 species: the first species - *Ditylenchus destructor* Thorne 1945, which is also the main obligatory parasite of the potato crop *Solanum tuberosum* in the Republic of Moldova (tab. 1; fig. 1). The species *D.destructor* differed both in frequency (100%) in all varieties studied and by density (thousands of individuals/gram of infested tissue), able to provoke dithylenosis disease, as well as epiphytotics - mass infestation of potato from warehouses.

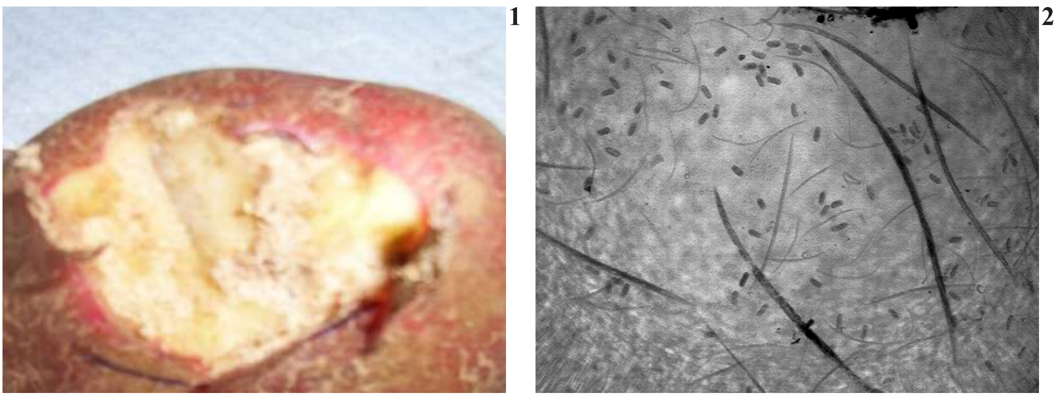


Figure 1. Infested Roko potato (storage period) infested, phase 2 of ditylenchosis (1); population *D.destructor (*2) (females, males, larvae, eggs), which was extracted from the infested tissue

It is necessary to mention that the second phytoparasitic species - *D.dipsaci*, is quite rare in potato tubers. Of the 10 varieties investigated, this species was found in association with the *D.destructor* species only at 3 of them - Bellarosa, collected from Ştefan-Vodă district and Desiree and Irga, collected from the north of the republic (Edinet district).The species T*ylenchus davainei* was common in some varieties of tubers. This is a migrating root ectoparasitic species, which accidentally hit the potato tubers. All 30 species, detected in potato tubers, according to the trophic-ecological characteristic [11], are included in the following 5 groups:

plant feeding (Pf); *hiphal feeding* (Hf); *bacterial feeding* (Bf); *animal predation* (Ap); *omnivorous* (Om) (tab. 1; fig. 2).

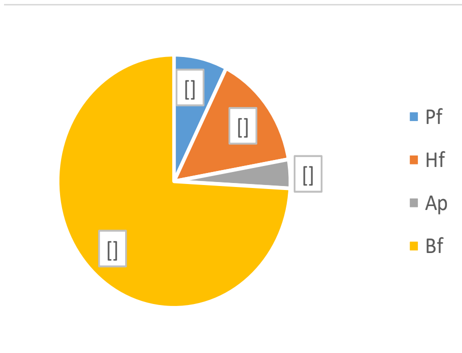
The group of phytophagous includes the species *Ditylenchus destructor, D.dipsaci* and T*ylenchus davainei* which account for 7.41%. D. destructor species is the only species of obligatory phytoparasites of potato tubers in the Republic of Moldova, which causes them dithylenosis (fig. 2). Because it parasitizes mainly only in the potato tubers, causing their disease of dithylenhosis, it was also called the tuber nematode. Other species of obligatory parasitic nematodes were not detected in the researched tubers, which confirms that in the conditions of the Republic of Moldova, namely *D. destructor* is the pathogen of dithylenhosis in potatoes in autumn-winter- spring.

Table 1. Biodiversity of phytonematodes in potato tubers of different varieties, infested by D.destructor, according to the phases of dithylenosis and trophic-ecological groups

No.	Nematode species	Phases 1, 2 of dithylenosis	Phases 3, 4 of dithylenosis	Trophic-ecological groups
	Class SECERNENTEA:1.Order Tylenchida			
1.	*Ditylenchus destructor*	+++	++	***Pf***
2.	*Ditylenchus dipsaci*	-	+	***Pf***
3.	*Tylenchus davainei*	-	+	***Pf***
	2. Order Aphelenchida			
4.	*Aphelenchus avenae*	-	+	***Hf***
5.	*Aphelenchus eremitus*	-	+	***Hf***
6.	*Aphelenchoides asterocaudatus*	-	+	***Hf***
7.	*Aphelenechoides parietinus*	-	+	***Hf***
8.	*Seinura demani*	-	+	***Ap***
	3. Order Rhabditida			
9.	*Bunonema* sp.	-	+	***Bf***
10.	*Acrobeles ciliatus*	-	+	***Bf***
11.	*Acrobeloides buetschlii*	-	+	***Bf***
12.	*Cephalobus persegnis*	-	+	***Bf***
13.	*Chiloplacus propinguus*	-	+	***Bf***
14.	*Chiloplacus symmetricus*	-	+	***Bf***
15.	*Eucephalobus mucronatus*	-	+	***Bf***
16.	*Eucephalobus striatus*	-	+	***Bf***
17.	*Heterocephalobus elongatus*	-	+	***Bf***
18.	*Diplogasteroides spengelii*	-	+	***Bf***
19.	*Diploscapter coronatus*	-	+	***Bf***
20.	*Pristionchus lheritieri*	-	+	***Bf, Ap***

21.	*Panagrolaimus rigidus*	-	+	***Bf***
22.	*Caenorhabditis elegans*	-	+	***Bf***
23.	*Mesorhabditis signifera*	-	+	***Bf***
24.	*Pelodera teres*	-	+	***Bf***
25.	*Rhabditis longicaudata*	-	+	***Bf***
26.	*Protorhabditis filiformis*	-	+	***Bf***
27.	*Protorhabditis* sp.	-	+	***Bf***
	Class ADENOPHOREA 1.Order Plectida			
28.	*Anaplectus granulosus*	-	+	***Bf***
	2. Order Dorylaimida			
29.	*Aporcelaimellus obtusicaudatus*	-	+	***Om, Ap***
30.	*Mesodorylaimus bastiani*	-	+	***Om***
Total species:		**1**	**30**	

a



b

Figure 2. Distribution of nematode species in the Desiree potato tuber, sick of ditylenchosis (phases 3, 4) (a), by trophic-ecological groups (b): Pf -7.41%; Hf -14.81%; Ap - 3.7%, Bf - 74.1%

It is necessary to mention that the presence of the nematode that forms cysts – *Globodera rostochiensis*, in the samples we investigated, was not detected. From the group of *animal predation* (Ap, type 5b) was detected only one species - *Seinura demani* (3,7%), the order Aphelenchida, the family Seinuridae which, as it is known, is able to devour and feed on other species of nematodes, including those in the group of bacteriophages - *Acrobeloides, Chiloplacus*, Plectus and mycelium-hypophagous group - some species of the genus *Aphelenchoides*, whose presence is quite common in the macerated tissue of tubers. The trophic-ecological group of *hiphal feeding* (Hf) includes species, which are directly dependent on the presence of fungal mycelium, where they find the right habitat and with which they feed - A*phelenchus avenae, Aphelenchus eremitus, Aphelenchoides asterocaudatus, Aphelenchoides sp*., permanently common being *Aphelenchus avenae*. According to our recalculations, they account for 14,80% of the total species (fig. 2b). Unlike bacteriovore species, which do not have a stylet, *hiphal feeding* species of the order Aphelenchida are endowed with a small stylet, which in the process of nutrition pierces the cell wall of the hyphae of fungi and absorbs their contents. *Aphelenchus avenae* is listed as one of the most common nematodes in the soil. According to its geographical distribution, it is a ubiquitous species. According to some authors, *A.avenae* is able to feed and reproduce in mycosis-infested tissue caused by pathogenic fungi - *Verticillium, Fusarium (Fusarium solani var coeruleum, F.sambucinum, F.oxysporum), Alternaria, Rhizoctonia solani* and a., which causes serious diseases, both in plants and in potato tubers, being permanently frequent in potato tubers in warehouses, infested by D.destructor in the advanced stages of dithylenhosis [8, 13, 18].

Finally, the most numerous and frequent in the affected tubers are the species from the group of bacterial feeding - 19 species, which have the highest percentage -74.1%. These are included in 17 genera: *Bunonema, Acrobeles, Acrobeloides, Cephalobus, Eucephalobus, Chiloplacus, Heterocephalobus, Diplogasteroides, Diploscapter, Pristionchus, Panagrolaimus, Caenorhabditis, Pelodera, Rhabditis, Mesorhabditis, Protorhabditis, Anaplectus* (Table 1). Most of the species of bacterial feeding nematodes, detected in the potato tubers researched by us -C*ephalobus persegnis, Chiloplacus propinguus, Eucephalobus striatus, Acrobeles ciliatus, Panagrolaimus rigidus, Diploscapter coronata* etc., as well as some mycophagous species - Aphelenchus existence are ubiquitous and euribionts.

It is necessary to mention that the saprophytic nematodes do not have a stylet, and the mode of nutrition takes place by swallowing the primary products, which result under the action of the saprobiotic microflora, so it does not pose a danger to the potato tubers, but in association with the species of parasites mandatory - nematodes *Ditylenchus destructor, D.dipsaci* and bacteria and fungi increase the putrefaction processes of the affected tissue and its total destruction. In such dead plant tissue, phytoparasitic nematodes, which feed only on live plant tissue, do not resist and move to the soil, where they are kept for a long time - 3-4 years. Along with the seed tubers, which are the first source of potato infestation, the infested soil is also a way of transmitting parasitic nematodes to the potato culture, which is also a moment of widening of the infested surfaces. The nematode *Ditylenchus destructor* is considered, globally, among the most dangerous pests in potato culture, being included in the list of quarantine species [7]. At the same time, it is known that potato tubers, compared to other vegetative organs of this crop, contain an increased amount of water, so in dry years they are primarily attacked by various pests, including bacterial infections, fungal infections, parasitic nematodes and saprophytes, mites, etc.

The studies were carried out within the research project no. 20.80009.7007.12.

Conclusions:

1. Nematological analysis of potato tubers of 10 varieties, collected in the autumn-winter-spring periods, showed the presence of 30 species of nematodes, most - 19 species, being of the order Rhabditida. The species Pristionchus lheritieri was distinguished by dominance, family Neodiplogasteridae.
2. The species, detected in the tubers of potatoes diseased with dithylenhosis, the advanced

phases - 3, 4, according to the trophico-ecological characteristic, are included in all 5 groups: plant feeding, hiphal feeding, bacterial feeding, animal predation, omnivorous.

3. Ditylenchus destructor Thorne 1945, which is also the main parasite in the potato crop in the Republic of Moldova, the cause of the disease, was distinguished by frequency (100%) in all 10 researched varieties and by density (thousands of individuals/gram of infested tissue), able to provoke ditylenchosis disease, as well as epiphytotics - mass infestation of potato from deposit.

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ABOUT CHEMICAL COMPOSITION OF THE NEMATODE DITYLENCHUS DIPSACI

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Abstract. In the article are presented data on the quantitative variations of bound amino acids in the tissue protein of the nematode Ditylenchus dipsaci Kuhn, 1857, parasite of Allium sativum crops. It was revealed that the largest share is: glutamic acid + glutamine -21.0% of the total amount, aspartic acid + asparagine - 11.0%, glycine -12.6% and alanine -10.5. In smaller quantities was evidenced: tryptophan (0.8%), histidine (0.8%) and methionine (0.1%). According to the distribution by groups, it was determined that non-essential amino acids have the highest percentage of the total -31.9%, followed by immunoactive amino acids - 25.7% and glycogen - 21.5%.

Introduction

Nematode *Ditylenchus dipsaci* Kuhn, 1857 is one of the primary migratory endoparasites, associated with crops from genus Allium (*Allium cepa, Allium sativum*). Due to their small size and the association with the host plant and the easy adaptation to survive in the soil for a long time in the absence of the host plant, creates major problems for agricultural specialists in most growing areas around the world. Under unfavourable environmental conditions, the larvae of the 4th stage of development, have a long-term condition of anabiosis - about 23 years, until the temperature and humidity values will be optimal to restore the state of activity [10]. The authors mention that *D.dipsaci* is an anhydrobiotic species, with a constant dehydration, having necessary water for survival in a slow rhythm. According to OEPP / EPPO data [9], it is a species of phytosanitary quarantine at international level. Nematode *D.dipsaci* penetrate and settle in the bulb crops of the genus *Allium*, both in field and in storage conditions. The damage caused are appreciable, sometimes compromising production [2; 3; 6; 8; 16]. In the parasitized plants takes place a number of biochemical changes [1; 5]. The physiological and biochemical modifications, caused to the host plants by *D.dipsaci*, are highly dependent on the chemical composition of the parasite itself, therefore the infested plant tissue is exposed to analysis together with nematodes. In the literature sources are indicated data on the content of free amino acids and ammonia in the exudate and homogenate of the species *D.dipsaci* and *D.destructor* [1; 4; 5; 15]. In the present research, the chemical composition of the species *D.dipsaci* tissue, the breed that parasitizes crops of the genus *Allium*, the nematodes being extracted from the bulbs of *A. sativum* plants, collected during the vegetation period.

Materials and methods

The amounts of protein, nitrogen and proteinogenic amino acids in the protein hydrolysate on *D.dipsaci*, was determined. For research, were selected live nematodes, pure culture (mature individuals, females, males + larval forms L2 - L4 + eggs), extracted from the bulbs of *Allium sativum*, infested in the initial phase of dithylenchosis, which were collected during the vegetation period (may - June). Nematode extraction took place by the modified Baermann funnel method [13]. The presence of other species of nematodes or microorganisms is excluded, therefore in the research process total preparations were performed to verify the purity of *D.dipsaci* individuals. The nematode suspension was thoroughly washed before analysis with distilled water by the centrifugation-suspension method [5]. After washing, the nematodes were dried in a thermostat at 600C for 24 hours, during which time the water evaporated completely and the nematode portion dried. Qualitative and quantitative determination of amino acids was performed by ion exchange chromatography on the automatic amino acid analyser, hydrolysis method with hydrochloric acid (HCl) 6 n [11]. The total protein was recalculated according to Scurihin [14]: N x 6.25 (N - total amount of nitrogen).

Results and discussions

The laboratory analysis of the nematode suspension, intended for research, determined the presence of a pure culture only of *D.dipsaci* (females, males, larvae of different ages, eggs) (fig. 1).

a

b

Figure 1. a. Garlic bulbs infested with *D.dipsaci* populations in the initial stage; b. nematodes *D.dipsaci* after washing

The results of the qualitative analyses showed that the in protein of the nematode tissue of *D.dipsaci* contains 19 bound amino acids - cysteic acid, aspartic acid + asparagine, threonine, serine, glutamic acid + glutamine, proline, glycine, alanine, valine, cysteine, methionine, isoleucine, leucine, tyrosine, phenylalanine, tryptophan, lysine, histidine, arginine (Table 1). Highest share has: glutamic acid + glutamine -21.0% followed by aspartic acid + asparagine - 11.0%, glycine -12.6% and alanine -10.5 of the total amount of amino acids, which also corresponds to an increased amount of nitrogen (N) (0.3044, 0.5200, 0.6084 and 0.4296 mg/100 mg). Usually the amount of these amino acids is higher in parasitic host plants [14]. Smaller quantity has: methionine (0.1% of total amino acids), tryptophan (0.8%) and histidine (0.8%). From previous data [1; 5] we notice that in the *D.dipsaci* homogenate are contained approximately the same free amino acids, with the difference of Pro, Gly and Ile, the presence of which has not been detected.

The percentage distribution of the amounts of amino acids, according to the main groups [11], is indicated in table 1 and figure 2. In the group of non-essential amino acids, which is also the largest group, 12 amino acids are included: Asp + Asn, Ser, Glu + Gln, Pro, Gly, Ala, Val, Cys, Ile, Tyr, His, Arg, which account for the highest percentage of the total amount of amino acid groups -31.9%. Followed by immunoactive amino acids (group 3), 8 amino acids: Asp + Asn, Thr, Ser, Glu + Gln, Ala, Val, Trp, Cys, which have 25.7% and glycogen (group 4), 6 amino acids: Asp-Asn, Thr, Ser, Gly, Ala, Val, which account for 21.5%.

Table 1. Variations in the amounts of proteinogenic amino acids, nitrogen (N) and protein in tissue of nematode *D.dipsaci*, according to their distribution by groups

Ord/ nr	Amino-acids (AA)	Letter code	mg/100 mg	% from total	N, mg/100mg
1.	Cisteine Acid	Cys	0,6628	2,3	0,0496
2.	Aspartic Acid + Asparagine	Asp + Asn	2,8936	11,0	0,3044
3.	Threonine	Thr	1,2169	4,5	0,1430
4.	Serine	Ser	1,7197	6,0	0,2291
5.	Glutamic Acid + Glutamine	Glu + Gln	5,4650	21,0	0,5200
6.	Proline	Pro	2,4161	9,3	0,2938
7.	Glycine	Gly	3,2623	12,6	0,6084
8.	Alanine	Ala	2,7340	10,5	0,4296
9.	Valine	Val	0,9609	3,5	0,1148
10.	Cysteine	Cys	0,0517	0,2	0,0121
11.	Methionine	Met	0,0354	0,1	0,0033
12.	Isoleucine	Ile	0,4922	1,8	0,0525
13.	Leucine	Leu	1,1072	4,3	0,1182
14.	Tyrosine	Tyr	0,4225	1,5	0,0326
15.	Phenilalanine	Phe	0,3279	1,3	0,0278
16.	Tryptophane	Trp	0,2202	0,8	0,0302
17.	Lysine	Lys	2,0751	8,0	0,3975
18.	Histidine	His	0,2078	0,8	0,0563
19.	Arginine	Arg	0,3724	1,4	0,1197
Σ:	Amino acids and Nitrogen		25,9809	100	3,4933
	Total amount of protein				3,4933x6,2= 22,1425
Distribution of amino acids, according to the main groups					
Σ 1.	Nonessential amino acids		18,9648	31,9	2,4300

Σ 2.	Essential amino acids		7,0161	11,8	1,0633
Σ 3.	Immunoactive amino acids		15,2620	25,7	1,7832
Σ 4.	Glycogenic amino acids		12,7874	21,5	1,8293
Σ 5.	Ketogenic amino acids		4,6452	7,8	0,6588
Σ ***6.***	Sulphur containing amino acids		0,7499	1,3	0,0650
	Total		59,4254	100,0	7,8296

By lower amounts, essential AA (group 2) - 8 amino acids: Lys, Met, Thr, Trp, Val, Phe, Leu, Ile, which have 11.8% and ketogen (group 5 - a), 6 amino acids: Ile, Phe, Thr, Leu, Lys, Trp - 7.8%, were distinguished. Sulphur-containing amino acids (group 6) - Cys, Met, have the minimum percentage - 1.3% of the total. Similar data were obtained by other authors [4], who determined the same amino acids in the composition of nematodes D.dipsaci, the race, which parasitizes alfalfa.

According to the data obtained by Taracanov [15], in the composition of D.destructor protein, which were cultivated on the fungus Alternaria tenuis, was determined 19 bound amino acids, by increased quantity were distinguished, 4 amino acids: Asp + Asn, Glu + Gln, Gly and Ala - 5.0; 19.4; 10.2 and 8.4 mg/100 mg investigated material, respectively, similar in our case. In our research it was also observed that D.dipsaci tissue also contains a high amount of protein - 22.1425 mg/100 mg.

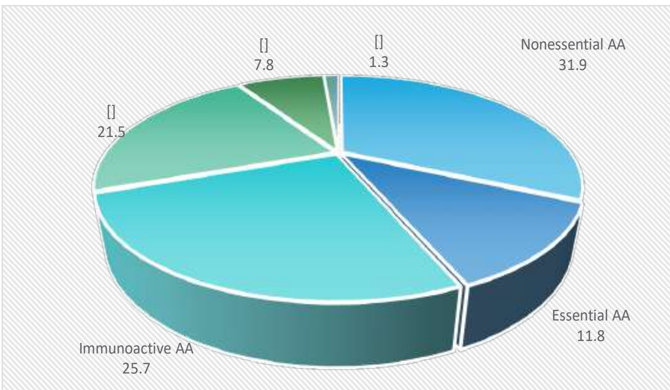

Figure 2. Percentage distribution of the main groups of amino acids in *D.dipsaci* homogenate

According to the obtained results in previous research [1, 5, 7], performed on garlic and onion bulbs infested by *D.dipsaci*, as well as on potato tubers infested by another parasite of the genus *Ditylenchus – D.destructor*, it was determined that in the infested tissue the amount of dry mass decreases compared to the uninfested tissue, which is a confirmation that the parasite in the process of nutrition consumes the cytoplasmic content of plant cells, leaving empty dissociated cells.

Other researches [12] shown that data obtained by histo- and cytochemical methods, determined that in the plant cells of the potato pulp infested in the second phase, the beginning of the 3rd phase of ditylenchosis where nematode *D. destructor* penetrated, decreased of proteins and amino acids content, because these substances, are absorbed by the parasite in the process of nutrition, according to the author.

The research was carried out within the project: 20.80009.7007.12

Conclusions

1. It was determined that in the tissue of populations *D.dipsaci* (mature forms, larvae and eggs), which were extracted from the bulbs of *Allium sativum*, infested in the early stages of ditylenchosis, contains an increased amount of protein - 22.1425 mg/100 mg product.
2. In the composition of proteins the presence of 19 amino acids: cysteic acid, aspartic acid + asparagine, threonine, serine, glutamic acid + glutamine, proline, glycine, alanine, valine, cysteine, methionine, isoleucine, leucine, tyrosine, phenylalanine, tryptophan, lysine, histidine, arginine, were detected.
3. It was found that the largest share has: glutamic acid + glutamine -21.0%, aspartic acid + asparagine - 11.0%, glycine -12.6% and alanine -10.5%, of the total amount of amino acids, and nitrogen increased with: 0.3044, 0.5200, 0.6084 and 0.4296 mg/100 mg, respectively.
4. According to the distribution of AA by groups, were calculated that nonessential amino acids (12 amino acids), which is also the largest group, have the highest percentage of total amino acids -31.9%, followed by AA immunoactive - 25.7%, glycogen - 21.5%, AA essential - 11.8% and ketogen - 7.8%.

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TACHINUS LATICOLLIS GRAV. (COLEOPTERA, STAPHYLINIDAE, TACHYPORINAE) – A NEW REPRESENTATIVE OF TACHIPORINS IN THE FAUNA OF THE REPUBLIC OF MOLDOVA

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Abstract. The paper presents the approach of researches on the species Tachinus laticollis Gravenhorst, 1802 (Coleoptera, Staphylinidae, Tachyporinae) identified as a new species for the fauna of the Republic of Moldova. The tachiporin specimen was collected at 05.03.2016 in the cattle manure in the pasture from Cocieri village, Dubasari District. The principal method of collecting was extraction by applying the flotation method. In the context of this study, the paper refers in more detail to the species in the collection of Staphylinidae within the Museum of Entomology of the Institute of Zoology, during the applied research (2015-2017). Also, to the analysis of rove beetles material extracted from samples accumulated during study, data on the systematic classification of the species, geographical distribution, bioecology, morphology, illustrative presentation.

Introduction

The species *Tachinus laticollis* Gravenhorst, 1802 (Coleoptera Order, Staphylinidae Family, Tachyporinae Subfamily) constitutes the completion of the chapter with new species found in our country. It also constitutes the maintaining records with the potential for expansion in certain territories, peak of occurrence in various ecosystems, preferred habitat and substrate for population, etc. Researches effectuated during 2015-2017 in the Cocieri village, Dubasari district allowed accumulation of staphylinides material abundant in species and specimens and diverse in population structure. In this context, the highlighting of tachiporins through new species status for the fauna of the Republic of Moldova continues with the sequential presentation of the some aspects like the existence of representatives from Tachyporinae subfamily into Staphylinidae collection; the period of initiation of research in the accumulation of staphylinids material; presentation of the species by systematic framing, synonymous, geographical spread, bioecology, aspects of morphology, presentation of illustrative material.

Materials and methods

The applied researches in the study of natural and anthropogenic points were carried out quarterly using the classic entomological methods of collecting staphylinides and materials that made work easier for storage in time.

The *tools* used – polyethylene bags, Eppendorf tubes and Petri plates, rubber gloves, secateurs, entomological needles, magnifier, etc.

The *methods* used in the working process were: 1) manual collection; 2) shaking through collection on a cloth with a texture resistant to application to various surfaces; 3) accumulation of material by raking; 4) flotation methods; 5) *insect* aspirator also known as a pooter, etc.

In the field the research and accumulation of staphylinides material included several practical steps from various types of biotopes and substrates:

- were applied collections, fixations and labeling of staphylinides from dead wood on standing trees, dry and/or in the drying phase, within fallen (felled) trees, within middle of whole and/or chipped trunks, stumps and wood in different stages of decomposition, trunks, branches, etc.;
- sectioning of wood elements of different antiquities and bark detachments;
- shaking the mushrooms with the concave hats in the blades where the insects accumulated;
- exploration of the litter in forests, forest strips, unmaintained gardens by taking samples and selecting them;
- direct observations on the habitats and behavior of staphylinides observed and then co lected;
- photographing of the materials and of studied points, etc.

In the laboratory conditions were done:

- sorting of the captured biological material, preparation, structuring and storage;
- identification according to the specific characters of determination; with the approach of the taxonomic classification and nomenclature according to the system accepted on the WEBSITES Fauna Europaea [5] and BioLib.cz [6]; with data analysis by compari staphylinide signals from the researched points and their numerical estimation.

Results and discussions

In the result of analysis and determining of collected materials during the years species, summaries in workbooks and statistical tables, a new species of rove beetles from the subfamily Tachyporinae for the fauna of our country was identified. The *T. laticollis* species is discussed for the first time and is an element through which the author expresses his contribution in completing the database of staphylinides for the Republic of Moldova.

Tachyporines group collection. The tachyporines group currently is represented by 31 species. The number is completed with a new species *T. laticollis* addressed in the paper, in total listing 32 species. Tachyporinae specimens are stored in boxes located on the upper floors of the entomological wardrobe, where the entire collection of staphylinides is kept, these being marked with the numbers 1, 2 and 3. For each specimen are established labels with the name of the subfamily and the genus to which they belong. The species *T. laticollis* is stored in the box with no. 2, the place where all the representatives of the genus Tachinus Gravenhorst, 1802 are positioned. The box is made of durable glass, thus the specimens being protected from dust and the penetration of foreign organisms inside. For accessibility in the work process and/or record keeping, the boxes are accompanied by lists of stored representatives (figure 1).

The faunal material studied. The research included several species of staphylinids collected during 2015, 2016 and 2017 in the pasture area of Cocieri village, Dubasari district and the apple orchard not maintained over time. Out of accumulated samples were extracted specimens of staphylinides by the genera Philonthus, Ocypus, Gabrius, Sepedophilus, *Tachinus*, Tachyporus, Bisnius, etc. Based on the identifications made according to the classical keys of determination, was highlighted the presence of staphylinid *Tachinus laticollis* Gravenhorst, 1802 of subfamily

Tachyporinae. The species was collected on 05.03.2016 on the pasture from Cocieri village, Dubăsari district, being extracted from cattle manure.

Systematic framing. The species *T. laticollis* belongs to Hexapoda subphylum, Insecta class, Coleoptera order, Polyphaga suborder, Staphyliniformia infraorder, Staphylinoidea superfamily, Staphylinidae family, Tachyporinae subfamily, Tachinus Gravenhorst, 1802 genera [2, 5, 6].

Synonymous. The synonyms given during the study for *T. laticollis* treated in foreign specialized sources are *Tachinus immaturus* Gravenhorst, 1802, Tachinus laticollis Gravenhorst, 1802, *Tachinus nigripes* Stephens, 1832, *Tachinus pastoralis* Veselova, 1993, *Tachinus ergates* Gistel, 1857, *Tachinus intermedius* Mannerheim, 1830 [4, 7].

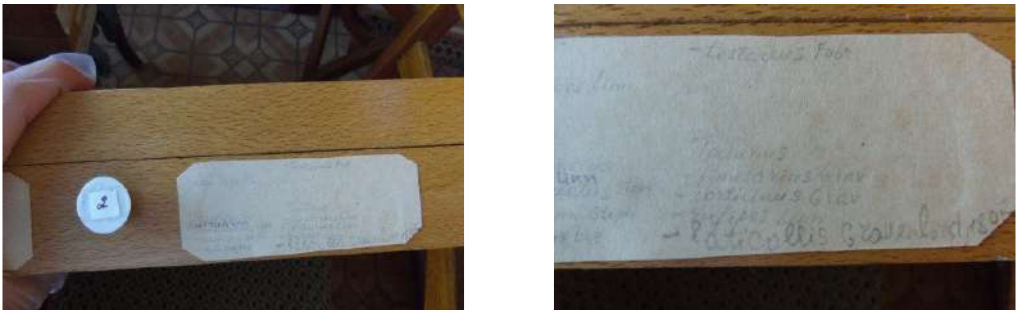
Bioecology of the species. The *T. laticollis* prefers moderate temperature and humidity conditions. It falls into mesophilic species group. Is a predator, eurytopic and coprobiont species [1].Geographical spread. The *T. laticollis* species is found in the fauna of European countries: Austria, Republic of Belarus, Belgium, Bulgaria, Czech Republic, Denmark, Estonia, Finland, France, Germany, Italy, Hungary, Lithuania, Slovakia, Poland, Romania, Ukraine, and Spain. Russian Federation: European side, Caucasus, Ural, Western and Eastern Siberia [8]. Asia: Armenia, Georgia, Turkey, Republic of Tajikistan, Republic of Turkmenistan, Uzbekistan, Republic of Kazakhstan, Mongolian People's Republic of [4, 5, 6]. Palearctic element.

Morphology. The adult of *T. laticollis* has a convex body, smooth surface, size 10-12 mm, with brown-brown coloration. Small head, slightly rounded, more wide is found below the basal segments of the pronotum. Eyes are quite accentuated, the temples exceed in length the surface of the eyes. The antennae have filiform form, are elongated, exceed the cephalic capsule and pronotum, the last antennal segment having more convex shape. Pronotum smooth, with rounded lateral edges. The rectangular and elongated elytra cover the first abdominal segment. The legs are reddish yellow with thickened tarses. The forelegs are wider compared to the middle and hind legs. The abdomen is wide in the basal part and narrow in the distal part. The larva, is white-brown and has a length of 6-7 mm [3].

The figure of the species *T. laticollis* is the proof of identification and storage of the species in Staphylinidae collection which is deposited in the Museum of Entomology of the Institute of Zoology (fig. 1).

a) adult displayed on cardboard plate

b) labeling of the species



c) storage in the collection box **d) framing in the collection list**

Figure 1. ***Tachinus laticollis*** **Gravenhorst, 1802 a new species for the fauna of the Republic**

Conclusions

The faunal researches on the pastures from Cocieri village, Dubăsari district and other biotopes allowed carrying out researches during three successive years (2015-2017) in order to follow the faunistical potential of the rove beetles in this field and to extract the species specific to this point.

Among the target staphylinid specimens collected from Cocieri village, Dubăsari district the was identified *T. laticollis* which has status as a new species for the fauna of the Republic of Moldova.

In the paper for *T. laticollis* was analysed the aspects of systematic classification, bioecology, geographical spread, storage in the Staphylinidae collection.

The investigations were performed within the project 20.80009.7007.02.

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A PITFALL TRAPPING SURVEY OF BEETLES IN STEPPE ECOSYSTEMS OF THE REPUBLIC OF MOLDOVA

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Abstract. A pitfall survey of beetles in steppe ecosystems of the Republic of Moldova was conducted. Five locations were investigated, two from the Balti steppe and three from the Bugeac steppe. As a result, a total of 563 beetle specimens, belonging to 98 species, 51 genera, and 15 families were collected. Among families recorded Carabidae was the one with the highest number of species. The ecological features of collected beetle species were revealed. The results show that steppes are still important biodiversity reservoirs within the boundaries of the country and urgent conservation activities are required.

Introduction

The biodiversity of a region is mainly determined by three factors: climate, geology, and evolutionary processes that occur in that territory. The influence of these factors and their relationship leads to the establishment of a territorial formation or unit called today by scientists as „ecoregions" (Dinerstein et al. 2001). Now there are 142 terrestrial, 53 freshwater and 43 marine ecoregions recognized across the globe.

The Republic of Moldova is a small landlocked country situated in south-eastern Europe between Ukraine and Romania. Vegetal coating and mottled landscapes composed of grassland interspersed with areas of woodland are defined by the unique positioning of the country at the crossroad of three terrestrial ecoregions Central European mixed forests, East European Forest steppe, and Pontic-Caspian steppe.

The Central European mixed forest ecoregion extends from eastern Germany to the northern part of the Republic of Moldova and north-eastern Romania. A distinct aspect of this ecoregion is the presence of thermophilous plant species common for warmer and drier habitats, particularly in the south-eastern part of the ecoregion, which borders the forest-steppe zone of Ukraine, the Republic of Moldova, and Romania.

The East European Forest steppe ecoregion is a patchwork of broadleaf forest stands and grasslands (steppe) that stretches across eastern Europe from the Ural Mountains through Central Russia to the middle of Ukraine, isolated areas of a similar character being found in eastern Romania, Republic of Moldova, and Bulgaria.

The Pontic-Caspian steppe ecoregion stretches from the northern shores of the Black Sea to the northern area around the Caspian Sea. It starts from Dobruja in the north-eastern corner of Bulgaria and south-eastern Romania and continues through the Republic of Moldova and southern and eastern Ukraine, across the Russian Northern Caucasus, the Southern and lower Volga regions to western Kazakhstan, both forming part of the larger Eurasian Steppe (www.worldwildlife.org).

In the Republic of Moldova, the steppe ecosystems are situated in the northern part of the country, the Balti steppe, with a total surface of 1920 km², and in the southern part, the Bugeac steppe covering a surface of 3210 km², divided by Codrii forest in the middle.

Specific for Bugeac steppe are primary vegetation communities consisting of feather grass (*Stipa capilata L.*, *S. lessingiana* Trin. et Rupr., *S. ucrainica* P. Smirn.), and fescue grass (Festuca valesiaca Gandin), degraded or secondary steppe sectors comprising yellow bluestem (*Botriochloa ischaemum* (L.) Keng), bluegrass (*Poa angustifolia L.*), and couch grass (*Elytrigia repens* (L.) Nevski). The phytocenoses of the Balti steppe are mainly composed of feather grass (*Stipa pulcherrima* C. Koch, *S. lessingiana* Trin. et Rupr.), and are spread over a third of the territory, less common are formations of fescue grass (*Festuca valesiaca* Gandin), while the sunnier slopes prone to landslides are usually suitable for yellow bluestem (*Botriochloa ischaemum* (L.) Keng) (Lazu, Teleuţă & Chirtoacă, 2006).

Grasslands are considered regional biodiversity hotspots and consequently of high conservation value. Unfortunately, the steppe is one of the most transformed and overworked ecosystems, and at the same time the most underrated of landscapes. In ecosystem processes, insects have an important role as pollinators, food chain elements, maintenance, and improvement of soil quality. Being sensitive to the changes of local resources, they are good indicators at the landscape level. Insects are of increasing interest to conservation practitioners, among which beetles have been widely proposed as a model for biodiversity inventory and monitoring, as they satisfy all the criteria necessary in ecological research and biodiversity survey (Eyre & Luff, 1990, Halffter & Favila, 1993, Ohsawa, 2010).

Despite the conservation value of the steppe-like grassland of the Republic of Moldova, little is known about the beetle diversity and community structure. This study aims to survey the fauna of beetles in steppe ecosystems.

Material and methods

The study presented was conducted in steppe ecosystems of the Republic of Moldova. Field trips took place between August and October 2015, sampling covered two sites from Balti steppe: Pelinia (47.8780°N, 27.8344°E) and Vranesti (47.6233°N, 28.1009°E) and three from Bugeac steppe: Stefanesti (46.4459°N, 29.6892°E), Bugeac (46.3658°N, 28.6633°E), and Ciumai (45.7822°N, 28.5697°E).

Specimens were captured out using pitfall traps. By ten traps were placed on each of the investigated sites, samples were collected every seven to ten days, due to unforeseeable circumstances. Traps were made of a 700 cm3 jar, set up at the ground level, and filled with saturated NaCl solution. Recovered specimens were transported to the laboratory for further counting and identification.

Taxonomic identification of collected beetles was carried out using Keys to Insects of the European Part of the USSR, vol. 2 (1965), and some additional online resources (Mike's Insect Keys and Käfer Europas).

Results and discussions

As a result of the survey from five localities, a total of 563 beetle specimens, belonging to

98 species, 51 genera, and 15 families were caught (table below). Among families recorded Carabidae was the one with the highest number of species (51), followed by Curculionidae (9), Scarabaeidae (6), Tenebrionidae and Dermestidae (by 5 species), Histeridae, and Chrysomelidae (by 4), Staphylinidae and Silphidae (by 3), Cerambycidae and Trogidae (by 2), and Buprestidae, Endomychidae, Cryptophagidae and Coccinellidae – by one species each. Of all collected beetles, ground beetles (Carabidae) represented the greatest proportion in terms of the number of individuals (368 specimens), followed by Tenebrionidae family with 50 specimens, Staphylinidae (37 specimens), Curculionidae (30), Dermestidae (22), Trogidae (16), Histeridae (12), Scarabaeidae (11), Silphidae and Chrysomelidae (by 5), Cerambycidae and Buprestidae (by 2) and Endomychidae, Cryptophagidae and Coccinellidae – by one specimen.

Table. Taxonomic composition and ecological features of beetle species collected

Taxa	Samples					Humidity preference	Trophic specialization	Distribution
	Balti		Bugeac					
	Pelinia	Vranesti	Stefanesti	Bugeac	Ciumai			
Carabidae								
Acinopus ammophilus Dej.					2	Mzx	Omn	E-Asn
A. laevigatus Mén.			1			Mzx	Omn	E-Asn
A. picipes (Ol.)			2		1	Mzx	Omn	E-Med
Amara aenea DeG.					1	Xr	Ph	T-Pal
A. brunnea Gyll.			1			Ms	Omn	Ola
A. consularis (Duft.)	1					Mzx	Omn	E-Si
A. equestris (Duft.)	2					Mzx	Omn	E-Si
A. littorea Thoms.	1					Ms	Omn	Eur
A. sabulosa (Serville)	1					Ms	Omn	Pal
Brachinus crepitans (L.)		1	1	14	1	Mzx	Z	W-Pal
Calathus ambiguus (Payk.)	1			1		Mzx	Z	E-Asn
C. distinguendus Chaud.					1	Mzx	Z	Med
C. erratus (Sahlb.)	1					Mzx	Z	E-Si
C. fuscipes (Goeze)	51		12	7		Ms	Omn	W-Pal
C. melanocephalus (L.)				2		Mzx	Z	T-Pal
Carabus nemoralis Müller			1			Ms	Z	Eur
Cryptophonus tenebrosus (Dej.)	1				3	Ms	Omn	E-Med
Cymindis axillaris (F.)				1		Xr	Z	W-Pal
Dolichus halensis (Schall.)					1	Ms	Z	T-Pal

Harpalus atratus Latr.	1	1				Msh	Omn	Pal
H. cephalotes Fairm., Laboul.			1		1	Hl	Ph	Eur
H. dimidiatus (P. Rossi)		2				Xr	Omn	E-Asn
H. dispar splendens Gebl.					6	Hl	Omn	W-Pal
H. distinguendus (Duft.)			1			Ms	Omn	T-Pal
H. hospes Sturm		14		26		Hl	Omn	T-Pal
H. melancholicus Dej.		2		4	4	Xr	Omn	Pal
H. modestus Dej.				1		Ms	Z	E-Asn
H. politus Dej.		2				Msh	Omn	W-Pal
H. pygmaeus Dej.	16		1	4		Xr	Omn	E-Med
H. rubripes Duft.		1				Ms	Omn	T-Pal
H. serripes (Quens.)				1		Mzx	Omn	E-Med
H. smaragdinus (Duft.)					1	Ms	Omn	E-Asn
H. tardus (Panz.)		3		1	1	Ms	Omn	E-Asn
Laemostenus terricola Hbst.			5	1		Msh	Z	E-C
Licinus cassideus (F.)			4			Ms	Z	E-Med
Ophonus azureus (F.)	2	4	4	1	12	Mzx	Omn	E-Med
O. convexicollis Men.					1	Mzx	Omn	E-Med
O. diffinis (Dej.)		2				Ms	Ph	E-Med
O. laticollis Mnrhm.		2				Xr	Ph	Pal
O. rufibarbis (F.)	1		8	1	6	Xr	Ph	Pal
O. sabulicola (Panz.)	1	4	6		1	Ms	Omn	E-Med
Paradromius linearis (Ol.)	1				1	Ms	Z	E-Med
Parophonus hirsutulus (Dej.)		1				Hg	Z	T-Med
Pseudoophonus griseus (Panz.)	1	1	3			Ms	Omn	T-Pal
P. rufipes (DeG.)	8	1	28	1	1	Ms	Omn	E-Si
Pterostichus macer (Marsh.)	1	2		1		Ms	Z	E-Asn
P. melas (Creutz.)				9		Ms	Omn	E-Med
Sphodrus leucophthalmus (L.)					1	Msh	Z	W-Pal
Trechus quadristriatus (Shrnk.)	1			1		Ms	Z	E-Asn
Zabrus spinipes (F.)				10	1	Ms	Omn	E-Med
Z. tenebrioides (Goeze)	18		2			Ms	Omn	E-Med
Histeridae								
Gnathoncus disjunctus suturifer Rtt.				2		Mzx	Omn	Eur
Hister quadrimaculatus L.				1		Ms	N	E-Asn

Saprinus maculatus (Rossi)	1				1	Mzx	N	E-Asn
S. semistriatus (Scriba)				5	2	Mzx	N	E-Si
Silphidae								
Nicrophorus antennatus (Rtt.)	1					Xr	N	E-Si
N. germanicus (L.)				1	1	Xr	N	Pal
N. interruptus Steph.					2	Xr	N	T-Pal
Staphylinidae								
Lathrobium brunnipes (F.)	20	2				Hg	Z, p	T-Pal
Ocypus ophthalmicus (Scop.)			8			Xr	Z, p	Pal
Platydracus stercorarius (Ol.)			7			Xr	Z	Pal
Trogidae								
Trox hispidus Pontop.		3		3	7	Xr	N	Eur
T. sabulosus (L.)	2			1		Xr	N	Eur
Scarabaeidae								
Onthophagus furcatus F.				4		Xr	C	T-E-Med
O. ovatus (L.)					2	Xr	C	Pal
O. ruficapillus Brulle				1		Xr	C	Pal
O. semicornis (Panz.)	2					Xr	C	Pal
Pentodon idiota (Hbst.)	1					Xr	Ph	E-Med
Sisyphus schaefferi (L.)				1		Xr	C	Eur
Buprestidae								
Trachys fragariae Bris.			2			Xr	Ph	Eur
Dermestidae								
**Dermestes intermedius* Kalik				1		Xr	N	Eur
D. laniarius Ill.	2	1	5	1	2	Xr	N	Pal
D. maculatus DeG.					1	Xr	N	Cos
D. mustelinus Er.					8	Xr	N	Pal
D. undulatus Brahm					1	Xr	N	Ola
Tenebrionidae								
Asida lutosa Solier				1		Xr	Ph	S-E Eur
Crypticus quisquilius (L.)	2		6	8	2	Xr	Ph	E-Si
Oodescelis polita (Sturm)			5			Xr	Ph	Med
Opatrum sabulosum (L.)			1	5	17	Xr	Ph	E-Si
Pedinus femoralis (L.)			3			Xr	Ph	P-Med
Endomychidae								
Lycoperdina succincta (L.)				1		Msh	F	Pal
Cryptophagidae								

Cryptophagus pilosus Gyll.				1		Mzx	F	Ola
Coccinellidae								
Hyperaspis campestris (Hbst.)					1	Xr	Z	Eur
Cerambycidae								
Dorcadion fulvum (Scop.)				1		Mzx	Ph	Eur
Neodorcadion bilineatum (Germ.)					1	Mzx	Ph	Med
Chrysomelidae								
Chrysolina haemoptera (L.)		1				Xr	Ph	Pal
Ch. limbata (F.)		2				Mzx	Ph	Pal
Coptocephala unifasciata (Scop.)					1	Mzx	Ph	W-Pal
Galeruca tanaceti (L.)	1					Mzx	Ph	Ola
Curculionidae								
Archeophloeus inermis (Boh.)				1		Xr	Ph	Pal
Cleonis pigra (Scop.)	2		4			Mzx	Ph	Ola
Otiorhynchus fullo (Schrnk.)				7		Ms	Ph	Eur
O. ligustici (L.)					7	Ms	Ph	Ola
O. ovatus (L.)				1	3	Ms	Ph	Ola
Pseudocleonus cinereus (Schrnk.)		1				Mzx	Ph	W-Pal
Sitona lateralis Gyll.				1		Mzx	Ph	Eur
S. suturalis Steph.				2		Mzx	Ph	T-Pal
Trachyphloeus alternans (Gyll.)					1	Ms	Ph	Eur

Humidity preference: **Hg** - hygrophilous, **Hl** - halophilous, **Msh** - mesohydrophilous, **Xr** - xerophilous, **Ms** - mesophilous, **Mzx** - mesoxerophilous; Trophic specialization: **Omn** - Omnivorous (carnivorous + partly granivorous in case of Carabidae, carnivorous + partly fungivorous in case of Histeridae), **F** - fungivorous, **Ph** - Phytophagous, **Z** - Zoophagous (p - predators), **N** - Necrophagous, **C** - coprophagous. Distribution: **Ola** - Holarctic, **Pal** - Palaearctic, **W-Pal** - West-Palaearctic, **T-Pal** - Trans-Palaearctic, **Eur** - European, **E-C** - Euro-Caucasian, **E-Si** - Euro-Siberian, **E-Asn** - Euro-Asian, **C-Asn** - Central-Asian, **S-E Eur** - South-East European, **E-Med** - Euro-Mediterranean, **T-E-Med** - Turanic-Euro-Mediterranean, **T-Med** - Turanic-Mediterranean, **Med** - Mediterranean, **P-Med** - Ponto-Mediterranean, **Cos** - Cosmopolitan. *First record of the species for RM fauna.

According to hygropreference the typical xerophilous species were represented by 34 species, followed by mesophilous (28), mesoxerophilous (26); mesohydrophilous (5), halophilous (3), and hygrophilous (2).

By trophic specialization, six groups were distinguished, of which omnivorous counted 31 species, followed by phytophagous (27), zoophagous (20), necrophagous (13), coprophagous (5), and fungivorous (2).

Giving their current distribution, the 98 identified species from all five investigated sites can be classified into 15 zoogeographical categories: Palaearctic (17 species), European (14), Euro-Mediterranean (14), Euro-Asian (11), Trans-Palaearctic (10), West-Palaearctic (8), Euro-Siberian (8), Holarctic (7), Mediterranean (3), Euro-Caucasian, South-East European, Turanic-Euro-Mediterranean, Turanic-Mediterranean, Ponto-Mediterranean, and Cosmopolitan by one species.

The natural ecosystems of the Republic of Moldova are highly degraded due to human impact. Today, in the Republic of Moldova, nearly two-thirds of the territory is used for agricultural purposes, with natural ecosystems covering only about 15% of the country. Of that, native steppe, and steppe-associated wet meadows are almost inexistent, making around 1.9%, those are usually areas unsuitable for agriculture (Biodiversity Assessment for Moldova, 2001, Postolache & Ghendov, 2020). Around 1200 areas, within 1 to 300 ha, with steppe and meadow vegetation, are known to be preserved in small, isolated patches, and most of them are being used as pastures (Postolache, 1995).

Over the years much of steppe ecosystems have been converted into arable land for intensive agriculture, suffering from soil depletion and degradation (Mordkovich, 2014). Therefore, steppes have been identified as a major conservation priority for both biodiversity value and landscapes. This is a first attempt to uncover the beetle diversity in the steppe ecosystems of the Republic of Moldova. Obtained results confirmed our expectation that steppes are still important biodiversity reservoirs within the boundaries of our country. There is an urgent need towards rising attention of the local and central public authorities, and population on the priority of the biodiversity conservation measures in the steppe region. The most important areas from steppe ecosystems of the Republic of Moldova must be identified and conserved as natural reserves within the National Ecological Network. Future investigations should focus on extending the number of sampling localities for better coverage of the steppe ecosystems of the Republic of Moldova, and the application of additional sampling methods to extend the range of insect groups.

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EFFECTS OF INVASIVE JAPANESE KNOTWEED ON DIVERSITY AND STRUCTURE OF SOIL NEMATODE COMMUNITIES

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Abstract. In this study we investigated the communities of soil nematodes in the forest habitats invaded and uninvaded by *Fallopia japonica (*Houtt.) Ronse Decr., in Tatra National Park, Slovakia. We found that invasion by *F. japonica* altered nematode communities and their structures. Total nematode abundance, species number and nematode biomass were significantly lower in invaded than uninvaded plots, but species diversity remained unaffected throughout the study. The overall abundance of all nematode trophic groups well represented the negative impact of *F. japonica* invasion on soil food webs, supported by low values of all maturity indices, a structural index and the Jaccard index of faunal similarity. A weighted faunal analysis similarly characterized the food webs of invaded plots as poorly developed or highly disturbed, with bacterial decomposition and a low C/N ratio. Our findings thus suggest that dense plots of knotweed simplify the structural complexity of the soil environment by reducing the richness of plant species, which may have contributed to the negative changes in the structures of the nematode communities.

Introduction

Fallopia japonica (Japanese knotweed) invasions have repeatedly been demonstrated to have a negative impact on the species richness and diversity of native plants [6, 7]. Studies of knotweed invasions, however, rarely address impacts on soil metazoans such as nematodes [2], which are probably the most well-studied group of soil organisms with excellent indicative characteristics [3].

We used soil nematodes as representatives of soil invertebrates since they are considered to the most abundant metazoans that can be found in almost all environments, under diverse climatic conditions and habitats [8].

Soil nematodes utilise plant resources from both litter inputs and root exudates, drive important ecosystems functions and comprise several specific trophic groups, including bacteria, fungi and plant feeding as well as carnivores and omnivores. Therefore, nematodes serve as valuable biological indicators and could provide interesting information on how Japanese knotweed invasion affects soil food webs.

Materials and methods

We established ten independent plots and collected soil core samples for characterizing the nematode communities in ruderal habitats of Tatra National Park invaded (five plots, Fig. 1a - INV) and uninvaded (five plots, Fig. 1b - UNI) by F. japonica. The soil samples were randomly collected from the 10-15 cm layer in each plot using a hand spade on 17 June 2017, 2018 and 2019, for a total of 30 samples (five plots × two sites × three sampling years). Extracted nematodes were heat killed, fixed and microscopically identified to the genus level. The evaluation of nematode communities was based on assignation of nematode species to the trophic groups (bacterivores, fungivores, plant feeders, predators and omnivores) [8]; to the "colonizer-persister (c-p) scale" (1-5) based on their r and k characteristics [1] and computation of several community indices e.g. the Shannon-Weaver diversity index (H´spp) [5], the Jaccard similarity index (Js) [4], the maturity index (MI) [1] or the enrichment index (EI) [3]. The statistical analyses were performed separately for each plot, and data were compared between the INV plots and the UNI control plots. All nematological data, including the indices, were calculated as means for the individual plots and sampling years. A factorial analysis of variance was used, and means were compared using Tukey's honestly significant difference (HSD) post hoc test ($P<0.05$; $P<0.01$) of the PlotIt program Ver 3.2 (Stat Soft). Data were log-transformed before analysis to improve normality.

a **b**

Figure 1. Plot invaded (a) and uninvaded (b) by *F. japonica* in the High Tatras

Results and conclusion

The response of the nematode communities to *F. japonica* invasion did not differ among sampling years but differed considerably between the INV and UNI plots. The mean number of species was significantly lower in the INV than in the UNI plots, but species diversity did not differ significantly between the plots (Table 1). Total nematode abundance also differed significantly between the INV and UNI plots (HSD, $P<0.01$; $P<0.05$) (tab. 1). Mean nematode abundance was significantly higher in the UNI than in the INV plots in all sampling years.

Additionally, The HSD test indicated that the establishment of *F. japonica* was negatively associated with all maturity indices (MI, PPI and ∑MI), SI and total nematode biomass in the INV plots throughout the study ($P<0.01$; $P<0.05$) (Table 1). The Jaccard index of faunal similarity ranged from 55.8 to 63.2%.

The nematode communities of the INV and UNI plots were represented by all trophic groups. Bacterivores were the most diverse trophic group in the study, followed by plant feeders, omnivores, predators and fungivores.

The total abundance of all trophic groups was generally lower in the INV than the UNI plots throughout the study (tab. 2)

Table 1. Mean total nematode abundance, species number, ecological and functional indices associated with knotweed invaded and uninvaded sites during study period (n=5)

	Uninvaded	Invaded	Significance HSD test
2017			
Nematode abundance	1093.3±114.5	484.9±64.2	**[1]
Number of species	48.4±3.6	39.0±1.9	*
Species diversity index (H´spp)	3.14±0.09	3.11±0.05	--
Maturity index	2.26±0.15	1.97±0.14	**
Plant parasitic index	2.58±0.10	2.18±0.17	**
Summ maturity index	2.34±0.14	2.06±0.13	*
Enrichment Index	69.9±8.6	66.7±5.1	--
Structure Index	67.5±2.1	42.5±11.3	**
Channel index	16.7±6.7	13.7±6.6	--
Total nematode biomass (mg)	3.44±0.96	0.94±0.32	**
Jaccard index of similarity (%)	63.2		
2018			
Nematode abundance	1171.5±157.9	492.9±205.2	*
Number of species	39.6±2.7	32.2±2.0	*
Species diversity index (H´spp)	3.08±0.08	2.91±0.11	--
Maturity index	2.24±0.17	2.02±0.05	*
Plant parasitic index	2.65±0.18	2.21±0.11	**
Summ maturity index	2.33±0.13	2.15±0.14	*
Enrichment Index	73.3±4.5	69.9±5.4	--
Structure Index	68.3±8.5	52.6±5.7	*
Channel index	15.5±7.3	7.05±5.1	--
Total nematode biomass (mg)	3.79±1.42	1.11±0.51	**
Jaccard index of similarity (%)	55.8		
2019			
Nematode abundance	924.8±90.5	507.4±86.6	*
Number of species	48.8±3.7	38.0±4.2	--
Species diversity index (H´spp)	3.27±0.14	3.05±0.10	--
Maturity index	2.30±0.13	1.99±0.08	**
Plant parasitic index	2.64±0.08	2.15±0.24	*
Summ maturity index	2.37±0.10	2.10±0.19	--
Enrichment Index	68.4±4.2	69.6±9.3	--
Structure Index	67.5±6.4	49.8±11.8	*
Channel index	16.6±2.7	12.9±77.2	--
Total nematode biomass (mg)	3.20±2.70	1.15±0.46	**
Jaccard index of similarity (%)	56.1		

In conclusion, our study found that *F. japonica* invasion had a negative impact on the native plant communities and on the structure of soil nematode communities during the three years of our study. The impact of knotweed on these communities did not differ among sampling years, indicating a persistent disturbance of the ecosystem and soil food webs in comparison to the uninvaded control plots. Overall nematode abundance and nematode biomass were significantly lower in invaded than in uninvaded plots, a weighted faunal analysis characterized the food webs of invaded plots as poorly developed or highly disturbed with prevailed bacterial decomposition pathway.

Table 2. Mean total abundance of nematode trophic groups associated with knotweed invaded and uninvaded sites during study period (n=5)

	Uninvaded	Invaded	Significance HSD test
2017			
Bacterial feeders	2504.9±345.2	1329.8±159.2	**[1]
Predators	183.0±35.9	61.0±29.9	*
Fungal feeders	420.5±111.3	292.1±88.7	--
Omnivores	550.6±126.7	172.2±53.8	**
Plant feeders	1807.7±428.8	569.7±188.4	**
2018			
Bacterial feeders	2787.4±259.7	1307.2±199.5	**
Predators	231.1±59.7	134.9±31.7	*
Fungal feeders	681.3±156.7	116.3±47.6	**
Omnivores	498.1±121.0	184.2±36.4	**
Plant feeders	1659.8±321.5	721.9±166.6	**
2019			
Bacterial feeders	2187.2±108.6	1455.8±161.7	*
Predators	215.5±44.4	103.0±26.6	*
Fungal feeders	455.9±91.4	164.3±60.5	*
Omnivores	438.8±124.9	212.2±99.2	*
Plant feeders	1325.3±333.9	727.4±88.7	**

(1) Different from uninvaded control according the Tukey´s (HSD) post hoc test (* for P<0.05; ** for P<0.01).

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ESTABLISHING OF THE MONO- AND POLYINVASION IMPACT ON SOME MORPHO-FUNCTIONAL INDICES IN WILD BOARS

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Abstract. In the paper is described the mono- and poly-invasions impact on some morpho-functional indices in wild boars. So, in result of the investigation of hematological indices in uninfested mono- and poly-parasitized wild boars, it was established that both the indices of hemoglobin content, of hematocrit, erythrocyte's number, thrombosis time and ESR (erythrocyte sedimentation rate) vary and are more increased in the I group with uninfested wild boars compared to mono - and poly-parasitized ones. It has been established that at infested boars with S. papillosus from the I group, and in those infested with D. lanceolatum from the II group there is a decrease of hemostatic indices, but their maximum decrease is highlighted in the IV group with wild boars infested with Dicrocoelium lanceolatum, Strongyloides papillosus, Metastrongylus elongatus and Eimeria debliecki. This decrease is due to eliminated exotoxins by parasites, which contain anticoagulants and hemolyzers and which neutralize the fibrinogen, thrombin, Ca+ ions and vitamin K properties from the body.

Introduction

According to data from the bibliographic references the parasitic agents can directly influence the host's biochemical reactions, which represent practical and theoretical interest, because they determine the consequences of their interaction with the host, they disrupt metabolism, increasing the blood serum ferments activity, it's changing the proteinogram, immunogram, plasma hemostasis indices [1-4, 9-12, 20-22].

The evaluation of the disease, caused by the pathogenic action of the larvae in the migration phase, predominates the cellular defense factor. The hemostasis indices show about the organism's protective reaction that happens in stopping the hemorrhages. An optimal physiological blood clotting function maintaining is essential. The blood clotting deficiencies have a major impact on its intravascular fluidity. Excessive coagulation can lead to a blood vessel occlusion of vital importance, and the deficient one can cause a hemorrhagic condition, very difficult to fight [5-8, 13- 19].

Also, it is known that in order to be developed effective measures in stabilizing hemostasis indices it is necessary to study their disorders level in mono- and polyparasite infestations and after that, can be taken measures for their normalization [5-8].

Materials and methods

The parasitological researches were carried out in the laboratory of Parasitology and Helminthology of the Institute of Zoology on 82 biological samples, collected during 2019 year from wild boars, raised at hunting from the "Pădurea Domnească" Nature Reserve forest ecosystem.

In the order to achieve the proposed objectives, were used coproovoscopic methods (*Fulleborn, Darling*), coprolarvoscopic methods (*Popov, Baermann*) and successive washing method. The invasion's intensity with nematodes was established in 5 g of fetuses, and the oocysts of *Eimeria* spp., eggs of *Fasciola hepatica*, *Dicrocoelium lanceolatum*, etc., in 10 visual microscopic fields (10x40).

The mono- and polyinvasions impact on some morpho-functional indices in wild boars was identified by determining hematological indices, proteinogram indices and by plasma hemostasis indices in the reference laboratories.

Results and discussion

A special section of the investigations was to establish the impact of parasitic agents on the host - body to wild boars, through the analysis prism of hematological and biochemical indices. The collection of blood samples in order to establish some hematological and biochemical indices, have been taken from wild boars with different levels of infestation from various natural and anthropogenic biotopes of the Republic of Moldova.

The main objective of the investigations was to evaluate the consequences of mono- and polyparasitosis on the physiological status of wild boars. In order to achieve this objective, was initially studied the parasitofauna in wild boars, after which were selected 20 specimens and distributed in 4 groups with 5 wild boars in each lot: the I group - uninfested, the II group - spontaneously infested with *Strongyloides papillosus*, the III group - spontaneously infested with Dicrocoelium lanceolatum and the IV group - spontaneously polyinfested with *Dicrocoelium lanceolatum, Strongyloides papillosus, Metastrongylus elongatus și Eimeria debliecki*.

Determination of hematological indices (hemoglobin, erythrocytes, leukocytes, hematocrit, prothrombin, thrombosis time, erythrocyte sedimentation rate (ESR)) was performed at uninfested, mono- and polyparasitized wild boars (tab. 1).

Table 1. Mono- and polyinvasions impact on hematological indices in wild boars

Groups	Hb (g/100ml sânge)	Erytrocytes, 10^6mm^3	Leukocytes, thousand / mm^3	Hematocrit (%)	Prothrombin %	Thrombosis time (sec.)	ESR (ml/sec)
I	13,0±1,5	7,2±0,6	11,2**±1,2**	41,5±3,6	86,7±4,2	42,2±2,3	0,8±0,14
II	8,0±0,4	5,5±0,4	13,8**±1,6**	32,2±3,2	85,3±3,5	36,6±1,8	0,5±0,12
III	9,0±0,7	6,5±0,7	13,5**±1,4**	35,5±2,5	85,7±3,6	34,5±1,3	0,6±0,11
IV	7,4±0,5	4,2±0,3	14,4**±2,5**	28,6±2,0	84,2±3,3	32,4±1,1	0,5±0,10

In the result of the hematological indices investigation in mono- and polyparasitic uninfested wild boars, it was established that both the hemoglobin content indices, hematocrit, erythrocytes number, thrombosis time and ESR (erythrocyte sedimentation rate) vary and are higher at the I group with uninfested wild boars, compared to mono- and polyparasitic ones. The leukocyte content, being opposite, decreased in the I group - uninfested, comparative to its values at the II group - spontaneously infested with Strongyloides papillosus, the III group - spontaneously infested with Dicrocoelium lanceolatum and the IV group - spontaneously infested with Dicrocoelium lanceolatum, Strongyloides papillosus, Metastrongylus elongatus și

Eimeria debliecki.Therefore, the index of hemoglobin content is more increased in the I group with uninfested wild boars, by 38,5% (P> 0,05), comparative to the II group - spontaneously infested with *Strongyloides papillosus*, by 30,8% (P> 0,05) comparative to the III group - spontaneously infested with *Dicrocoelium lanceolatum* and by 43,0% (P> 0,05) comparative to the IV group - wild boars spontaneously infested with *Dicrocoelium lanceolatum, Strongyloides papillosus, Metastrongylus elongatus și Eimeria debliecki.*The erythrocytes number also varies from group to group and being in the I group by 23,6% (P> 0,05) higher comparative to the II group, by 9.7% (P> 0,05), comparative to the III group and by 41,7% (P> 0,05), comparative to the IV group.The values of hematocrit and ESR indices are also higher in the I group by 22,4% (P> 0,05), comparative to the II group, by 14,5% (P> 0,05), comparative to the III group and by 31,0% (P> 0,05), comparative to the IV group for hematocrit content, and respectively higher in the I group by 37,5% (P> 0,05), comparative to the II and the IV group, and by 25,0% (P> 0,05), comparative to the III group for ESR.Thrombosis time indices, was highlighted higher for the I group of uninfested wild boars by 15,9%, comparative to the II and the III groups with monoparasitized wild boars (*Strongyloides papillosus, Dicrocoelium lanceolatum*) and by 23,3% (P> 0,05) comparative to the IV group with polyparasitized wild boars (*Dicrocoelium lanceolatum, Strongyloides papillosus, Metastrongylus elongatus și Eimeria debliecki*).

The leukocyte content shown in wild boar groups was on average lower in the I group, by 17,7% (P> 0,05), comparative to the II and the III groups and by 22,3% (P> 0,05), comparative with the IV lot. Also, it was studied the impact of monoinvasions (*Strongyloides papillosus, Dicrocoelium lanceolatum*) and polyinvasions (*Dicrocoelium lanceolatum, Strongyloides papillosus, Metastrongylus elongatus și Eimeria debliecki*) on the lymphocyte indices in wild boars (tab. 2).

Table 2. Mono- and polyinvasions impact on leukogram in wild boars

Groups	Leukocytes (thousand /mm^3)	Thrombocytes (mii/ mm^3)	Leukocyte formula (%)						
			Basophils	Eosinophils	Neutrophil			Lymphocytes	Monocytes
					Young	Stick	Segmented		
I	11,2±**1,2**	284±**10,4**	0,5±**00,6**	3,2±**0,12**	1,6±**0,4**	2,5±**0,26**	40,6±**5,3**	47,9±**4,2**	3,7±**0, 34**
II	13,8±**1,6**	272±**12,0**	0,4±**00,4**	4,5±**0,24**	2,2±**0,7**	3,7±**0,34**	42,4±**5,6**	41,7±**4,4**	5,7±**0,62**
III	13,5±**1,4**	274±**12,3**	0,4±**00,3**	3,9±**0,28**	2,8±**0,6**	3,4±**0,32**	45,8±**6,4**	38,6±**3,7**	5,4±**0,76**
IV	14,4±**2,5**	265±**12,6**	0,2±**00,2**	5,4±**0,26**	3,7±**0,8**	4,8±**0,38**	45,4±**5,8**	35,5±**3,8**	6,0±**0,74**

In the analysis result of the leukocyte formula in wild boars, was found a decrease of the eosinophils number from the I group - uninfested by 29,0% (P> 0,05), comparative to the II group - spontaneously infested with *Strongyloides papillosus*, by 18,0% (P > 0,05), comparative to the III group - spontaneously infested with *Dicrocoelium lanceolatum* and respectively with 40,8% (P> 0,05) decreased, comparative to the IV group, with polyparasitized wild boars (*Dicrocoelium lanceolatum, Strongyloides papillosus, Metastrongylus elongatus and Eimeria debliecki*).Depending on the parasitic species and the level of wild boar infestation, there are variations in the leukocyte formula of the young neutrophils content, which are also decreased in the I group - uninfested wild boars by 27,3% (P> 0,05), comparative to those from the II group, by 42,9% (P> 0,05), comparative to those from the III group and by 56,8% (P> 0,05), comparative to the IV group.The lymphocyte indices analysis shows variations in the lymphocyte content, which is higher in the I group with uninfested wild boars by 13,0% (P> 0,05), comparative to those from the II group, by 19,5% (P> 0,05), comparative to those from the III group and by 25,9% (P> 0,05), comparative to polyparasitized wild boars from the IV group.

In the study's result of the impact of mono- and polyinvasions on lymphocyte indices in wild boars, it was possible to highlight the fact that both their invasion with Strongyloides papillosus from the II group, and with *Dicrocoelium lanceolatum* from the III group, and polyinvasion with *Dicrocoelium lanceolatum, Strongyloides papillosus, Metastrong elongatus* and *Eimeria debliecki*, ultimately, leads to the substances release with immunotoxic action on B, T and Th lymphocytes, affecting them morphofunctionally and increasing the amount of null and Ts lymphocytes (tab. 3).

In the study's result of the mono- and polyinvasions impact, on the proteinogram's indices in wild boars, was established in them the content of total proteins, albumins and globulins (tab. 4).

Table 3. Mono- and polyinvasions impact on lymphocyte indices in wild boars

Groups	Total lymphocytes %	Lymphocytes				
		B %	T %	null %	Th %	Ts %
I	47,9±**4,2**	17,4±2,4	52,0±2,6	22,1±3,2	22,1±1,1	18,3±1,2
II	41,7±**4,4**	16,2±1,3	28,1±1,7	47,4±3,4	12,0±1,1	27,3±1,4
III	38,6±**3,7**	12,2±1,5	21,4±1,5	54,2±2,3	9,8±2,2	25,3±2,4
IV	35,5±**3,8**	10,2±1,5	20,2±1,4	58,3±2,6	10,2±2,4	28,4±3,6

Table 4. Mono- and polyinvasions impact on proteinogram indices in wild boars

Groups	Total protein, g/100ml	Albumins, %	Globulins			
			α_1 %	α_2 %	β %	γ %
I	7,7±1,16	35,4±1,62	8,0±0,34	9,5±0,52	11,9±0,45	27,8±1,45
II	5,1±1,12	30,2±1,12	6,4±0,44	10,4±0,34	13,5±0,82	33,3±0,78
III	6,2±0,99	32,8±0,77	6,9±0,43	11,6±0,37	14,6±0,54	32,2±1,13
IV	4,8±0,86	27,6±0,86	5,4±0,32	12,4±0,34	15,8±0,32	36,4±1,62

In the wild boars from the II group, infested spontaneously with Strongyloides papillosus, was observed a total protein's content of 33,8% (P> 0,05), in those from the III group - by 19,5% (P <0,05), and in those from the IV group - by 37,7% (P <0,05) decreased, comparative to the I group. The albumin decreases by 14,7% (P <0,01) in the II group, by 7,4% (P <0,01) in the III group and by 22,0% (P <0,01) in the IV group, comparative to the I group, uninfested.

The α1 globulins, decreased by 20,0% (P <0,05) in the II group, in the III group - by 13,8%, and in the IV group - by 32,5%, comparative to the I group. The globulins α2 level from the II group was with 8,7% (P> 0,05), in the III group with 18,1% (P> 0,05), and in the IV group - with 23,4% (P> 0,05)) increased, comparative to the I group. β globulins in the II group was by 11,9% (P> 0,05), inthe III group by 18,5% (P> 0,05), and in the IV group - by 24,7% (P> 0,05), also increased, comparative to the I group, and the globulin γ level, in the II group was by 16,5% (P> 0,05), in the III group by 13,7% (P> 0,05), and in the IV group - with 23,6% (P> 0,05) higher at infested groups, comparative to the I group, uninfested.

The obtained results indicate that the infestation with *Strongyloides papillosus* causes a proteinogram indices decrease, characteristic for the type I electrophoregram changes, specific to the acute inflammatory processes, due to the mechanical and spoiling action of the rhabditoid larvae, which circulate in the pathogenic microflora in the host organism. Infestation with *Dicrocoelium lanceolatum* leads to the proteinogram's index change and causes respective changes, wich expressed the VIII electrophoregram type, specific for the hepatobiliary symptomatic complex, due to the toxic action, mechanical and spoliating action of dichroceles from the liver's bile ducts.In the study's result of the mono- and polyinvasions impact on the plasma hemostasis indices in wild boars, it was established that the prothrombin index's level (PI) in animals from the II group was lower by 16,4% (P> 0,05), in those from the III group by 14,2% (P> 0,05) and in those from the IV group by 18,2% (P> 0,05), comparative to those from the I group. The activated recalcification time (TRA), in wild boars from the II group, has increased compararative to the I group by 16,8% (P> 0,05), from the III group by 9,5% (P> 0,05) and from the IV group by 18,5% (P> 0,05). The activated partial thromboplastin time (aPTT) also increased in wild boars from the II group by 18,7% (P> 0,5), in the III group by 11,4% (P> 0,05) and in the IV group with 19,1% (P> 0,05), comparative to those from the I group. Thrombin time (TT) is in decreasing at infested groups, by 21,3% (P> 0,05) in the II group, with 14,9% (P <0,05) in the III group and with 24,6% (P <0,05) in the IV group, comparative to the I group - uninfested wild boars. The fibrinogen content from the I group with uninfested wild boars is higher with 21,2% than in the II group and with 9,7% (P> 0,05) than in the III group with monoinfested wild boars, and by 36.6% (P> 0, 05) than in the IV group with polyinfested wild boars. Ca2+ content, is also decreased by 35,5% (P> 0,05) in the II group, in the III group by 23,5% (P> 0,05) and in the IV group by 44,2% (P> 0,05), comparative to the I lot. (tab. 5).

Table 5. Mono- and polyinvasions impact on the plasma hemostasis indices in wild boars

Groups	PI,%	TRA,seconds	aPTT,seconds	TT,seconds	Fibrinogen,g/l	Ca^{2+},mmol/l
I	98,3±3,35	65,4±2,4	46,6±1,3	30,4±1,2	5,2±0,21	3,4±0,18
II	82,2±3,21	78,6±2,6	57,3±1,4	38,6±1,4	4,1±0,32	2,2±0,12
III	84,4±2,79	72,2±2,1	52,6±2,1	35,7±1,3	4,7±0,33	2,6±0,24
IV	80,4±2,21	80,2±2,6	57,6±2,6	40,3±0,30	3,3±0,18	1,9±0,12

The obtained results indicate that, both in boars infested with S. papillosus from the I group and in those infested with *D. lanceolatum* from the II group, indices was established a hemostatic decrease, and their maximum decrease is highlighted in the IV group with polyinfested wild boars with *Dicrocoelium lanceolatum, Strongyloides papillosus, Metastrongylus elongatus* and *Eimeria debliecki*. This decrease is due to exotoxins eliminated by parasites, which contain anticoagulant and hemolyzer substances, and which neutralize the fibrinogen, thrombin, Ca+ ions and vitamin K properties.

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STUDY OF ECTOPARASITIC FAUNA DIVERSITY IN WILD BIRDS FROM VARIOUS ANTHROPIC BIOTOPES OF THE REPUBLIC OF MOLDOVA

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Abstract. The results of the complex parasitological studies conducted in the wild birds of hunting interest as well as domestic birds demonstrates that the phenomenon of the poliparasitism is a static one, although the poliparasitic quantitative and qualitative structure is continuously changing. This is due to instantaneous contact of the wild birds with the domestic ones, irregular deparasiting of domestic animals and their crates, reduced areas for animals breading and maintenance that ensures the permanent contact with the wild birds as the infestation source.

The findings of the study on the diversity of the ectoparasitic fauna in wild birds from various anthropic biotopes of Moldova revealed that the wild and domestic birds are infested with multiple parasites and that the most identified parasitoses are common for major birds of hunting interest as well as for the domestic birds.

Introduction

The parasitoses represent the most frequent diseases in wild and domestic birds that cause essential economic losses [8]. Some authors indicate that among the birds, gallinaceans are especially sensible to infestation with ectoparasites, compared to palmipeds [1, 3-8].

The effective evaluation of animal population species of the hunting interest implies awareness about behavior, relationships among the species and components of the ecosystems they belong to. The parasitic fauna of wild birds can influence the birds' population dynamics especially in the long term timeframe [10-13, 19].

The gamasid mites and some species of mallophaga parasiting on domestic and wild birds, serve also the role of vectors of some viral and bacterian pathogens. Therefore it is imperious to identify the mixt invasions with ectoparasites in wild birds continuously spread off and representing one of the actual problems of parasitology and contemporary ecology [9, 14-18, 20].

Materials and methods

The parasitologic research conducted during years 2015-2019 on ectoparasitic fauna in 257 fancy poultry of hunting interest hold in captivity in various zoological collections in Moldova allowed to track the diversity of their ectoparasitic species.

The ectoparasites were collected from the live birds in line with the new, more informative protocol. The collection and further qualitative research of the ectoparasites in various stages of development reaches the level of app 100,0%, comparatively with classic collection methods applied on dead birds, since the parasites leave the dead hosts [2].

The collected material was further studied with the help of lens MBS-9 (ob.14x2) and microscope Novex Holland B (ob. 20-40) WF 10x Din/20mm.

Results and discussions

The conducted studies on the diversity of parasitic fauna in pheasants (*Chrysolophus amherstiae and Lophura nycthemera*) allowed identifying the diverse ectoparasites from: Family Philopteridae - 3 species (*Cuclotogaster cinereus, Goniocotes gallinae, Cuclotogaster heterographus*); Family Menoponidae - 2 species (*Menacanthus stramineus, Menopon gallinae*) and Family Ceratophyllidae - one species (*Ceratophylus hirundinis*) (see Table 1).

There were three species of ectoparasites identified in silver pheasant, all belonging to Family Philopteridae (*Cuclotogaster cinereus, Cuclotogaster heterographus Lipeurus caponis*) and one species from Family Menoponidae (*Eomenacanthus stramineus*).

The ordinary pheasant kept in the Zoo from Chişinău city revealed the high diversity of ectoparasites: Family Philopteridae - 2 species (*Goniocotes gallinae, Cuclotogaster cinereus*); Family Menoponidae - 2 species (*Menacanthus stramineus, Menopon gallinae*); Family Ceratophyllidae - one species (*Ceratophylus hirundinis*) and Family Dermanyssidae - 2 species (*Dermanyssus gallinae, D. hirundinis*).

The study on the ectopasites diversity in quails kept in the Zoo of Chişinău city revealed the high diversity of ectoparasites. Totally, 10 species of ectoparasies have been identified in quails being systematized to the following families: Family Philopteridae - 4 species (*Cuclotogaster cinereus, Goniocotes chrysocephalus, Goniodes astrocephalus, Lipeurus caponis*); Family Menoponidae - 2 species (*Menacanthus abdominalis, Menopon gallinae*); Family Ceratophyllidae – 2 species (*Ceratophylus gallinae, Ceratophylus hirundinis*) and Family Dermanyssidae - 2 species (*Dermanyssus gallinae, D. hirundinis*).

The studies on identifying the diversity of ectoparasitic zoofauna in ashy partridges allowed tracking 5 species of ectoparasites from the following families: Family Philopteridae - one species (*Goniodes dispar*); Family Menoponidae - 2 species (A*myrsidea perdicis, Menopon gallinae*); Family Ceratophyllidae – one species (*Ceratophylus hirundinis*) and Family Dermanyssidae - 2 species (*Dermanyssus hirundinis*).

Table 1. Ectoparasitic fauna in birds of hunting interest from the Zoo of Chişinău city

N/o	Host of parasites	Examined birds (exemplars)	Species of identified parasites
	Lady Amherst's pheasant	12	*Cuclotogaster cinereus (Nitzsch, 1866);* *Goniocotes gallinae (De Geer, 1778) ;* *Cuclotogaster heterographus (Nitzsch, 1866);* *Menacanthus stramineus (Nitzsch,1818);* *Menopon gallinae (Linnaeus, 1758);* *Ceratophylus hirundinis (Curtis, 1826)*
	Silver pheasant	16	*Cuclotogaster cinereus (Nitzsch, 1866);* *Goniocotes gallinae (De Geer, 1778) ;* *Cuclotogaster heterographus (Nitzsch, 1866);* *Menacanthus stramineus (Nitzsch,1818);* *Menopon gallinae (Linnaeus, 1758);* *Ceratophylus hirundinis (Curtis, 1826)*

	Golden pheasant	9	*Cuclotogaster cinereus (Nitzsch, 1866);* *Cuclotogaster heterographus (Nitzsch, 1866);* *Lipeurus caponis(Linné. 1758);* *Eomenacanthus stramineus (Nitzsch, 1818).*
	Common pheasant	18	*Goniocotes gallinae (De Geer, 1778);* *Cuclotogaster cinereus (Nitzsch, 1866);* *Menacanthus stramineus (Nitzsch,1818)* *Menopon gallinae (Linnaeus, 1758);* *Ceratophylus hirundinis (Curtis, 1826);* *Dermanyssus gallinae (De Geer, 1778) ;* *D. hirundinis (Duges,1834).*
	Quail	22	*Cuclotogaster cinereus (Nitzsch, 1866);* *Goniocotes chrysocephalus (Giebel,1874);* *Goniodes astrocephalus (Burmeister, 1838);* *Lipeurus caponis(Linné. 1758);* *Menacanthus abdominalis (Piaget,1880);* *Menopon gallinae (Linnaeus, 1758);* *Ceratophylus gallinae (Schrank, 1803);* *Ceratophylus hirundinis (Curtis, 1826);* *Dermanyssus gallinae (De Geer, 1778);* *D. hirundinis (Duges,1834).*
	Grey partridge	8	*Goniodes dispar (Burmeister,1838);* *Amyrsidea perdicis (Denny,1842);* *Menopon gallinae (Linnaeus, 1758);* *Ceratophylus hirundinis (Curtis, 1826);* *Dermanyssus hirundinis (Duges,1834)*
	Peacock	7	*Goniocotes chrysocephalus (Giebel,1874);* *Cuclotogaster cinereus (Nitzsch, 1866);* *Menopon gallinae (Linnaeus, 1758);* *Eomenacanthus stramineus (Nitzsch, 1818);* *Amyrsidea perdicis (Denny,1842);* *Ceratophylus hirundinis (Curtis, 1826);* *Dermanyssus gallinae (De Geer, 1778);* *D. hirundinis (Duges,1834);* *Ornithonyssus sylviarum (Canestrini et Fanzago,1877).*
	Chucar partridge	6	*Goniocotes chrysocephalus (Giebel,1874);* *Goniocotes microthorax (Stephens,1829);* *Cuclotogaster heterographus (Nitzsch, 1866);* *Ceratophylus hirundinis (Curtis, 1826);* *Menopon gallinae (Linnaeus, 1758);* *Menacanthus stramineus (Nitzsch,1818)*
	Mandarin duck	12	*Menopon obscurum, Trinoton querquedulae.*
	Wild goose	14	*Anaticola crassicornis, Anatoecus dentatus, Anatoecus icterodes.*

The study of ectoparasitic fauna diversity in peacocks allowed systematizing to the following families: Family Philopteridae - 2 species (*Goniocotes chrysocephalus, Cuclotogaster cinereus*); Family Menoponidae - 3 species (*Amyrsidea perdicis, Menopon gallinae, Eomenacanthus stramineus*); Family Ceratophyllidae – one species (*Ceratophylus hirundinis*) and Family Dermanyssidae - 3 species (*Dermanyssus gallinae, Dermanyssus hirundinis, Ornithonyssus sylviarum*).

The study of ectoparasitic fauna diversity in 6 species of partridges kept in captivity allowed systematizing of the identified ectoparasites to the following families: Family Philopteridae - 3 species (*Goniocotes chrysocephalus, Goniocotes microthorax, Cuclotogaster heterographus*); Family Menoponidae - 2 species (*Menopon gallinae, Menacanthus stramineus*) and Family Ceratophyllidae – one species (*Ceratophylus hirundinis*).

The study on diversity of ectoparasites in Mandarin ducks kept in captivity along with other fancy poultry allowed tracking two species of mallophaga from Family Menoponidae (*Menopon obscurum, Trinoton querquedulae*).

The study on ectoparasitic diversity in wild gooses allowed identifying three species of ectoparasites from Family Menoponidae (*Anaticola crassicornis, Anatoecus dentatus, Anatoecus icterodes*).

In this way, the parasitological research revealed that the domestic birds as well as the wild ones are infested with multiple parasites and that the majority of identified parasitizes are common for majority of birds of hunting interest as well as for those domestic ones.

The results of the complex parasitological studies in wild birds of hunting interest and in domestic birds revealed that the poliparasitism phenomenon has a permanent character although the poliparasitism structure is in continuous quantitative and qualitative dynamic. The reasons are the continuous contact of wild birds with those domestic ones, irregular disinfestation measures, reducing birds' area for breading and maintenance that ensures the permanent contact with the wild birds as the infestation source.

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SEASONAL DYNAMICS OF PHLEBOTOMUS PAPATASI (SCOPOLI, 1786) (DIPTERA: PSYCHODIDAE) POPULATION IN SOUTHERN REPUBLIC OF MOLDOVA

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Abstract. Phlebotomine sand flies are vectors of several infectious pathogens, including parasitic protozoans of the genus Leishmania and phleboviruses. Increasing sand fly biting nuisance reported by residents from southern Republic of Moldova since 2011 initiated this study. Ceadir-Lunga, a semi-urban locality in southern Republic of Moldova was selected for seasonal sand fly collections outdoors and indoors in 2015 and 2017 using CDC light traps and manual aspirators. Continuous trapping showed markedly longer activity of P. papatasi indoors. Specimens were collected from first aspirations in the second half of June until last collections in mid-September, suggesting that the actual indoor activity of P. papatasi may have been longer. Low numbers of trapped specimens do not allow make accurate conclusions regarding the seasonal dynamics.

Introduction

Phlebotomine sand flies (Diptera: Psychodidae) are vectors of several infectious pathogens, including parasitic protozoans of the genus *Leishmania* and phleboviruses [1]. Republic of Moldova is considered a country non-endemic for leishmaniasis, there are no records of autochthonous human or canine cases [2]. In the past, presence of three *Phlebotomus* species was reported in Moldova: Phlebotomus papatasi (Scopoli, 1786), P. perfiliewi Parrot, 1930 and *P. chinensis* (Newstead, 1916) [4, 5]. However, the historical studies provided scarse information about their abundance and seasonal dynamics. Historical data mentioned low numbers of *P. papatasi* (5 females and 2 males) in Giurgiulesti village, Cahul district, southern Moldova in 1946. In 1947, sand fly surveillance revealed the presence of low numbers of *P. papatasi* in other villages located in Cahul district (Chislita-Prut, Colibasi, Vadul-lui-Isac, Manta, Crihana Veche villages and Cahul town), but no exact numbers were given. Only one *P. papatasi* male was caught in Chisinau city in 1947 [6]. After several decades, increasing sand fly biting nuisance reported by residents from southern Moldova since 2011 initiated this study.

Materials and methods

Ceadir-Lunga (WGS84 coordinates: 46o06549N, 28o84219E), a semi-urban locality in southern Moldova was selected for seasonal sand fly collections in 2015 and 2017 using Centre for Disease Control (CDC) light traps (Trappola per Monitoraggio Zanzare, IMT Original 2002, Italy), miniature CDC light traps (John W. Hock Company, model 512, Gainesville, Florida, U.S.A.) and manual aspirators. Two CDC light traps operated on two permanent sites in Ceadir-Lunga close to two poultry houses between June 22 and September 26, 2015. Each site was sampled 2-3 times per week. Collection by manual aspirators inside houses (n=13) and animal shelters (n=14) was conducted several times per week between June 26 and September 14, 2015. Every time one house and/or one animal shelter were visited. In 2017, two CDC light traps operated every week on the permanent sites between July 6 and September 25. Additionally, sand flies were collected manually inside a chicken coop and a house (July 30-31, 2017). The collected insects were killed by freezing at -20 oC and preserved in 96% ethanol. For morphological identification, head and genitalia of each specimen were dissected and mounted on slides using Berlese mounting medium. Species identification was performed using published key [3].

Results and discussions

A total of 225 and 47 sand flies were sampled in 2015 and 2017, respectively, in the locality Ceadir-Lunga. In total, 18 specimens were trapped using CDC traps between June 22 and September 26, 2015. The majority of specimens belonged to *P. papatasi* (4 males, 5 females). First specimens of P. papatasi were trapped in mid-July and last in mid-August (Figure 1). Collection by manual aspirators inside houses and animals shelters between June 26 and September 14, 2015 yielded 207 sand flies (140 specimens in the houses and 67 specimens in animal shelters). The highest number of *P. papatasi* (49 males, 108 females) was collected in July in hen houses. Indoor sand fly activity was markedly longer, first specimens being collected at first aspiration in June 24 and last in September 14.

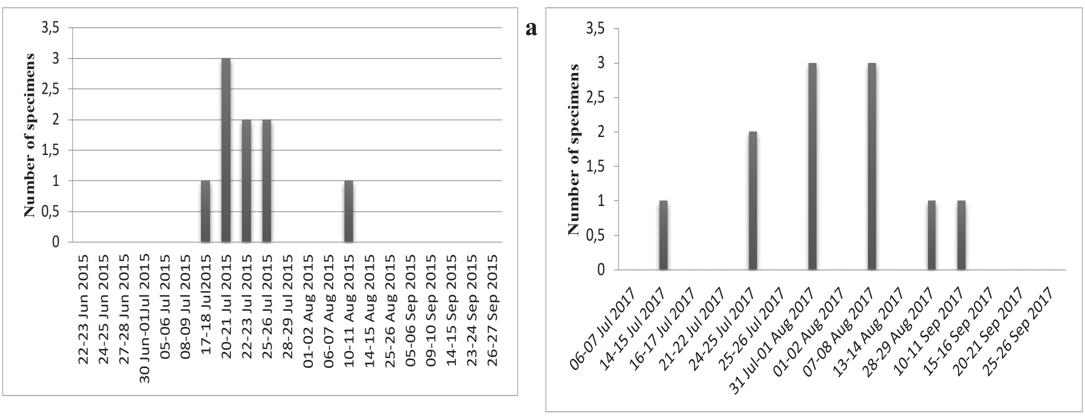

Figure 1. Seasonal collections of P. papatasi using CDC light traps in Ceadir-Lunga: b - collections conducted in 2017

In 2017, a total of 18 sand flies were collected by two CDC light traps, operated on the permanent sites between July 6 and September 25. The majority of specimens belonged to P. papatasi (3 males, 3 females) was collected in July. Four specimens of *P. papatasi* were sampled in August and one specimen in September 2017. Manual collection of sand flies inside a chicken coop and a house (July 30-31, 2017) provided additional 29 *P. papatasi* specimens (12 males, 17 females). Numbers of specimens trapped by the CDC traps in 2015 and 2017 are shown on graphs (Figure 1).

Continuous trapping in Ceadir-Lunga in 2015 and 2017 showed markedly longer activity of P. papatasi indoors. Specimens were collected from first aspirations in the second half of June until last collections in mid-September, suggesting that the actual indoor activity of *P. papatasi* may have been longer. First specimens from outdoor trapping were collected in early July, despite earlier trapping effort. In 2015, no specimens were trapped outdoors after mid-August and in 2017, outdoor activity ceased by mid-September, despite later trapping effort. Low numbers of trapped specimens do not allow to make accurate conclusions regarding the seasonal dynamics.

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NEW AND RARE DIURNAL BUTTERFLIES (LEPIDOPTERA: PAPILIONOIDEA) FROM THE "COBÎLENI" NATURAL RESERVE (REPUBLIC OF MOLDOVA)

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Abstract. In the present paper data on seven rare species of diurnal butterflies (Lepidoptera: Papilionoidea) in the fauna of the "Cobîleni" Natural Reserve (Orhei district) are given. The investigations were carried out during 2012-2020 in different habitats of the Reserve: like forest, forest edge, meadows and calcareous canyons. The species Nymphalis xanthomelas (Esper, 1781) and Papilio machaon (Linnaeus, 1758) are reported as new for the fauna of the "Cobîleni" Natural Reserve. Thus, the number of diurnal butterflies in the fauna of the "Cobîleni" Natural Reserve reached 64 species.

Introduction

In the Republic of Moldova, nature reserves are protected areas, which represent natural spaces, valuable from a scientific point of view, intended to preserve and restore one or more components of nature to maintain ecological balance. According to Law no. 1538 of 25.02.1998 on the fund of natural areas protected by the state, 51 forest reserves, 9 medicinal plants and 3 mixed ones are registered, in total 63 nature reserves with a total area is 8,009 ha, one of them being and the "Cobîleni'' Natural Reserve [7].

Despite of small territory (33.5 ha), the "Cobîleni" Natural Reserve includes many types of biotopes such as landscapes of flooded meadows, mixed forests and limestone rocks. The rock forest, specific to the steep slopes of the Dniester valley, are dominated by *Quercus robur* and *Fraxinus excelsior* species.

The study of Lepidoptera fauna in the „Cobîleni" Natural Reserve started in 2012. Until then, the entomological fauna of the reserve has not been researched. So far, 298 species of diurnal butterflies and moths have been reported on the territory of the reserve [10, 11, 12, 14]. Faunal diversity of diurnal butterflies in the reserve consists of 62 species belonging to 39 genera and taxonimic 6 families: Hesperiidae (6 species), Pieridae (10), Papilionidae (2), Nymphalidae (19), Riodinidae (1) and Lycaenidae (24 species) [10].

During the research, several new lepidopteran species for the fauna of the Republic of Moldova were reported in the "Cobileni" Natural Reserve such as *Acontia candefacta* (Hübner, 1831), *Eucarta amethystina* (Hübner, 1803), *Cucullia fraterna* Butler, 1878, *Chersotis margaritacea* (Villers, 1789), *Ch. rectangula* (Denis & Schiffermüller, 1775), *Euxoa cos* (Hübner, 1824), *E. birivia* (Denis & Schiffermüller, 1775), *Noctua tertia* Mentzer & al., 1991, *Dasypolia templi* (Thunberg, 1792) and *Aedophron rhodites* (Eversmann, 1851) [11-14]. Many of the reported species are endangered and protected by law both in Europe and Republic of Moldova, being cited in various Red Lists as well Habitats Directive (Annex II and IV), Bern Convention, European Red List of Butterflies, Red Book of the Republic of Moldova, etc.

The purpose of the study was to identify the faunal diversity of rare diurnal butterflies on the territory of the "Cobîleni" Natural Reserve.

Materials and methods

The study was conducted in the “Cobîleni” Natural Reserve (47°30´51´´N, 29°1´20,37´´E) of the Republic of Moldova, which is taken under protection since 1998. The Reserve belongs to the Forest District Susleni, of State Forestry Enterprise Orhei [8]. The “Cobîleni” Natural Reserve is a natural forest area with a surface of 33.5 ha, situated near Lopatna village, Orhei district on the bank of the Dniester River.

According to the geomorphological regionalization, the Reserve is located on the west Dniester’s hills and its terraces, at 250-300 m altitude, characterized by a vertical fragmentation. Flooded meadows, mixed forests and limestone rocks, specific to the steep slopes of the Dniester valley, covered by *Quercus robur* and **Fraxinus excelsior**, represent the studied Reserve.

There are also ephemeral and ephemeroid plants growing under the shade of the trees – *Convallaria majalis, Anemone ranunculoides, Ficaria verna*, but especially of rare species: *Fritillaria montana, Lunaria annua and Galanthus nivalis*. Numerous species of ferns, mosses, lichens (*Cladonia pyxidata, C. fimbriata,* etc.) and some angiosperms are growing on the limestone rocks in the Dniester valley, which, combined, create a mosaic, similar to that of the mountainous areas [1]. The vegetation of steep section in the middle part of the Reserve, exposed to the east, consist of *Stipa pulcherrima, Amygdalus nana, Rhamnus cathartica, Thalictrum minus, Silene fabaria, etc*.

The research was carried out during the vegetation period of 2012-2020. The entomological material were collected in different habitats of the “Cobîleni” Natural Reserve like forest, forest edge, meadows and calcareous canyons.

Collecting of diurnal butterflies was done with entomological net, manually, or only photos were taken for rare species. For species identification we have used the work by L. Rakosy [9].

Results and discussions

During the research seven species of diunal butterflies rare in the fauna of the Republic of Moldova, have been reported in “Cobîleni” Natural Reserve taxonomically classified in five families: Nymphalidae – 2 species, Lycaenidae – 1, Riodinidae – 1, Papilionidae – 2 and Pieridae – 1 species. Of these, all species are included in the Red Book of the Republic of Moldova [2], one species are mentioned in the Habitats Directive (Annex IV) [5], one species is cited in the Bern Convention [4] and another three species – in the European Red List of Butterflies [3]. The species *Polyommatus daphnis* are included in the IUCN Red List (LC category) for Europe [6].

The species *Nymphalis xanthomelas* (Esper, 1781) and *Papilio machaon* (Linnaeus, 1758) represent new taxa for the fauna of the “Cobîleni” Natural Reserve. The species are protected by law, included in the European Red List of Butterflies and in the Red Book of the Republic of Moldova.

In the list of butterflies shown below, we mention the most important rare species, their records, some ecological and distribution data as well the conservation status.

Family NYMPHALIDAE

1. ***Neptis sappho* (Pallas, 1771)** (fig. 1, a)

   Collected material: July 20, 2016, 1 spec.

   Geographical spread: it is spread in temperate zone of Eurasia.

   Ecological preferences: meadows and humid forest edges.

   Protection and conservation: the species is protected by law, included in European Red List of Butterflies and in the Red Book of the Republic of Moldova.

2. ***Nymphalis xanthomelas* (Esper, 1781)**

   Collected material: June 24, 2016, 1 spec.

   Geographical spread: it is spread in temperate zone of Eurasia.

   Ecological preferences: meadows and moist forest edges, especially those in the floodplains.

Protection and conservation: the species is protected by law, included in European Red List of Butterflies and in the Red Book of the Republic of Moldova.

Family LYCAENIDAE

3. ***Polyommatus daphnis* (Denis & Schiffermüller, 1775)** (fig. 1, b)

   Collected material: May 28, 2017, 3 specs.

   Geographical spread: it is spread in Europe and to the east of the Black Sea, including Iran.

   Ecological preferences: sunny and dry meadows, with a large amount of florigenic vegetation on the limestone slopes; rich pastures and soil containing limestone.

Protection and conservation: the species is protected by law, included in the IUCN Red List (LC category) for Europe and in the Red Book of the Republic of Moldova.

Family RIODINIDAE

4. ***Hamearis lucina* (*Linnaeus, 1758*)** (fig. 1, c)

   Collected material: July 12, 2012, 5 specs.; July 08, 2014, 8 specs.

   Geographical spread: it is spread in Cental and Southern Europe and Asia Minor.

   Ecological preferences: meadows and forest edges.

Protection and conservation: the species is protected by law, included in the Red Book of the Republic of Moldova.

Family PAPILIONIDAE

5. ***Zerynthia polyxena* (Denis & Schiffermüller, 1775)** (fig. 1, d)

   Collected material: the species was observed in all years of research in May.

   Geographical spread: it is spread in Central, South and South-Eastern Europe, Western Caucasus and Asia Minor.

   Ecological preferences: shrubberies, orchards, agricultural fields.

Protection and conservation: the species is protected by law, included in the Annex II of the Berne Conventions, 1979; the Annex IV of Habitats Directive and in the Red Book of the Republic of Moldova.

6. ***Papilio machaon* (Linnaeus, 1758)**

   Collected material: the species was observed in all years of research in May.

   Geographical spread: it is spread in Europe, Asia, North Africa, North America, including some tropical regions.

   Ecological preferences: open biotopes – meadows, sectors of steppe vegetation, groves.

Protection and conservation: the species is protected by law, included in the Red Book of the Republic of Moldova.

Family PIERIDAE

7. ***Leptidea morsei*** **(Fenton, 1882)**

Collected material: June 06, 2012, 2 specs.; July 02, 2014, 1 spec.

Geographical spread: it is spread in Eastern Europe, temperate Asia, Sakhalin Island and Japan.

Ecological preferences: humid meadows and shady forest roads.

Protection and conservation: the species is protected by law, included in European Red List of Butterflies and in the Red Book of the Republic of Moldova.

a b c d

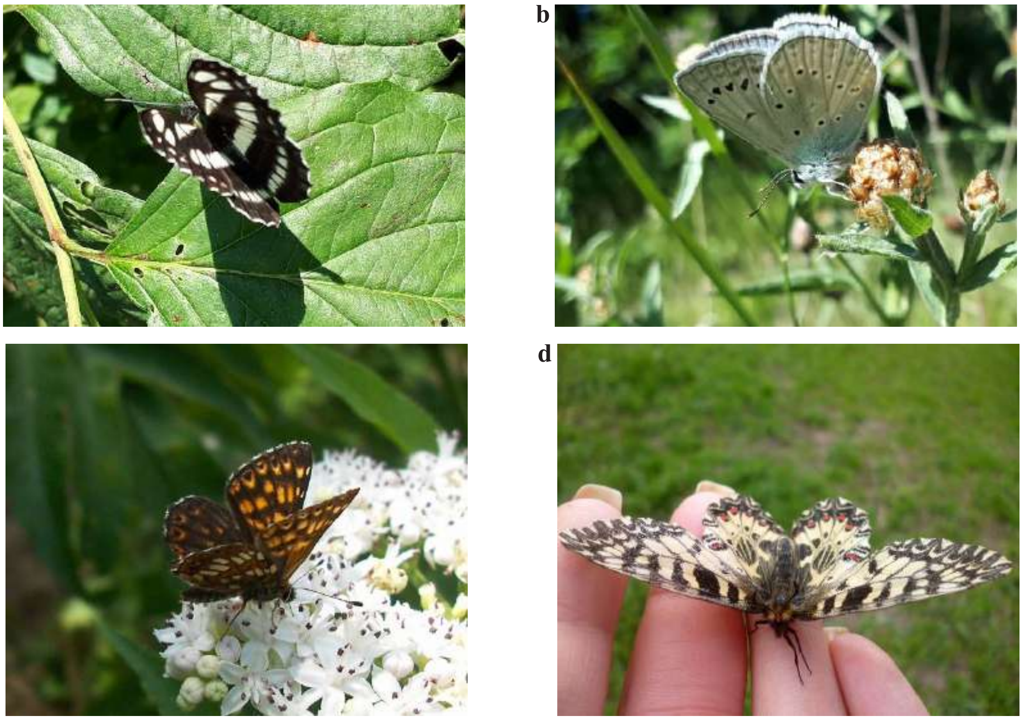

Figure 1. The species of diurnal butterflies reported in „Cobîleni" Natural Reserve: a – *Neptis sappho*, b – *Polyommatus daphnis*, c – *Hamearis lucina*, d – *Zerynthia polyxena* (orig.).

Conclusions

So far, 62 species of diurnal butterflies have been identified in the „Cobîleni" Natural Reserve. The species *Nymphalis xanthomelas* (Esper, 1781) and *Papilio machaon* (Linnaeus, 1758) represent new taxa for the reservation's fauna. Thus, the number of diurnal butterflies in the fauna of the „Cobîleni" Natural Reserve reached 64 species.

The species *Zerynthia polyxena* (Denis & Schiffermüller, 1775), *Polyommatus daphnis* (Denis & Schiffermüller, 1775), *Neptis sappho* (Pallas, 1771), *Nymphalis xanthomelas* (Esper, 1781), *Hamearis lucina* (Linnaeus, 1758), *Papilio machaon* (Linnaeus, 1758) and *Leptidea morsei* (Fenton, 1882) are endangered and require protection and conservation.

The large number of rare and new species proves that the „Cobîleni" Natural Reserve represents an area of particular importance for the conservation of biological diversity, and research in Reserve needs to be continued.

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VARIATION OF SOME MEAT QUALITY INDICES IN ECTOPARASITE POLYPARASITIZED AND ANTIPARASITIC TREATED HENS

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Abstract. As a result of the conducted research it was established, that the meat quality of hens polyparasitized with malophages (Cuclotogaster heterographus, Eomenacanthus stramineus, Goniocotes gallinae, Goniocotes maculatus, Goniodes dissimilis, Lipeurus caponis, Menopon gallinae, Menacanthus cornutus, Menacanthus pallidulus) fleas (Ceratophylus gallinae, C. hirundinis) and mites (Dermanyssus gallinae, D. hirundinis), then treated with Ectostop T5%, Ectostop P5%, showed a protein content of 20.5±0.20%, a pH of 5.3±0.12 and a moisture content of 65.4±0.21%, these indices reaching the level of the non-infested batch (control).

The protein level is low in the meat samples of batch II (untreated infested), constituting 16.22±0.03%, and in batch VII treated with Ivomec chemical preparation 17.02±0.87%. The meat moisture index is characterized by a greater difference for batch II and VII constituting 74.52±0.18% and 70.1±0.05% respectively. The pH assessed in untreated infested and Ivomec-treated hens is alkaline, which will lead to decreased keeping capacity.

Introduction

One of the main directions of the technical and scientific policy of the state, in the direction of maintaining a decent food supply of the population, is to provide it with quality food products. In the Republic of Moldova, a number of legislative documents have been adopted in recent years to protect the domestic consumer [10]. Moreover, bibliographical data show that the impact of parasitosis and antiparasitic preparations on the bacteriological status, vitamin, micro- and macroelement content of the parasitized organism is considerable [3, 4].

Poultry meat has numerous organoleptic and nutritional properties, is low in calories and high in protein (21.0-22.0%), and occupies a special place in human nutrition because of its quality. The muscle tissue is more compact, the muscle fibres are finer, the sarcolemma is very thin and the meat is thinner, the connective tissue in it is less, blood irrigation is minimal. All these structural properties give it a good digestibility, making it a dietary meat. It contains a higher percentage of vitamins than mammalian meat [5, 7, 11].

The main physical characteristic of meat is its acidity (pH), which determines and reflects the state of its chemical components. The importance of the pH of meat is determined by the fact that it is directly or indirectly related to: colour, tenderness, aroma and taste, water holding capacity. It is appreciated that meat pH is influenced primarily by non-genetic factors (physical activity, diet duration, maintenance status, parasite stress, temperature, etc.), as well as some genetic factors (species), individual specificity of response, etc. [2, 12].

In addition to the aforementioned characteristics, some authors highlight the importance of meat moisture. The greater or lesser moisture content of meat is related to its capacity to retain its own water and, in particular, so-called ‘free water’, which depends on a large number of factors after slaughter [13].

At the same time, according to the World Health Organisation, animal production must be ecologically pure and harmless to the environment. International trade of animals, meat and meat products, the active movement of tourists and globalisation exacerbate and increase the risk of parasitic zoonoses [9]. An effective compliance programme ensures that the requirements of EU legislation and, where applicable, national legislation and other international standards, which are related to the production of food for human consumption, are met [1].

Materials and methods

The research was carried out during 2015-2020 in the Parasitology and Helminthology laboratory of the Institute of Zoology. Malophagia, fleas and gamasid mites were collected from live birds, according to a new procedure for collecting ectoparasites from live birds [6], and from hen houses, by applying rubber systems placed in cages and nests (mite hiders). The collected material was further examined using the МБС-9 magnifying glass (ob. x 4) and the МБИ -3 microscope (ob. x 10). The population structure of ectoparasites (developmental stages: eggs, larvae, nymphs and imago) was studied, determining the quota, share of eggs, larvae, nymphs and imago. The method applied is effective for collecting various groups of ectoparasites from live birds.

In order to elucidate the indices of meat chemical composition (pH, protein, lipid and moisture content), 35 6-month-old Adler’s Silver breed chickens were trained in the experiment. Initially the chickens were polyparasitized and then treated with plant preparations Ectostop T5% (extract from dried aerial parts of tobacco (Nicotiana rustica L.), Ectostop P5% (natural biologically active extract obtained from plant raw material of wormwood (Artemisia absinthium L.). and the chemical preparation Ivomec. The birds were divided into seven equal groups: group I - control (non-infested); groups II-VII - infested with malophagous (*Cuclotogaster heterographus, Eomenacanthus stramineus, Goniocotes gallinae, Goniocotes maculatus, Goniodes dissimilis, Lipeurus caponis, Menopon gallinae, Menacanthus cornutus, Menacanthus pallidulus), with fleas (Ceratophylus gallinae, C. hirundinis*) and mites (*Dermanyssus gallinae, D. hirundinis*).

Batch I - control (uninfested chickens); batch II - untreated, batch III - treated with Ectostop T5% at a dose of 50 ml per bird by spraying, (aqueous solution); batch IV - treated with Ectostop T5% at a dose of 50 ml per bird by spraying (aqueous solution); lot V - with Ectostop P5%% in a dose of 50 ml per bird by spraying (hydroalcoholic solution); lot VI - with Ectostop P5% in a dose of 50 ml per bird by spraying (aqueous solution); lot VII - with Ivomec 1% in a dose of 0.2 ml administered subcutaneously in one dose. At the end of the experiment (after 35 days) the chickens were sacrificed. Meat samples of 100-150 g were collected individually from each bird. Protein, lipid, pH value and moisture content were determined in the meat samples according to classical methods in the laboratory of I.P. Republican Centre for Veterinary Diagnosis (I.P.C.R.D.V.).

Results and discussion

The analysis of the obtained data reveals that the pH level in the chickens of batch I was 5.3±0.08, for batch II this index increased and was 7.2±0.15 or 35.8% (p>0.9) higher than batch I (tab. 1). In batches III, IV and V the pH values were identical, 5.5±0.17, but 30.9% (p<0.9) lower than batch II and 3.7% (p>0.9) higher than batch I. The pH value in batch VI was at the same level as batch I index of 5.3±0.12. In batch VII this index reached 6.3±0.13, was 18.8% (p>0.9) higher than batch I, but 12.5% (p<0.9) lower than batch II, and 14.2% (p>0.9) higher than batches III, IV and V (fig.1).

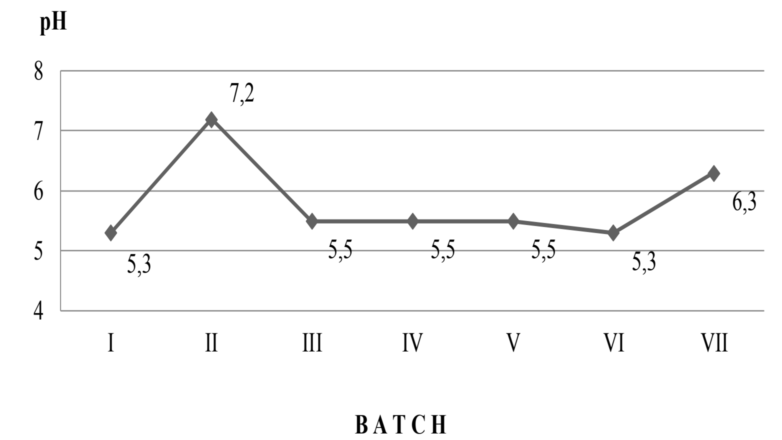

Figure 1. Chick pH levels in muscle tissue of polyparasitized and treated chickens

Another index characterising meat quality is the moisture content. This index reached a level of 63.78±1.52% in batch I or 16.8% (p<0.9) lower than batch II. In batch II the meat moisture index has the highest level of 74.52±0.18%, 14.2% (p>0.9) higher than in batches III, IV V and VI. In batch VII this index reaches 70.1±0.05%, 7.5% (p>0.9) higher than in batches III, IV, V and VI, and 9.9% (p>0.9) higher than in batch I, but 6.0% (p<0.9) lower than in batch II (tab. 1).

Table 1. Chemical composition of muscle tissue in chickenspolyparasitized and after antiparasitic treatment

Batch	Probe numb.	pH	Protein, %	Lipid, %	Moisture, %
I	5	5.3±0.08	21.0±0.01	6.22±0.14	63.78±0.52
II	5	7.2±0.15	16.22±0.03	2.54±0.15	74.52±0.18
III	5	5.5±0.17	20.36±0.27	9.16±0.06	65.1±0.05
IV	5	5.5±0.16	20.5±0.20	9.24±0.11	65.0±0.05
V	5	5.5±0.17	20.3±0.10	9.20±0.13	65.0±0.05
VI	5	5.3±0.12	19.0±0.10	8.50±0.21	65.40±0.21
VII	5	6.3±0.13	17.02±0.87	7.22±0.13	70.10±0.05

The result of the analysis shows that the protein level in the meat was 21.0±0.01% in batch

I and 16.22±0.03% in batch II, or 22.8% ($p<0.9$) lower than in batch I. In batches III, IV and V this index was approximately at the same level of 20.36±0.27%, 3.1% ($p<0.9$) lower than in batch I and 25.5% ($p>0.9$) higher than in batch II. This index reached 19±0.01% in group VI, 9.6% lower ($p<0.9$) than group I but 17.1% higher ($p>0.9$) than group II. In group VII this index reached 17.02±0.87% and was 19.1% lower ($p<0.9$) than group I, but 4.9% higher ($p>0.9$) than group II (fig. 2).

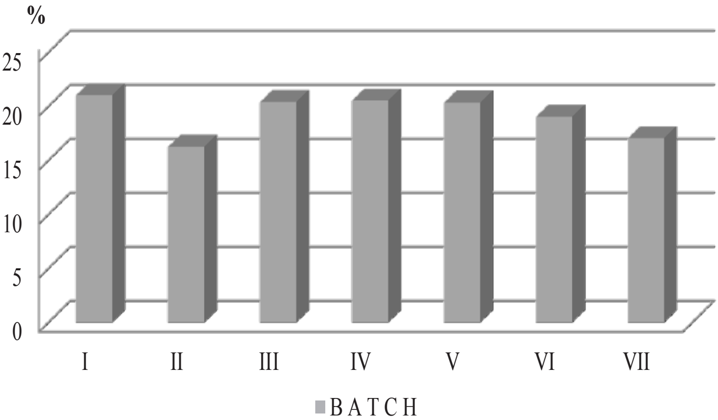

Figure 2. Protein content in muscle tissue of polyparasitized and treated chickens

Analyses carried out to determine the lipid level in chickens after antiparasitic treatment show that in group I - control, this index reached 6.22 ± 0.14% or 2.5 times higher than in group II (infested, untreated). An insignificant difference in this index was recorded between batches III, IV and V, with an average of 9.24±0.11%, 48.5% ($p>0.9$) higher than batch I, and a significant increase of 72.6% ($p>0.9$) compared to batch II. In batch VI the lipid content reached 8.5±0.21%, 36.6% ($p>0.9$) higher than batch I and 70.6% ($p>0.9$) higher than batch II. In group VII the lipid level was 7.22±0.13%, 16.0% ($p>0.9$) higher than group I and 64.9% ($p>0.9$) higher than group II (fig. 3).

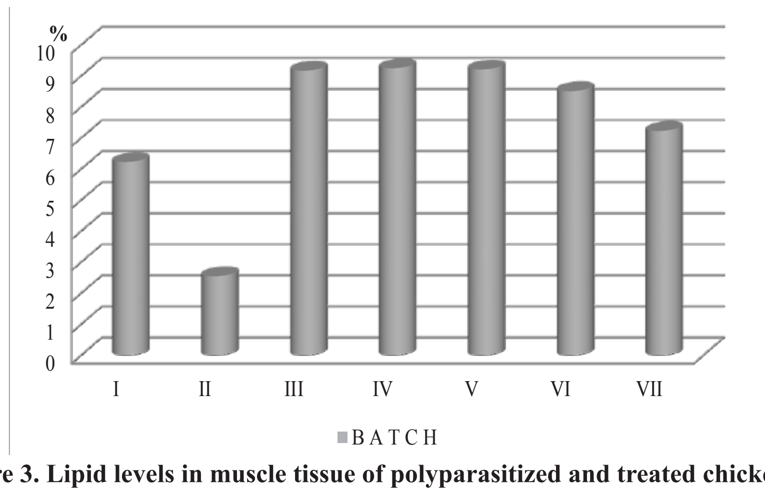

Figure 3. Lipid levels in muscle tissue of polyparasitized and treated chickens

Therefore, the analyses carried out show that the meat quality of birds polyparasitised with malophages: (*Cuclotogaster heterographus, Eomenacanthus stramineus, Goniocotes gallinae, Goniocotes maculatus, Goniodes dissimilis, Lipeurus caponis, Menopon gallinae, Menacanthus cornutus, Menacanthus pallidulus*) fleas: (*Ceratophylus gallinae, C. hirundinis) and mites (Dermanyssus gallinae, D. hirundinis*), then treated with Ectostop T5% , Ectostop P5% plant preparations, showed a higher protein content (20.5±0.20%), a lower pH level (5.3±0.12) and a lower moisture content (65.4±0.21%), these indices being within the level of the non-infested batch (control).Protein content is low in the meat samples in batch II (untreated infested), constituting 16.22±0.03% and in batch VII treated with Ivomec preparation constituting 17.02±0.87%. The meat moisture index is characterized by a greater difference for batch II and VII constituting 74.52±0.18% and 70.1±0.05% respectively.The PH level determined in both infested, untreated (batch II) and Ivomec-treated chickens is alkaline and constitutes 7.2±0.15 and VII 6.3±0.13 respectively, which will lead to a decrease in keeping capacity.Based on the results obtained, the meat of the chickens in batches III-VI has a higher protein content, lower pH level and lower moisture content, which allows it to be preserved for a longer period of time and is therefore of a much higher quality compared to batch II (untreated) and batch VII (treated with Ivomec).The use of extracts of natural origin with anti-parasitic action ensures that the preparation is not toxic to the animal organism and does not impose restrictions on the use of products and by-products The studies were carried out within the research project no. 20.80009.7007.12.

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SECTION 3. TERRESTRIAL VERTEBRATES

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THE INVENTORY OF THE ORNITOFAUNA OF SARATA NOUA LAKE, LEOVA COUNTY, REPUBLIC OF MOLDOVA FROM 2016 – 2021

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Abstract. The Republic of Moldova is a small European country where long-term studies have been more of an exception than a rule. Being an agrarian country, the mosaic of natural and artificial habitats is found on a smaller scale, namely in the Sărata river meadow, and in Sărata Nouă lake and surrounding. The Sărata River is a right tributary of the Prut River in the Republic of Moldova, having a quiet plain character, with a mosaic of natural and artificial habitats which are traditionally managed. The area overlaps with the East-Elbic migration route, the short distance to the Prut River and being located in the northern part of the Bugeac steppe, ensures a great diversity both in winter and nesting, and especially in the migration period. During 2016-2021, we registered the presence of 126 bird species, which represent 46% of the total species encountered in the Republic of Moldova.

Introduction

The Sărata Nouă Lake is an average-sized lake located in the middle course of the river Sărata, which is a tributary of the Prut river in the Republic of Moldova. The Sărata river has a total length of 59 km [2], while the Sărata Nouă lake has a water surface of 139.50 hectares, according to „Apele Moldovei" Agency [9].

The Sărata Nouă lake is located near the village of the same name, situated in the Leova country. In the Sărata river meadow and on the related slopes there are 14 villages with a total population of over 16 thousand people [8]. The river basin is located in the northern part of the Bugeac steppe, having its springs from the village Sarata-Mereșeni (Hîncești county). The upper course of the river has a southern direction, while the middle is oriented southeast. Near the place where it flows into the Prut river (Nicolaevca village, Leova county) it turns sharply to the west [3]. The lake is located on the middle course of the river Sărata.

In the Sărata river basin there are over 60 artificial ponds, which are used mainly for irrigation and fish farming. The largest artificial lakes are Sărata Nouă, Cneazevca, Caracui and Sărata Galbenă. The fact that the area overlaps with the East-Elbic migration route [4], the short distance to the Prut River and being located in the northern part of the Bugeac steppe [5], ensures a great diversity both in winter and nesting, and especially in the migration period.

There are no exhaustive studies describing the location and geographical features of the Sărata Nouă lake, and also there is no study regarding the ornitofauna of the Sărata river basin or particularly on Sărata Nouă Lake.

Materials and methods

Because wetlands are among the most threatened ecosystems [7] it is very important to have permanent monitoring of biodiversity in general and of waterbirds in particular, due to their capacity to indicate the changes in wetlands. This is why we selected as a study area an average-sized lake on the Sarata River in the Republic of Moldova. This study is based on regular observations on the Sărata Nouă Lake during the last 6 years (2016 to 2021), covering all phenological periods: migration, breeding, and wintering. The field observations were carried with a monthly frequency during the migration and breeding season, while the wintering observations were carried out every two or three months.

During breeding seasons we carried out point counts and line transects, which were equally distributed in all types of habitats to meet 100% coverage. To estimate the migration and wintering population, we used point observations in order to cover the entire surface of the lake. During the field studies we used binoculars (Zeiss, 8X45), field scope (Swarovski, 20x - 60x) and specific bird identification books (Collins bird guide, 2nd edition, 2009; Ghid pentru identificarea păsărilor, 2017).

Results and discussions

During the 2016-2021, we documented the occurrence of 126 bird species, out of which 109 were migratory, 75 breeding and 37 wintering. These 126 species represent around 46% of the total number of bird species registered in the Republic of Moldova. Taxonomically, the 126 bird species recorded during our study are distributed in 16 orders of the Aves class, the Passeriformes order being the most well represented (37 species) (tab. 1).

Table 1. List of birds observed in the study area structured on phenological seasons (xR - regular breeder, xS - sporadic breeder)

No	Species	Phenological season		
		Migration	Breeding	Wintering
	ord. Anseriformes			
1	*Cygnus olor*	x	xS	x
2	*Tadorna ferruginea*	x	xS	
3	*Anas platyrhynchos*	x	xR	x
4	*Anas crecca*	x		x
5	*Anas acuta*	x		
6	*Spatula querquedula*	x		
7	*Spatula clypeata*	x		
8	*Mareca penelope*	x		x
9	*Mareca strepera*	x		
10	*Aythya ferina*	x		
11	*Aythya fuligula*	x		x
	ord. Galliformes			
12	*Phasianus colchicus*		xR	x
	ord. Podicipediiformes			
13	*Podiceps cristatus*	x		x

	ord. Columbiformes			
14	*Columba palumbus*	**x**	**xR**	
15	*Streptopelia turtur*	**x**	**xR**	
16	*Streptopelia decaocto*	**x**	**xR**	
	ord. Cuculiformes			
17	*Cuculus canorus*	**x**	**xR**	
	ord. Apodiformes			
18	*Apus apus*	**x**	**xS**	
	ord. Gruiformes			
19	*Fulica atra*	**x**	**xR**	**x**
20	*Gallinula chloropus*	**x**	**xR**	
21	*Rallus aquaticus*	**x**	**xR**	
22	*Crex crex*	**x**	**xR**	
23	*Grus grus*	**x**		
	ord. Chradriiformes			
24	*Himantopus himantopus*	**x**	**xS**	
25	*Vanellus vanellus*	**x**	**xR**	**x**
26	*Calidris pugnax*	**x**		
27	*Calidris alpina*	**x**		
28	*Calidris ferruginea*	**x**		
29	*Calidris minuta*	**x**		
30	*Actitis hypoleucos*	**x**		
31	*Tringa nebularia*	**x**		
32	*Tringa glareola*	**x**		
33	*Tringa totanus*	**x**		
34	*Tringa stagnatilis*	**x**		
35	*Tringa ochropus*	**x**		
36	*Tringa erythropus*	**x**		
37	*Charadrius alexandrinus*	**x**		
38	*Charadrius hiaticula*	**x**		
39	*Charadrius dubius*	**x**	**xS**	
40	*Gallinago gallinago*	**x**		
41	*Limosa limosa*	**x**		
42	*Phalarobus lobatus*	**x**		
43	*Chroicocephalus ridibundus*	**x**		**x**
44	*Ichthyaetus melanocephalus*	**x**		
45	*Larus cachinnans*	**x**		**x**
46	*Chlidonias hybrida*	**x**		
	ord. Ciconiiformes			

47	*Ciconia nigra*	x		
48	*Ciconia ciconia*	x	x	
	ord. Suliformes			
49	*Phalacrocorax carbo*	x		x
	ord. Pelecaniformes			
50	*Pelecanus onocrotalus*	x		
51	*Ardea alba*	x		x
52	*Ardea cinerea*	x	xR	x
53	*Ardea purpurea*	x	xR	
54	*Ardeola ralloides*	x	xS	
55	*Ixobrychus minutus*	x	xR	
56	*Nycticorax nycticorax*	x	xR	
57	*Egretta garzetta*	x	xS	
58	*Botaurus stellaris*	x	xR	
59	*Platalea leucorodia*	x	xS	
60	*Plegadis falcinellus*	x		
	ord. Accipitriformes			
61	*Accipiter gentilis*		xR	
62	*Accipiter nisus*	x	xR	
63	*Milvus migrans*	x	xS	
64	*Circus cyaneus*	x		x
65	*Circus aeruginosus*	x	xR	
66	*Buteo buteo*	x	xR	x
67	*Buteo rufinus*		xR	
68	*Haliaeetus albicilla*	x		
69	*Circaetus gallicus*	x		
70	*Falco tinnunculus*	x	xR	
71	*Falco subbuteo*	x	xR	
72	*Pandion haliaetus*	x		
73	*Clanga pomarina*	x	xR	
	ord. Bucerotiformes			
74	*Upupa epops*	x	xR	
	ord. Coraciiformes			
75	*Alcedo atthis*	x	xR	
76	*Merops apiaster*	x	xR	
	ord. Piciiformes			
77	*Dendrocopos syriacus*		xR	x
78	*Dendrocopos major*		xR	x
79	*Dryobates minor*		xR	x

	ord. Passeriformes			
90	*Oriolus oriolus*	**x**	**xR**	
91	*Lanius collurio*	**x**	**xR**	
92	*Lanius excubitor*		**xS**	**x**
93	*Lanius minor*	**x**	**xR**	
94	*Corvus frugilegus*	**x**	**xR**	**x**
95	*Coloeus monedula*	**x**	**xR**	**x**
96	*Corvus cornix*	**x**	**xR**	**x**
97	*Garrulus glandarius*	**x**	**xR**	**x**
98	*Cyanistes caeruleus*	**x**	**xR**	**x**
99	*Parus major*	**x**	**xR**	**x**
100	*Panurus biarmicus*	**x**	**xR**	**x**
101	*Galerida cristata*	**x**	**xR**	**x**
102	*Acrocephalus arundinaceus*	**x**	**xR**	
103	*Acrocephalus scirpaceus*	**x**	**xR**	
104	*Locustella luscinioides*	**x**	**xR**	
105	*Hirundo rustica*	**x**	**xR**	
106	*Delichon urbicum*	**x**	**xR**	
107	*Riparia riparia*	**x**	**xR**	
108	*Sturnus vulgaris*	**x**	**xR**	**x**
109	*Turdus philomelos*	**x**	**xR**	
110	*Turdus merula*	**x**	**xR**	
111	*Lullula arborea*	**x**	**xR**	
112	*Alauda arvensis*	**x**	**xR**	
113	*Luscinia luscinia*	**x**	**xR**	
114	*Phylloscopus collybita*	**x**	**xR**	
115	*Troglodytes troglodytes*	**x**		**x**
116	*Motacilla alba*	**x**	**xR**	
117	*Motacilla flava*	**x**	**xR**	
118	*Saxicola rubicola*	**x**	**xR**	
119	*Oenanthe oenanthe*	**x**	**xR**	
120	*Coccothraustes coccothraustes*	**x**	**xR**	**x**
121	*Fringilla coelebs*	**x**	**xR**	**x**
122	*Fringilla montifringilla*	**x**		**x**
123	*Carduelis carduelis*	**x**	**xR**	**x**
124	*Emberiza calandra*	**x**	**xR**	**x**
125	*Passer montanus*	**x**	**xR**	**x**
126	*Passer domesticus*	**x**	**xR**	**x**

Migration

Being on the East-Elbic migration route, the lake is a very important stop for an impressive number of migratory birds, which use the location as a feeding and resting site during the spring and autumn migration. In spring, the Sărata Nouă lake hosts regular migrants such as *Vanellus vanellus, Calidris pugnax, Spatula querquedula, Spatula clypeata, Mareca penelope, Anas acuta, Aythya ferina*, sometimes in big numbers. For example, on 31st of March, 2017, we encountered 250 individuals of Garganey (*Spatula querquedula*), the majority being males resting on the lake. On the same day we counted around 850 individuals of Black-headed gulls (*Chroicocephalus ridibundus*).

The autumn migration unfolds starting with middle August, in some years even late July, bringing the first individuals of various wader species that begin their journey towards south: Wood sandpiper (*Tringa glareola*), Common redshank (*T. totanus*), Common greenshank (*T. nebularia*), Common sandpiper (*Actitis hypoleucos*), Black-winged stilt (Himantopus himantopus), Ruff (*Calidris pugnax*) (tab. 1). Late July and August is also a suitable time for observing the Black stork (*Ciconia nigra*) migration: we recorded the presence of one individual in August 2017 and July 2020.

The peak of autumn migration is usually September, when various duck, wader and raptor species can be seen in small flocks: Eurasian teal (*Anas crecca*), Eurasian wigeon (*Mareca penelope*), Black-tailed godwit (*Limosa limosa*), Common snipe (*Gallinago gallinago*), Curlew sandpiper (*Calidris ferruginea*), Dunlin (*C. alpina*), Spotted redshank (*Tringa erythropus*) and Marsh sandpiper (*T. stagnatilis*) (Table no 1). In 2016, in late September, we encountered a big flock of grey wagtails (*Motacilla alba*), numbering 326 individuals, that were feeding on the ground near the lake shore. This period also brings less common and frequent species, such as Little Stint (*Calidris minuta*) and Common Ringed Plover (*Charadrius dubius*), that were noticed on September 29th, 2019. Also in September of 2019 we observed a single bird of Kentish Plover (*Charadrius alexandrinus*) and three individuals of Common Ringed Plover (*Charadrius hiaticula*), that were feeding on the shore in a muddy area. The white-tailed eagle (Haliaeetus albicilla) was repeatedly observed during autumn migration: 23th Sep 2017 and 10th Aug 2019.

Some of the late migrants, that arrive in October and late November, are represented by a small number of individuals. The most notable observation from this period is a flock of 32 Ruddy shelducks (*Tadorna ferruginea*) and 180 Mallards (*Anas platyrhynchos*) spotted on the 27th of November, 2019.

Every year, during the migration seasons, we've also encountered species that are considered rare in the Republic of Moldova and very rare for this particular location. In this regard, on 3rd September 2016 we noticed a Red-necked phalarope (*Phalaropus lobatus*) individual, feeding alone. This was the second observation of the species in the Republic of Moldova and the first for this region [1]. In March 2018 we observed a flock of 10 cranes (*Grus grus*) flying across the lake. In April 2017 we recorded an Osprey individual (*Pandion haliaetus*) hunting for fish above the lake. Another rare raptor species observed at the Sarata-Noua lake was the

Short-toed snake eagle (*Circaetus gallicus*) which was seen on the 23th of September, 2017.

We also encountered species that were never registered before in the Republic of Moldova, such as the Mediterranean Gull (*Ichthyaetus melanocephalus*). The bird was observed twice in late March (2017), feeding among 240 Black-headed Gulls on the lake.

Breeding season

Due to the multitude of habitats during the nesting period, we have registered various types of bird species: aquatic, woodland, farmland species, as well as birds adapted to open spaces, meadows and pastures. In summer, regular breeders are: Mallard (Anas platyrhynchos), Common coot (*Fulica atra*), Moorhen (*Gallinula chloropus*) (tab. 1). The marshes provide a suitable habitat for Great reed warbler (*Acrocephalus arundinaceus*), Eurasian Reed Warbler (A. scirpaceus) and Savi's warbler (*Locustella luscinioides*). In the forest strips along the lake hosts are breeding pairs of Wood pigeon (*Columba palumbus*), Turtle Dove (*Streptopelia turtur*), Eurasian golden oriole (*Oriolus oriolus*), Red-backed shrike (*Lanius collurio*) and Lesser grey shrike (*L. minor*) (tab. 1). The near-by poplar stripe hosts an important breeding colony consisting of 200 nests of Rook (*Corvus frugilegus*) and Hooded crow (*C. cornix*), in which the Eurasian hobby (*Falco subbuteo*) is known to occupy empty nests. We documented the breeding of this species in 2017 and 2019. In the neighbouring villages of Cazangic and Seliște there are several White stork (*Ciconia ciconia*) nests.

Among the birds which show a sporadic breeding character are the following species: Ruddy Shelduck (*Tadorna ferruginea*), Black-winged stilt (*Himantopus himantopus*), Little ringed plover (*Charadrius dubius*) and Mute swan (*Cygnus olor*) (tab. 1). A breeding pair of Ruddy Shelduck was documented in 2016, into a near-by meadow between Sărata Nouă and Romanovca [6]. The breeding evolution of this species needs to be further monitored since its current area is expanding in the south region of the Republic of Moldova. In the recent years (2020 and 2021) we documented the nesting of the Black Kite (*Milvus migrans*) on the strip of tree lines situated on the farmlands from the vicinity of the lake. However, several Black kite adults were spotted many times hunting on the lake or nearby.

The Great White pelican (*Pelecanus onocrotalus*) is a regular visitor in this area, but currently no population of this species is breeding on the lake or in its vicinity. We encountered various-sized flocks in the summer months of different years, but the highest number of great white pelicans was observed in the summer of 2016, when 120 birds were spotted for a few days feeding on the lake.

During the breeding season, the Sărata Nouă lake is also visited by various wader species which delay or still continue their spring migration: Ruff (*Calidris pugnax*), Common sandpiper (*Actitis hypoleucos*), Common greenshank (*Tringa nebularia*), Spotted redshank (*T. erythropus*), Wood sandpiper (*T. glareola*) (tab. 1). The birds observed at the beginning of the breeding season have probably not yet reached their sexual maturity, while the birds seen later in the breeding season could be the ones that start descending immediately after mating.

It is important to mention that due to the severe drought and lack of precipitation during the summer of 2020, the Sărata Nouă lake reduced its area by 50%. Although we don't know fully how this impacted the local ornitofauna, we observed that the water depth decreased significantly, which made it difficult for pelicans, cormorants and other diving species to obtain their food. Also, we encountered changes in the lake shores, where wader and duck species used to gather for food and shelter. Some of these areas were rapidly colonized by reedbeds, which made feeding difficult for many waders.

Wintering

The wintering season is characterized by the presence of few bird species that use the Sărata Nouă lake as a roosting site. In mild winters, when the lake`s water is not entirely frozen, big flocks of waterbirds can be seen, such as mallards (*Anas platyrhynchos*) - on the 1st of January 2020 there were observed up to 2400 individuals gathered in the middle of the lake. The local diversity of bird species is rather poor during the cold season, with average-sized flocks of Mute swan (*Cygnus olor*), Green-winged teal (*Anas crecca*), Northern lapwings (*Vanellus vanellus*), Yellow-legged gulls (*Larus cachinnans*), Ardea cinerea, Ardea alba, Chroicocephalus ridibundus, Podiceps cristatus. Due to the short distance to the Prut river, small flocks of Great cormorant (*Phalacrocorax carbo*) reach the lake to feed constantly. The highest number of great cormorants was registered on 28 february 2020 when 68 individuals were observed.

The presence of Hen Harrier (*Circus cyaneus*) and Common Buzzard (*Buteo buteo*) is common during the winter months, with few individuals spotted almost every year in the near-by area, probably preying on small passerines or rodents.

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RODENT SPECIES IN URBAN CEMETERIES OF CHISINAU CITY, REPUBLIC OF MOLDOVA

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Abstract. The research has been conducted in 3 biggest cemeteries of Chisinau city: Central ("Armenian"), *"Saint Lazarus"* ("Doina") and Jewish. We found 9 species of small mammals (7 species of rodents and 2 shrews). Species of rodents were *Clethrionomys glareolus, Microtus rossiaemeridionalis, Apodemus flavicollis, A. sylvaticus, A. uralensis, Rattus norvegicus and Mus musculus and 2 shrew species – Sorex araneus and Crocidura suaveolens*. A.sylvaticus was dominant species, followed by *M. rossiaemeridionalis* and *A. flavicollis.*

Introduction

In recent decades the world has undergone rapid change, including population explosions and massive urbanization [4] as a result of human activities has affected natural ecosystems [5, 7]. That is why there are more and more that refer to the ecological divergence in animal species. Small mammals are an important object of study in the sense of synanthropy, because they quickly adapt to the anthropogenic disturbances and to human presence. The importance of studying the synanthropy of small mammals in the urban environment explains, first of all, the conditions for their coexistence with humans.

Urban regions may be cities or towns and are characterized by closely located buildings and by higher human population density compared to areas surrounding it. Among rodents occupying urbanized territories, it is easy to distinguish two major groups. One group represented by two synanthropic species of rodents: rat *(Rattus norvegicus*) and house mouse (Mus musculus), which are highly adapted to survive within urban landscapes and can be found throughout the world. The other group is represented by rodent species that may be found within city limits most of them are more common outside of human dwelling and are adapted to a natural environment [6].

In the Republic of Moldova, more intense researches of urban fauna were performed after 2000 [1, 8, 12]. Thus, researches were carried out in the forests of the municipality and its surroundings [11], as well as in various types of more or less anthropized biotopes of Chisinau city. Cemeteries are exceptional places in the cities. These areas are cut off from the noise of the city and the night lights are not as bright. The old trees and even the graves constitute good shelters for the small mammals [2]. City cemeteries are often bordered by parks or green areas, creating more favorable shelters for small mammals. This part of the biota of Chisinau city remains poorly studied [8]. The aim of the study was to reveal the diversity and abundance of small mammal species in several cemeteries of Chisinau city.

Materials and methods

The research has been conducted in 3 biggest cemeteries: Central ("Armenian"), "Saint Lazarus" ("Doina") and Jewish in October–November 2008.

The Jewish Cemetery is one of the oldest cemeteries in Chisinau city, Republic of Moldova. It is a historical monument and art of national category, which has been operating since 1887, although the first tombs are attested from the beginning of the century XIX, and other sources attribute an age from 200 to almost 300 years (fig. 1). Different sources expose the dimensions of the preserved part of the cemetery. It would cover an area of 11, 12 or 15 hectares and would have about 24 thousand or over 40 thousand graves. Also here is a funeral synagogue, unique in Moldova, now ruined. In the cemetery there are a lot of tombs and monuments over a century old. A monument with a propeller is installed at the tomb of an aviator, which is still rotating today.

Figure 1. Location map of the Jewish cemetery

The central cemetery in Chisinau city, improperly called the Armenian cemetery, is one of the most famous cemeteries in the Republic of Moldova. Founded in 1811, it is the resting place of many prominent personalities in the history and culture of Moldova. The cemetery is located in the Center sector of Chisinau city (fig. 2). Although it is the oldest cemetery in Chisinau city, it is not found among other cemeteries with protected status, included in the "Register of Monuments of the Republic of Moldova".

Figure 2. Map of the location of the Armenian cemetery

St. Lazarus Cemetery, called colloquial Doina Cemetery, is a cemetery located in the Old Post sector in Chisinau city. It is one of the largest cemeteries in Europe, the area is about 2,000,000 m2 (fig. 3). It was opened in 1966 and currently has over 300.000 graves, including 600 graves, being divided into 266 sectors.

The material was collected with snap traps, the total effort was 390 trap-nights. The traps we placed in lines at the distance of 5 m between the traps and 25 to 50 m distance between the rows. As bait small pieces of bread imbued with sun-flower oil were used.

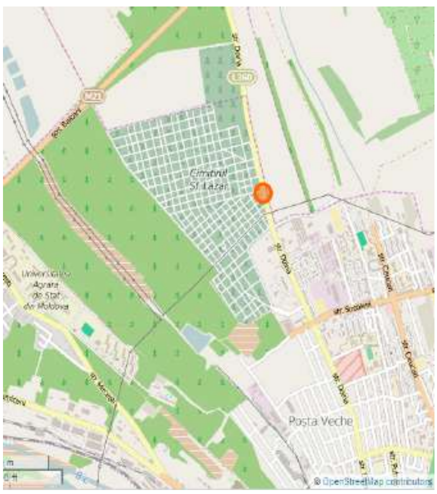

Figure 3. Location map of the "Doina" cemetery

The statistical analysis was performed using the programs Microsoft Excel and BiodiversityPro.

Results and discussions

During the study there were collected 78 individuals from 7 species of rodents (*Clethrionomys glareolus, Microtus rossiaemeridionalis, Apodemus flavicollis, A. sylvaticus, A. uralensis, Rattus norvegicus and Mus musculus*) and 2 shrew species (Sorex araneus and Crocidura suaveolens).

As result of the analysis of the total abundance and capture coefficient on the cemeteries, it was found that the cemetery with the richest fauna of small mammals was "Doina" cemetery with an abundance of 82.1% with total capture coefficient was 8.7%, followed by Jewish cemetery with abundance of 15.4% and the capture coefficient was of 3.1%. Due to the location of the Armenian cemetery in the center city and largely surrounded only by buildings, only one species was captured, *Rattus norvegicus* – typically synanthropic with 2.6% abundance and capture coefficient of 0.5%.

The dominant species was *A. sylvaticus*, a capture coefficient of 7.2% and an abundance of 35.9%, followed by species *M. rossiaemeridionalis* and *A. flavicollis* which had a capture coefficient of 3.8% and an abundance of 19.2% each, and *A. uralensis* with c.c. 2.1% and an abundance of 10.3%. The least abundant rodent species was C. *glareolus* and R. norvegicus which had abundence 3.8% and capture coefficient of 0.8% each. The typical synanthropic species M. *musculus* had an abundance of 1.3% and capture coefficient of 0.3%. In several European cities the species A. sylvaticus also dominates in similar cenoses (3, 9, 10).

Among the shrews, two species were identified: *C. suaveolens* with a total abundance of 3.8 and c.c. 0.8% and *S. araneus* had abundance – 2.6% and c.c. 0.5%.

In the Jewish cemetery, which is an old cemetery, 4 species of rodent and 1 shrew species were observed. The dominant species was *A. sylvaticus* with 41.7%, followed by A. uralensis – 33.3%, *C. glareolus* and *R. norvegicus* had the abundance of 8.3% each. The shrew species *C. suaveolens* had an abundance of 8.3% (fig. 4).

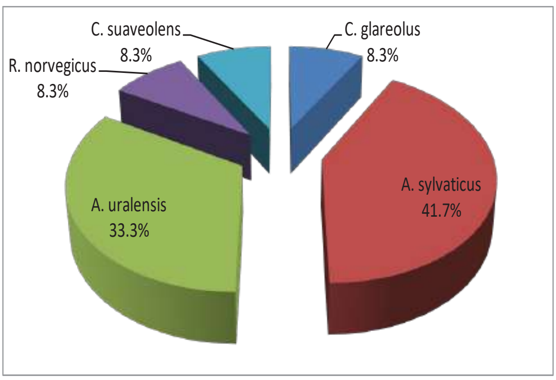

Figure 4. Abundance of small mammal species in the Jewish cemetery

Location of the "Doina" cemetery outside the city surrounded by different types of biotopes allows to support a richer fauna than the other studied cemeteries. In "Doina" cemetery A. sylvaticus was the most abundant species with 35.9% and catch coefficient of 10.9%, followed by *A. flavicollis and M. rossiaemeridionalis* with 23.4% and 7.1% catch coefficient for each species. The rodent species with a lower abundance were *A. uralensis* with 6.3% and catch coefficient – 1.9%, followed by *Clethrionomys glareolus*, which is a silvicolous species and had an abundance of 3.1%, a capture coefficient of 0.9%, due to the fact that the cemetery is surrounded by a recreational forest. The synanthropic species *M. musculus* had an abundance of only 1.6% and a capture coefficient of 0.5%.

The shrew's species *C. suaveolens* and *S. araneus* had abundance of 3.1% and capture coefficient of 0.9% for each species (fig.5).

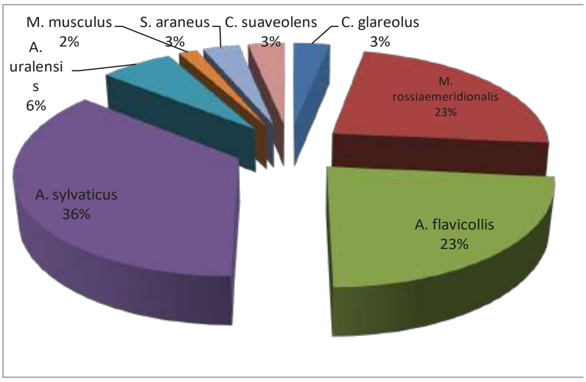

Figure 5. Abundance of small mammals in the "Doina" cemetery

In present conditions many small mammal species manifest remarkable adaptive capacities. Therefore, in the last years, in spite of the anthropic pressure, small mammals, especially common rodent species are constant in various types of anthropized ecosystems, even in cemeteries. The adaptive potential of small mammals consists of the use of a large trophic spectrum of resources, the high reproductive potential, as well as the extensive period of reproduction activity.

Conclusion

Nine species inhabit in the cemeteries of Chisinau city, of which A.sylvaticus dominated. The second most abundant species of rodents were *M. rossiaemeridionalis and A. flavicollis*.

In the cemeteries of Chisinau there was a fairly rich and variable fauna of small mammals due to the existence of various types of biotopes in "Doina" cemetery, including semi-natural ones, which favor the presence of stable rodent and shrew community, which in turn favors the existence of a series of vertebrates that consume them.

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ECOLOGICAL-EVOLUTIONARY ETHOLOGY OF THE AMPHIBIANS: CONCEPTUAL SYNTHESIS OF RESEARCH RESULTS AT REGIONAL AND EUROPEAN LEVEL

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Abstract. Following long-term investigations of over 50 amphibian populations in the Republic of Moldova and Italy, the ecological and evolutionary peculiarities of reproductive behavior in some species of the orders Caudata and Anura were elucidated. For the first time, at the autecological and synecological level, the evaluation of amphibian nuptial systems - parental input, reproductive success, "r" and "K" reproductive strategies and their role in the evolution of sexual selection and the realization of the reproduction potential as a fundamental mechanism for the survival of amphibian populations in various environmental conditions was emphasized.

Introduction

One of the primary ecological imperatives for humanity is the conservation and rational and sustainable use of the biological diversity of the planet. However, the existence of a considerable decline in amphibian populations caused by multiple factors and the need for the situation created, requires to effectively undertake urgent actions to investigate the reproductive systems, which assess as effective mechanisms for achieving reproductive success of species perpetuation [1, 2, 4, 5, 7, 14, 17]. The extinction of certain amphibian populations at international level is sometimes due to a negative set of demographic factors [16], although in most cases this is due to a complex action of negative anthropogenic factors and random demographic processes, or almost exclusively, only as a result of the considerable degradation of the reproductive aquatic basins [1, 2, 6, 12, 13] or of the natural habitats in general [14]. Several literature data have mentioned that the problem of establishing the true causes of the ecological decline of amphibian species is difficult (and sometimes almost impossible) to solve due to the lack of long-term ecological monitoring and often the use of inappropriate or ineffective methods of estimation of the population number [10, 17]. All mentioned eloquently demonstrates that the realization of long-term studies adequate to the batracological rigor-standards regarding the reproductive cycle and the reproduction strategies of this vulnerable group of vertebrates, have a special theoretical-scientific interest and, at the same time, they can be very useful in solving many problems related to the conservation of amphibian diversity and their rational use, both regionally and globally. The studies on estimation of the ethological and ecological-evolutionary peculiarities of amphibians in natural and anthropogenic ecosystems at local, regional and international level allowed the development of scientific-methodological support for assessing the ecological and conservation status of these vertebrates, which represent an indispensable component of the diversity of the animal world.

In the Republic of Moldova, because of the intensification of the anthropogenic impact on biological diversity, more than 240 species of animals are critically endangered, including

9 species of amphibians, which constitutes 69% of the national batrachofauna. The ecosystems of the country are damaged, and the natural areas are not able to fulfill their function of stabilizing the ecological systems. The recovery of the situation of animal world diversity requires essential efficiency of measures to conserve biodiversity and natural habitats, rational and sustainable use of biological resources, restoration of degraded ecosystems and endangered species, identification of valuable sectors in terms of biological diversity and promotion of biodiversity activities, extension of the fund of protected natural areas. In this sense, the Network of State Protected Areas is of particular interest for the conservation of biodiversity, including amphibians. These studies are in line with the criteria of the International Union for Conservation of Nature (IUCN) and the requirements of the Convention on Biological Diversity. The natural sites of the universal natural heritage are protected natural areas whose destination is the protection and conservation of natural habitat areas, including those populated by amphibians, on which territory there are natural elements whose value is recognized as being of universal importance.

Materials and methods

The methodology of scientific research is constituted by the theoretical-scientific conceptions exposed in the works elaborated by batrachological scientists [8, 9, 11, 13, 14, 15, 17], regarding the eco-ethological structure of the populations, nuptial systems and reproduction strategies, intra- and interpopulation mechanisms of reproductive systems, parental investment and reproductive success, ecological evolutionary peculiarities of selection „r„ and „K„, mechanisms of sexual selection, the role of acoustic communication in achieving intra- and intersexual relationships and in achieving maximum reproductive success.

The aim of the long-term investigations (over 30 years) focused on the autecological and synecological substantiation of an integrated and detailed conception of the nuptial systems and breeding strategies of amphibian species in natural and man-made ecosystems in order to establish evolutionary particularities of ecological and behavioral mechanisms of intrapopulation and interpopulation mechanisms of species perpetuation. For this purpose, over 50 populations from the Republic of Moldova and Italy were analyzed in 13 species of amphibians of the genera *Triturus, Bufo, Pelobates, Bombina, Hyla and Rana* from the orders Cautata and Anura.

Results and discussions

The results obtained have a fundamental theoretical significance, which is summarized by:

- At the autecological and synecological level, the nuptial systems of amphibians - parental input, reproductive success, "r" and "K" reproductive strategies and their role in the evolution of sexual selection and in the realization of reproductive potential as a fundamental mechanism of survival of amphibian populations in various environmental conditions;
- It has been established that amphibians are characterized by a relatively high level of ecological plasticity of reproductive strategies - as an evolutionary ecological complex and species-specific behavioral complex, which is aimed at ensuring an optimal level of sur-

vival of local populations in various conditions of natural and anthropogenic ecosystem;
- The results obtained contribute to the essential strengthening of the concept of reproductive systems and strategies in fluctuating conditions of ecological factors in natural and anthropized ecosystems;
- The intersexual mechanisms of communication of native amphibian species have been elucidated, the legities of acoustic communication has been established, which can be used to assess the problems of the emergence and evolution of perception and communication systems of individuals and to develop mathematical, ecological and ethological models of nuptial systems for this group of vertebrates;
- It has been established that amphibian calling songs are valued as important factors in sexual selection; which indicates that the phenomenon of female preference toward calling songs with a higher intensity could significantly influence the reproductive success of males within the same population.

Therefore, the results obtained, both experimentally and in natural conditions with amphibian populations of different species from the Republic of Moldova and Italy, allow us to conclude that the intensity of the calling songs of the males represent an important criterion in determining the reproductive success. The data on the mechanism of egg fertilization are fundamentally new to science and considerably expand the existing theoretical concepts regarding the process of fertilization of ecaudata amphibians - appreciated as an intrinsic external type (as previously considered). However, we have established that the fertilization of eggs is carried out in a strictly delimited aquatic space (created by the two conjugal partners through a specific positioning of the body and limbs) which ensures the complete fertilization of the eggs.

This mechanism of egg fertilization is, in fact, an intermediate fertilization mechanism (transient) between the external type (particular for fish - ancestors of amphibians) and the internal type (particular for reptiles - class of vertebrates evolved from amphibians). Thus, for the first time, we established that according to the reproductive system also, the ecaudata amphibians occupy an intermediate position between the class of fish and the class of reptiles - this being one of the fundamental evolutionary concepts of zoology regarding the origin of amphibians. For the first time, models of the nuptial systems of caudata and ecaudata amphibian species were developed. These ethological models have been developed for the following amphibian species: *Hyla arborea, Rana dalmatina, Triturus cristatus, Triturus vulgaris, the green frog complex – R. lessonae x R. ridibunda x R. esculenta*.

These complex studies carried out at regional (Republic of Moldova) and European level (five regions of Italy) with the use of high-performance contemporary methodologies (spectral analyzes of the sounds emitted by amphibians in the most diverse ecological and ethological conditions), allowed to elaborate the following theoretical concepts new for science:
- The effect of the complementarity of the structural and temporal factors in the acoustic communication of the individuals of the ecaudata amphibian species at intra- and inter-population level and in the realization of the sexual selection;
- Mimicking the opposite sex by non-territorial males in intrasexual relationships within

the populations - as a reproductive strategy and one of the forms of sexual selection in caudata amphibians;

- Male satellite-ism as an opportunistic form of sexual behavior in amphibians;
- The effect of the integrity of the sensory channels of chemical, optical and tactile communication in the realization of sexual selection in amphibians.

The main scientific results regarding the evolutionary, ecological and ethological peculiarities of amphibians obtained after long-term investigations in natural conditions lies in:

1. At the basis of the formation of amphibian complexes under diversified and variable ecosystem conditions are intra- and interpopulation co-adaptive mechanisms, labile in time and space, which allow amphibian populations to consistently and efficiently use the abiotic and biotic resources of habitats.
2. There is a very close correlation between the duration of the reproductive period and the specific manifestation of amphibian reproduction strategies. Thus, depending on the length of the breeding period, the caudata and ecaudata amphibians fall into two broad categories:

a) Amphibians with a short reproductive period (also called "explosive"), lasting 7-10 days;

b) Amphibians with a long reproductive period, lasting more than a month.

The main breeding strategy, used by males of short-breeding species, is not to attract females by acoustic signals and through the manifestation of territorialism, but by direct competition ("scramble competition") with other rivals, and their reproductive success depends entirely on their competitive ability.

On the contrary, the strategy of reproduction of species with a long reproductive period consists in:

a) The protection of an individual territory and the attraction of the female through chemical communication, nuptial coloration and certain demonstration postures - in caudata amphibians (genus *Triturus*);

b) The protection of an individual territory and the attraction of the female through the issuance of calling songs – in ecaudata amphibians (genera - *Bufo, Hyla, Rana* etc.).

An alternative reproductive strategy of the males of some species with a long reproductive period consists in the satellite behavior of the males, called by us the concept of satelliteism, which was first discovered and evaluated in the species *Hyla arborea*. Male satelliteism has emerged as an opportunistic form of sexual behavior in equatorial amphibian species and is geared toward achieving the reproductive success of non-territorial males that act as dominant male-territorial satellites.

The effect of satellite has a frequency of about 11% in breeding populations and is an important source of realization of the reproductive potential of populations of ecaudata amphibians.

1. For the first time, the principles of integrating the chemical (a), optical (b) and tactile (c) sensory communication channels into sexual selection in ecaudata amphibians were evaluated:

a) Chemical communication is used, in fact, to ensure the effective interaction of partners during reproduction - to protect the individual territory, attracting and conquering the female, communicating about the onset of spermat ophore deposition process, stimulating the female to capture sperm laid by the male. The essence of chemical communication in caudata amphibian species consists in: 1. Transmitting information about the physiological state of the conjugal partner (the fact that the sperm or eggs are already mature and ready for deposition and fertilization); 2. The arousal of the conjugal partner so that he initiates the realization of certain nuptial behaviors that are directed towards the deposition of gametes and the realizations of fertilization.

b) Optical communication also plays a fairly important role, manifesting as one of the effective mechanisms for recognizing the conspecific individual, but also for achieving nuptial behavior as a complex of nuptial demonstration postures designed to ensure sexual selection at the intrapopulation level;

c) Tactile communication has a secondary and / or complementary role, meant to strengthen the effect of the two main sensory communication channels - the olfactory and optical one.

2. The effective realization of the reproductive potential in amphibian populations is determined by the nuptial system and reproductive strategies, which establish the level of reproductive success in the given environmental conditions.
3. The role of the fundamental frequency of amphibian male calling songs was assessed, based on which females have the opportunity to choose larger males so that the dimensional difference between them and males would be minimal and thus the sexual partners' cloaca overlap; thus ensuring a high level of fertilization of the eggs during reproduction.
4. The phenomenon of the manifestation of female preferences toward calling songs with certain frequency is a complex one and, therefore, in order to determine the true causes of the its manifestation and role in sexual selection, it is necessary to analyze female preference not only according to a single sound parameter, but of several parameters, take together (such as, for example, intensity and frequency, duration and frequency, etc.). By doing so, it was possible to establish the presence of a new concept of acoustic communication in amphibians - the effect of complementarity (interdependence of sound parameters) of structural and temporal factors in acoustic communication of individuals intra- and interpopulation and sexual selection.
5. The nuptial systems of local amphibian populations are strongly influenced by certain environmental factors, in particular by the spatial and temporal distribution of breeding habitats and the length of the breeding period;
6. In the evaluated amphibian populations, the manifestation of the reproduction strategies "r" and "K" presents certain intra- and interpopulation features, which are determined by certain endogenous and exogenous factors. The inter- and intrapopulation variability of the "r" and "K" reproduction strategies of amphibians is evolutionary, it appeared and consolidated within the populations as an efficient mechanism of species-specific surviv-

al.

Research to estimate the ethological and ecological-evolutionary peculiarities of amphibians in natural and anthropogenic ecosystems at local, regional and international level allowed to develop a scientific-methodological support for assessing the ecological and conservation status of these vertebrates - which represent an indispensable component of the animal world diversity.

The studies were performed within the State Program project 20.80009.7007.02.

Conclusions

In amphibian populations the manifestation of reproduction strategies “r” and “K” have certain intra- and interpopulation features, which are determined by certain endogenous and exogenous factors.

The inter- and intrapopulation variability of the “r” and “K” reproduction strategies of amphibians is of evolutionary order, it appeared and consolidated within the populations as an efficient mechanism of species-specific survival.

An alternative reproductive strategy of the males of some amphibian species with a long reproductive period consists in the satellite behavior of the males, which was evaluated for the first time in the species *Hyla arborea*. Male satelliteism has emerged as an opportunistic form of sexual behavior, aimed at achieving the reproductive success of non-territorial males that act as satellites of dominant territorial male.

The effect of satelliteism has a frequency of about 11% in breeding populations and is an important source of realization of the reproductive potential of amphibian populations.

The effective realization of the reproductive potential in amphibian populations is determined by the nuptial system and reproductive strategies, which establish the level of reproductive success in the given environmental conditions.

A new principle has been established in the system of acoustic communication in amphibians - that of the complementarity effect (interdependence of sound parameters) of structural and temporal factors in the acoustic communication of individuals at intra- and interpopulation level and in sexual selection.

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A REAPPRAISAL OF THE "EAST CARPATHIAN TEMPERATE CLIMATE REFUGIUM" DURING THE LAST GLACIAL MAXIMUM

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Abstract. The concept of "East Carpathian Refugium" is largely based on reports on temperate-climate species from the Late Paleolithic sites of Moldova. The present report proposes new faunistic data from the key Paleolithic sites of Moldova that question the presence of some temperate species in the East Carpathian Region during the Last Glacial Maximum. The revision of archaeozoological material from Cosăuți did not confirm the presence of Capreolus capreolus and Alces alces in this palaeolithic site. Osteological remains previously ascribed to Cervus elaphus, according to new data, belong to Cervus canadensis and Ovibos moschatus.

Introduction

The Quaternary fauna of Europe was deeply influenced by the glacial cycles that started 2.58 million years ago and caused important faunal turnovers, extinctions, dispersals and retractions of cold- or temperate-adapted species [1]. The alternations of glacial and interglacial periods, as well as their duration greatly affected the evolution of European biota, speciation, and intraspecific diversification [2, 3, 4, 5]. During the Quaternary period, the southern regions of Europe (the Iberian, Italian, and Balkan Peninsulas) played an important role of glacial refugia for a large number of animals and plants that then recolonized northern parts of Europe during interglacial climate warmings [1, 6]. The problem of Quaternary refugia is of great interest to palaeontologists, palaeoecologists, population geneticists and conservation biologists and attracted great attention from the scientific community during the last decades [1, 4, 5, 7]. Special importance in the rapid interglacial recolonizations by temperate plants and animals is given to the so-called cryptic glacial refugia, which represented particular restricted biogeographic areas with the relatively mild local climate that permitted the isolated survival of temperate biota during glaciation peaks [5]. The East Carpathian area, including the territory of the Republic of Moldova, is often regarded as one of such northern cryptic refugium. The notion of the East Carpathian temperate climate refugium was introduced by Sommer and Benecke [8] based on the presence in the northern part of Moldova of subfossil remains of Ursus arctos and several forest-bound species (mostly cervids) during the Last Maximum Glaciation (LMG) dated back to 23 000–16 000 years BP (before present). The list of forest temperate climate species from the Late Paleolithic sites includes *Alces alces*, *Capreolus capreolus*, and *Cervus elaphus* that coexisted with such typical tundra-steppe faunal elements as *Rangifer tarandus, Mammuthus primigenius, Coelodonta antiquitatis, Bison priscus*, etc. [7, 9, 10]. Here should be also mentioned *Megaloceros giganteus* reported from the second "Magdalenian" layer of Duruitoarea Veche [10] that represented an exceptional isolated case of persistence of the giant deer during LGM in Europe. Stewart et al. [11] regarded the East Carpathian refugium as one of the cryptic northern refugium of temperate-adapted taxa. The present study presents results of the revision of cervid remains that supposedly were ascribed to the temperate-climate species and proposes to reconsider the concept of the East Carpathian cryptic glacial refugium.

Materials and methods

The material involved in the present study includes the archaeozoological Paleolithic material from Duruitoarea Veche, Rașcov-7, Cosouți, Climăuți II (curated at the Institute of Zoology, Chișinău), Rașcov-8, and the collection of Paleolithic artefacts from Cosăuți (curated at the National Museum of History of Moldova, Chișinău). The systematic revision of the archaeozoological material is based on the cranial, dental, and postcranial morphological characters of cervid and bovid species [12, 13, 14].

Results and discussions

The presence of giant deer remains in layer II of Duruitoarea Veche reported by David [9] conflicts with the data of Stuart et al. [15] that indicate a conspicuous absence of Megaloceros giganteus in Western and Central Europe during the time interval around 20–12.5 kyr. N.A. Chetraru (personal communication, 2008) believed that the older bones of giant deer from Duruitoarea Veche could be admixed during the material sorting and the preliminary study of the osteological remains [16]. This suggestion was recently confirmed by Lister and Stuart [17] who obtained the date 37050 ± 450 years BP for the giant deer molar sample said to be from the second layer of Duruitoarea Veche. Thus, the available data do not support the persistence of M. giganteus in the East Carpathian area.

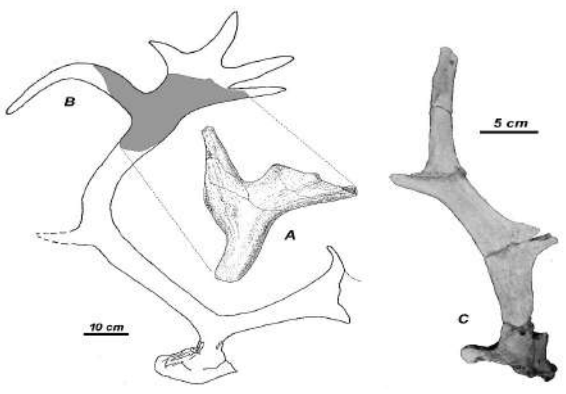

Figure 1. Reindeer (*Rangifer tarandus*) antlers from the Palaeolithic site of Cosăuți (Soroca District, Moldova): A, the fragment of distal palmed part from layer VII; B, the antler of a reindeer male from Cosăuți (redrawn from a protograph kindly provided by Dr. S. Covalenco) with a shaded part that corresponds to the antler fragment from layer VII; C, the juvenile antler of reindeer that shows the *Capreolus*-like bauplan

The reported occurrences of elk (*A. alces*), roe deer (*C. capreolus*) and red deer (*C. elaphus*) in the Late Paleolithic of Cosăuți [10] served as an important argument in favour of the concept of East Carpathian refugium of temperate species [7]. Cultural layers of Cosăuți were accumulated during the relatively short period between ca. 19 000 BP and 17 000 years BP, i.e. shortly after the peak of the LGM [10, 18]. Unfortunately, David et al. [10] did not provide any description or indications to specimens ascribed to temperate climate cervids. The single specimen of *A. alces*, according to David et al. [10], comes from layer VII of Cosăuți. There is only one specimen from this layer that could be ascribed to elk, a processed fragment of large palmated antler [19: fig. 5-5]. However, unlike *A. alces*, the specimen in question is characterised by an oval cross-section of antler beam below the palmation (not subrectangular as in *A. alces*) and its palmed part is not bowed as in elks [14: fig. 13]. According to the present study, the palmed fragment of antler from layer VII belongs to a very large specimen of reindeer, as is demonstrated in fig. 1, A-B.

The revision of the archaeozoological collections from Cosăuți did not reveal any specimen that belongs to C. capreolus. Nonetheless, it is necessary to indicate that some juvenile antlers of R. tarandus from Cosăuți are characterized by bauplan that reminds the structure of antlers of roe deer (fig. 1, C). Thus, according to our opinion, the faunal assemblage from Cosăuți has a typical tundra-steppe character [19].

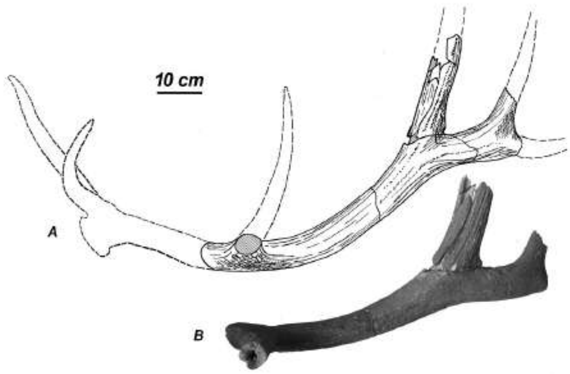

Figure 2. The reconstruction of antler of *Cervus canadensis* (A) based on a distal portion of antler (B) from the Palaeolithic site of Climăuți II (Șoldanești Discrict, Moldova)

The multiple reports of the presence of red deer *Cervus elaphus* in the East Carpathian area during the LGM [9, 20, 21] represent a complex problem largely based on the taxonomic issues and species definitions of the so-called "elaphine deer" group that includes two vicarious species, *C. elaphus* and *C. canadensis* [22, 23]. The recent genetic [24, 25] and morphological [22, 23] studies revealed the dispersal of Asian wapiti (*C. canadensis*) in Europe during the LGM. During cold phases of glacial cycles, *C. canadensis* substituted *C. elaphus* in most of Europe with exception of the southern glacial refugia where populations of red deer persisted [23, 24]. The antler fragments from Climăuți II (20 350 ± 230 years BP) originally reported as *C. elaphus* [20] belong to a significantly larger form of *C. canadensis* and are characterized by typical for this species morphology of distal portion of antler [22]. The poor antler fragments with specific for "elaphines" pearled antler surface from Raşcov 8 reported by Croitor and Covalenco [21] as *C. elaphus*, most probably belong to *C. canadensis*.

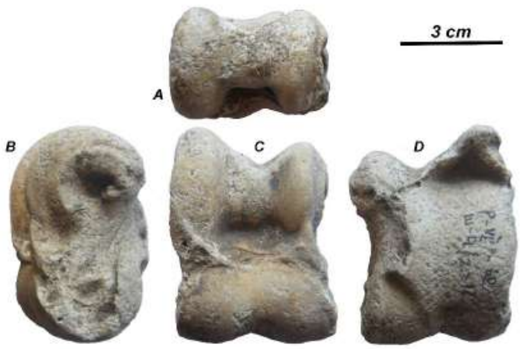

Figure 3. The right talus "P-VII-62 Ш-4/2-12" of *Ovibos moschatus* from Rașcov 7 (Camenca District, Moldova): A, proximal view; B, medial view; C, dorsal view; D, plantar view

A part of remains from Rașcov 7 (22 000 - 17 000 years BP: [26]) ascribed to C. elaphus [9] belong to musk ox *Ovibos moschatus.* The musk ox talus "P-VII-62 Ш-4/2-12" from Rașcov 7 is characterized by the size and proportions (the maximum bone length is 59.8 mm; the distal breadth is 41.2 mm) quite similar to those of red deer, however, morphological characters of the specimen in question (Fig. 3) correspond to the diagnostic features of *O. moschatus* provided by Gromova [13]. The finding of musk ox from Rașcov 7 is the first record of this species on the territory of Moldova and the southernmost record of *O. moschatus* in Eastern Europe. The presence of musk ox in the archaeozoological material from Rașcov 7 confirms the strong cold-adapted character to the fauna from this Paleolithic site. Generally, findings of musk ox in the southern part of Eastern Europe are extremely rare. According to the broadly accepted opinion, *O. moschatus* dispersed into southern regions of Eastern Europe only during the coldest phases of glaciations [27, 28 and references therein].

The above presented data do not support the concept of "East Carpathian glacial refugium", which, therefore, should be rejected. Nonetheless, the reports on the occurrence of temperate climate species (plants and animals) in the Late Pleistocene glacial phases of Hungary and Slovenian Karst in southern Slovenia [7] require further attention, since the Pannonian plane hypothetically could be protected from the glacial climatic influence by the Carpathian Mountains. The term "Pannonian Glacial Refugium" is more appropriate in this case.

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SCREENING METHODS OF COXOFEMURAL DYSPLASIA IN DOGS

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Abstract. The major desideratum of the screening action of the hip dysplasia is the early discovery of the disease and its highlighting at an early stage. Hip dysplasia is interpreted as an abnormal and / or defective development of the hip (hip joint), which can cause excessive wear of the articular cartilage due to the support of body weight leading to the development of arthritis, often called degenerative joint disease or simply osteoarthritis, these terms being used randomly. All these morphofunctional changes can be detected early, by different methods of observation, which necessarily involve X-ray radiological examination, performing a complex analysis of the images studied, according to the recommendations of various international organizations such as FCI Fédération Cynologique Internationale (FCI) - score which includes measuring the Norberg angle, the Orthopedic Foundation for Animals (OFA), the Penn-HIP Program - which involves measuring hip laxity, etc.

Introduction

Coxo-femoral dysplasia is a disease characteristic of dogs, categorized as hereditary, which can be transmitted to offspring within 14 generations [18]. This causes morphological changes in the coxo-femoral joint [1], which is manifested by wiping the acetabular cavity in different degrees, flattening the articular head of the femur, increasing the intra-articular distance [2], and functionally, evolving from lack of clinical signs to severe lameness, without a direct correlation with the severity of morphological lesions. Affected joints usually develop varying degrees of synovial inflammation, joint cartilage damage, osteophytes and sclerosis, and subchondral bone remodeling [2]. Also common changes characteristic of hip dysplasia are associated with environmental factors, such as nutrition [10], exercise [8], and skeletal ossification [12].

Canine hip dysplasia was first described in 1935-1937 by Dr. Gerry B. Schnelle, who called it “bilateral congenital subluxation of the hip joint” [14], considering it a rare but rare pathology which currently turns out to be the most common orthopedic condition in dogs. Examining a large number of dogs, he finds that joint deformities have different aspects from one animal to another, which determines the different forms of the disease, depending on the radiological severity of the lesions, in five degrees [15].

In 1966, Henricson, Norberg and Olsson refined the definition of coxofemoral dysplasia, describing it as follows “A different degree of laxity of the hip joint that allows subluxation during early life, giving rise to varying degrees of superficial acetabulum and flattening of the femoral head, eventually inevitably leading to osteoarthritis” [9].

The intensive breeding of purebred dogs makes coxo-femoral dysplasia one of the most studied diseases in this species, and the most intensely studied breed is the German Shepherd, due to its wide range [17].

Subsequently, the disease was diagnosed in almost all races, especially those of large size and robust constitution, in which the growth rate at a young age is very high. The most commonly affected are the Terra-nova, Saint-Bernard, Rottweiler, Kuvasz, Giant Schnauzer and Boxer [6], but the disease can also be found in some small breeds. However, there are breeds in which the disease has not been diagnosed, among them we list: Dwarf Pinscher, Borzoi, Greyhound, Bedlington-Terrier, Irish-Terrier, Lakeland-Terrier.

The disease affects both males and females, with coxo-femoral dysplasia being more important in service dogs because they cannot be subjected to prolonged exertion during training, they get tired in a short time and have severe joint pain. For this reason, the disease should be detected as early as possible before dogs are selected for training.

Today, the veterinary consensus is that hip dysplasia is a congenital condition manifested as joint laxity that leads to the development of osteoarthritis.

The aim of the paper was to visually familiarize with the measurement technique on an X-ray to diagnose and assess the degree of hip dysplasia in dogs, performing measurements, using the technique described by Norberg, and assessing the laxity index.

Materials and methods

The materials were radiographs of 2 dogs, who were diagnosed with X-rays based on images of the pelvis, with the capture of the knee joints. The examined subjects were placed on their backs in a supine position, with the hind limbs extended and parallel to each other, the knee joints being rotated internally with the symmetrical pelvis. The radiography was obtained in direct ventro-dorsal projection, using an ARIA veterinary digital X-ray device at the "Iasi Veterinary Imaging Center". The examined animals were anesthetized with SEDAM (acepromazine) in combination with VETASED (ketamine) according to the instructions.

The measurements were performed according to the protocols published on the websites: Animal Orthopedic Foundation (OFA) [16], Federation Cynologique Internationale (FCI) [6], Pennsylvania Hip Improvement Program (PennHIP) [13] - where the most exposed are widespread and detailed screening approaches for canine hip dysplasia (CHD).

Results and discussions

The study methods, imposed by various international organizations, were transposed on two radiographs submitted by colleagues from Iasi. Thus, we performed measurements of the Norberg angle [3] and evaluated the hip laxity - Pennhip [13]. Both techniques require mandatory sedation of dogs until muscle relaxation is achieved. The examination of an radiographic image for the diagnosis and assessment of the degree of hip dysplasia takes into account the acetabular cavity, the femoral head and the secondary changes. Of interest are 9 detailed features: Norberg angle, subluxation, cranial acetabular margin, dorsal acetabular margin, cranial acetabular lip, acetabular fossa, caudal acetabular margin, exostoses of the femoral head and neck and femoral head remodeling.

Theoretically, *the Norberg method* [3] consists in measuring the angle obtained between a line joining the geometric centers of the two femoral ends and a line starting from the center of the femoral head and passing tangentially to the anterior edge of the acetabular cavity. The technique is possible only on radiographic film of sufficient quality and obtained on the animal positioned in supine position with the limbs in forced extension, as far as possible to ensure a parallelism between the femoral bone rays, while achieving a slight internal rotation of the two limbs, a situation that will highlight the femoral heads more clearly (Fig.1 B).

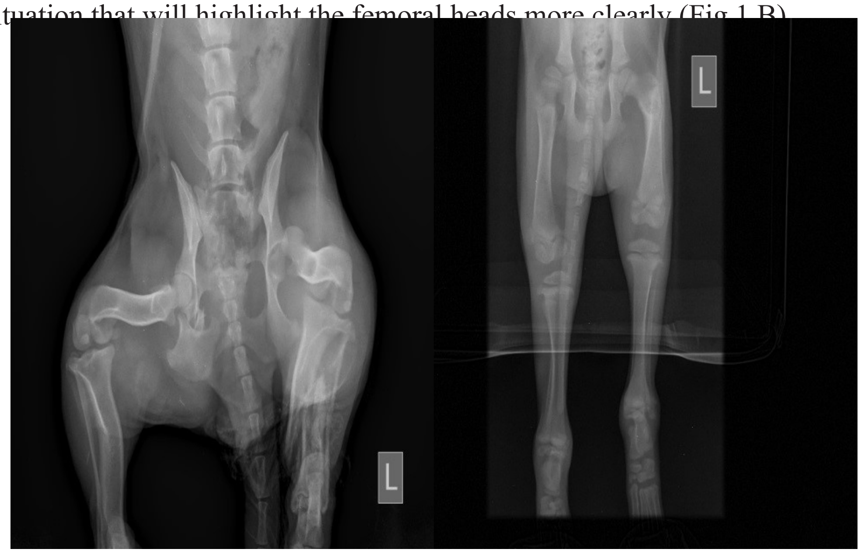

Figure 1. A - dislocation of the hip joint, B - dysplastic joint

The classification by degrees of dysplasia is made both after observing the osteoarticular structural changes and the value of the Norberg angle. 5 degrees of dysplasia are unanimously accepted, as follows:

Grade A (1) -lack of dysplasia. Signs of hip dysplasia are missing, femoral head and acetabulum are congruent. The craniolateral edges have clear contours and rounded ends. The joint space is narrow and smooth. The acetabular angle according to Norberg is approx. 1050 (as a reference). In the case of excellent hip joints, the craniolateral acetabular edge surrounds the femoral head somewhat more distant than laterocaudal.

Grade B (2) - approximately normal joint. The coxo-femoral joint is almost normal, the femoral head and acetabulum are slightly incongruent, the Norberg angle is about 1050, or the center of the femoral head is in a medial position relative to the dorsal edge of the acetabulum, the femoral head and acetabulum are congruent.

Grade C (3) - mild dysplasia. The femoral head and acetabulum are incongruent, the Norberg acetabular angle is approximately 1000 and / or the craniolateral edge of the acetabulum is slightly flattened. There may be irregularities or just fine signs of osteoarticular alterations of the cranial, caudal or dorsal acetabular margin or on the femoral head and neck.

Grade D (4) - moderate dysplasia. Obvious incongruity between the femoral head and the subluxated acetabulum. Acetabular angle according to Norberg greater than 900 (reference only). Flattening of the craniolateral margins and / or signs of osteoarthritis.

Grade E (5) - severe dysplasia. Marked dysplastic changes of the hip joints, such as dislocations or subluxations [5] (Fig. 1 A), Norberg's right acetabular angle less than 900, obvious flattening of the cranial acetabular margin, deformation of the femoral head (mushroom-shaped, flattened), or other signs of osteoarthritis.

The PennHip method [13] evaluates both the qualitative aspect and the quantitative aspect of the hip by measuring the degree of joint laxity. Joint laxity of the hip is the most important risk factor in the development of osteoarthritis as such it is very important to understand the difference between passive and functional laxity. Functional laxity is the pathological laxity that occurs during the normal season in the dysplastic dog. Passive laxity is the laxity determined by the PennHip method under sedation by measuring the degree of "extension".

The index method is calculated by digitally superimposing precision circle indicators on the cortical edges of the acetabulum and the femoral heads to identify the respective geometric centers. In the compression picture (Fig. 2. A), if the joint does not show osteoarthritis, the centers of the acetabulum and the femoral head should coincide, indicating that the joint is indeed concentric.

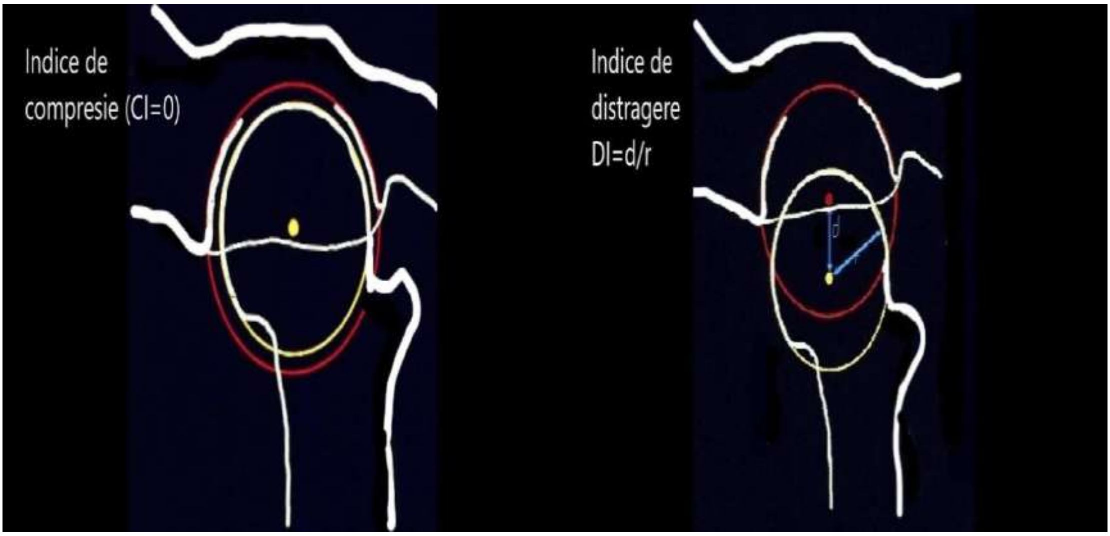

Figure 2. Graphical representation of the compression index (A) and distraction index (B)

From the point of view of distraction (Fig. 2. B) [17], the distracting force determines the separation between the centers. The distance "d" between the centers is a measure of the laxity of the hip joint. However, "d" also varies depending on the size of the dogs (larger dogs would probably have a larger "d" than smaller dogs). To circumvent these potential sources of variation, "d" is normalized to the size of the femoral head and acetabulum, dividing it by the radius of the femoral head - "r". The resulting index, Distraction Index = d / r, is a number without units between 0 and 1 (or more). The laxity index calculated for the compression view is called the compression index (CI), and the laxity index for the distraction view is called the distraction index (DI). Hips with distraction vision DI that are less than 0.3 are considered to be tight, while DIs close to 1 is considered to be extremely weak. DI is an indication of the "percentage of the joint" that the femoral head is displaced from the acetabulum. Therefore, laxity caused by DI is called passive hip laxity, as opposed to functional hip laxity, which is the pathological form of hip laxity that occurs in dysplastic hips during weight bearing [17].

Recently, the PennHIP stress index and OA scores have been found to have strong correlations with modified articular cartilage microstructures, potentially indicating a relationship between joint laxity measured by this technique and joint surface degeneration [11].

Table 1 categorizes the degrees of hip dysplasia in dogs depending on the requirements and standards of different international resource organizations.

Table 1. Degrees of coxofemoral dysplasia in dogs

OFA (USA)	FCI (Europa)	BVA (UK/Australia)	SV (Germania)
Excellent	A- 1	0-4	Normal
Good	A- 2	5-10	Normal
Fair	B- 1	11-18	Normal
Borderline	B -2	19-25	Fast Normal
Mild	C	26-35	Noch Zugelasser
Moderate	D	36-50	Mittlere
Severe	E	51-106	Schwere

The minimum age for performing the hip dysplasia test by the PennHip method [13] is 16 weeks and the forced extension method can be applied from the age of 5 months. In both cases it is recommended to repeat the radiographs to stage the changes in case of a positive diagnosis and for certification on the OFA system [16] it is accepted over the age of 24 months and on the British system [4,7] the minimum age should be 12 months.

Conclusions

Canine hip dysplasia affects millions of canine specimens each year and can result in debilitating orthopedic hip pathology. Many dogs suffer from osteoarthritis, pain and lameness causing material damage to owners through treatments, shortening the life of working dogs and thus reduced performance. The incidence of the disease is very well documented in terms of large breeds of dogs, but it can also occur in small dogs and toys and also in cats. Hip dysplasia is a complex congenital disease of a polygenic nature. Both veterinarians and breeders have tried to eliminate this condition through selective mating strategies, but still the frequency of reduction to purebreds is disappointing.

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DIVERSITY OF MAMMAL FAUNA FROM THE AREA CRICOVA-GOIAN OF ICHEL RIVER BASIN, REPUBLIC OF MOLDOVA

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Abstract. The studies were performed in 2017- 2021 in various types of ecosystems from the area Cricova-Goian of Ichel river basin. 45 species of mammals were registered: one hedgehog species, 5 species of Soricomorpha, 12 bat species, 18 rodent species, one hare species, 6 carnivorous species and 2 artiodactyl species. The multiannual dynamics of bat communities in Cricova and Goianul Noi stone mines was assessed. The rodent species were the most numerous and spread in the ecosystems of the studied area. 14 species are listed in the Red Book of the Republic of Moldova.

Introduction

The Ichel river is an affluent of Nistru river of the Republic of Moldova. The studied area presents a mosaic landscape, where the remaining natural ecosystems (woods, meadows, steppe sectors, slopes with rocky sites, gorges) alternate with the anthropized ones: shelter belts, agrocoenoses, localities, stone mines, roads. This natural complex is intensely subjected to the action of anthropogenic factors, such as grazing, limestone extraction, wastewater discharge, garbage storage etc. The building of dams and use of river waters for irrigation and aquaculture purposes disturbed the natural course of Ichel river and has affected the natural components of te area. Nevertheless, the terrestrial vertebrate fauna of the area Cricova-Goian from lower Ichel basin is rather rich and at initial fauna inventory there were indicated 97 species of terrestrial vertebrates [2] and up to 138 species in subsequent studies [3, 6]. Data on some bat species in the study area are presented in several studies [4, 5, 7, 8]. The aim of the study is to complete the data on diversity and some ecological peculiarities of mammal species the area Cricova-Goian of Ichel river basin.

Materials and methods

The studies were performed in 2017- 2021 in various types of ecosystems from the area Cricova-Goian of Ichel river basin. The ecosistems were represented by small woods, forest belts, rocky habitats, riverine and paludous biotopes, agrocoenoses and rural localities (fig. 1). The mammal species were recorded by direct observations, using traps (small mammals), counts on the route, after the traces and trophic activity (carnivorous mammals and artiodactyls), catch with nets and use of ultrasound detector (bats).


Figure 1. Ichel river and rocky ecosystem of Cricova mines and forest belt near Goianul Nou

In the ecological analysis of mammal communities, the indexes of abundance and frequency were used. Also, the ecological significance was evaluated (W), using the formula W = F·A/100, where F is frequency of the species and A – abundance index. The species with the significance lower than 1% in the studied biocenoses are considered accidental; 1.1 – 5% - accessorial; 5.1-10% – characteristic and W > 10% - constant in ecosystem.

Results and discussions

In the studied ecosystems of Crivova-Goian area 45 species of mammals were registered: one hedgehog species, 5 species of Soricomorpha, 12 bat species, 18 rodent species, one hare species, 6 carnivorous species and 2 artiodactyl species (tab. 1).

Table 1. Diversity and biotopic distribution of mammals species in the studied biotopes from the area Cricova-Goian of Ichel river

No	Species	Ecosystems					
		Forest	Pasture	Rocky sites	Wet	Agroce-noses	Locali-ties
	Erinaceomorpha						
1	Erinaceus roumanicus	+	+	+	+	+	+
2	**Soricomorpha**						
3	Talpa europaea	+	+	+	+	+	+
4	Sorex araneus	+	-	-	+	-	-
5	Sorex minutus	+	-	-	+	-	-
6	Crocidura leucodon	+	+	-	+	-	-
7	Crocidura suaveolens	+	+	-	+	+	+
	Chiroptera						
8	Rhinolophus hipposideros	+	-	+	-	-	-
9	Myotis myotis	-	-	+	-	-	-
10	Myotis blythii	-	-	+	-	-	-
11	Myotis bechsteini	-	-	+	-	-	-
12	Myotis daubentoni	+	-	+	+	-	+

13	Myotis dasycneme	+	-	+	+	-	-
14	Myotis mystacinus	+	-	+	+	-	-
15	Nyctalus noctula	-	+	-	+	+	+
16	Pipistrellus pipistrellus	-	-	+	+	+	+
17	Plecotus auritus	+	-	+	+	-	-
18	Plecotus austriacus	+	-	+	+	+	+
19	Eptesicus serotinus	+	+	+	+	+	+
	Rodentia						
20	Sciurus vulgaris	+	-	-	-	-	+
21	Dryomys nitedula	+	-	-	-	+	-
22	Muscardinus avellanarius	+	-	-	-	-	-
23	Nannospalax leucodon	-	+	+	-	+	+
24	Ondatra zibethicus	-	-	-	+	-	-
25	Arvicola terrestris	-	-	-	+	-	-
26	Cricetus cricetus	-	+	-	-	-	-
27	Rattus norvegicus	-	-	-	-	+	+
28	Mus musculus	-	-	-	+	+	+
29	Mus spicilegus	-	+	-	+	+	+
30	Micromys minutus	-	-	-	+	-	-
31	Apodemus sylvaticus	+	-	+	+	+	+
32	Apodemus uralensis	-	+	+	+	+	+
33	Apodemus flavicollis	+	-	-	-	+	+
34	Apodemus agrarius	+	+	-	+	+	+
35	Microtus arvalis	-	+	-	+	-	+
36	M. rossiaemeridionalis	+	-	-	+	-	+
37	Clethrionomys glareolus	+	-	-	-	-	-
	Lagomorpha						
38	Lepus europaeus	+	-	-	-	+	-
39	**Carnivora**						
40	Felis sylvestris	+	-	-	-	-	-
41	Lutra lutra	-	-	-	+	-	-
42	Mustela nivalis	+	-	+	-	+	+
43	Mustela putorius	+	-	+	-	-	-
44	Martes foina	+	-	+	-	-	-
45	Vulpes vulpes	+	+	+	+	+	+
	Artiodactyla						
46	Capreolus capreolus	+	-	-	-	-	-
47	Sus scrofa	+	-	-	-	+	-

The common and eurytopic mammals species, such as the northern white-breasted hedgehog, the mole, the mole-rat, the wood mouse, the fox are frequent and widespread in the studied territory, with a frequency of 47%-89% and a characteristic or constant ecological significance (W = 8.8 – 56,4%).

The shrew species were registered in low number mostly in forest and wet natural biotopes, with accidental or accessorial ecological significance (W = 0,6 – 4,7%). The white-toothed shrew is a vulnerable species, listed in the Red Book of Moldova (2015).

The bat fauna is represented by 12 species, of which 9 species were registered in Cricova lime stone mines and 10 species in Goianul Nou stone mines (tab. 2,3). The species N. noctula and *P. pipistrellus* were reregistered with the ultrasound detector in the evening period in localities, rocky sites, agrocoenoses. The monitoring of bat hibernation communities in Cricova mines allow to emphasize the multiannual dynamics of bat species (tab. 2).

Table 2. Dynamics of bat community structure (%) in Cricova mines in the hibernation period

No	Species	2013	2014	2015	2016	2017	2018	2019	2021
1	Rhinolophus hipposideros	-	12.73	7.97	5,77	10	22.54	9.09	3.33
2	Myotis blythii	-	-	-	-	2	1.41	3.03	-
3	Myotis daubentoni	51.22	41.82	65.49	71.15	50	45.07	63.64	60
4	Myotis dasycneme	29.27	5.45	12.39	13.47	22	14.08	9.09	6.67
5	Myotis mystacinus	2.44	16.36	2.65	3.85	2	1.41	-	-
6	Myotis bechsteini	2.44	12.73	8.85	1.92	12	9.86	12.12	20
7	Plecotus austriacus	14.63	5.45	0.88	-	2	-	-	6.67
8	Plecotus auritus	-	3.64	1.77	1.92	-	1.41	3.03	3.33
9	Eptesicus serotinus	-	1.82	-	1.92	-	4.23	-	-
	Total individuals	41	55	113	51	50	71	33	30

The most numerous during the entire study period was *M. daubentonii*, which represented more than half of the community, followed by *M. dasycneme* with the abundance of 6%-30%. Since these species prefer aquatic biotopes, their high proportion in Cricova mines is explained by the proximity of the Ichel river. The third most abundant species was the lesser horseshoe bat, followed by the Bechstein's bat and the whiskered bat. The Plecotus bats constituted 2-6% of the community, while *M. blythii* and *E. serotinus* single individuals.

In the Goianul Nou mines the number of individuals is rather low, due to the small surface of the mines, but the bat diversity is high, being registered 10 species. The diversity of bat community is the lowest at the end of hibernation period and the highest in september, when the individuals are intensely feeding and preparing for the hibernation. The most abundant was *M. daubentonii*, followed by *M. myotis* and *M. bechsteinii* (tab. 3).

The mines of Goian have a very low depth and only two species were recorded: *M. mystacinus* and *P. austriacus*, both protected by low.

Table 3. Dynamics of bat community structure (%) in Goianul Nou mines in the study years

No	Species	04.2016	09.2020	03.2021
1	Rhinolophus hipposideros	18.18	-	-
2	Myotis myotis	-	2.78	29.63
3	Myotis blythii	-	-	7.41
4	Myotis daubentoni	36.36	36.11	37.03
5	Myotis dasycneme	-	5.56	-
6	Myotis mystacinus	9.09	-	-
7	Myotis bechsteini	36.36	11.11	18.52
8	Plecotus austriacus	-	-	7.41
9	Plecotus auritus	-	5.56	-
10	Eptesicus serotinus	-	47.22	-
	Total individuals	11	36	27

All species identified in Cricova and Goianul Nou mines, except E. serotinus, are rare species, listed in the Red Book of the Republic of Moldova [1] with different rarity criteria. The presence of critically endangered species *M. myotis* and *M. bechsteinii* is of particular interest and emphasize the importance of these sites in bat diversity conservation in central area of the republic.

The rodent species were the most numerous and spread in the ecosystems of the studied area. The arboreal rodents – the dormouse species and the squirrel were recorded in the forest habitats and at forest edge, especially in forested canyons of the area. The semiaquatic species *Arvicola terrestris* and *Ondatra zibethicus* were registered only near Ichel river. A nest of *Micromys minutus* was found in the reed thickets on the river bank. The small rodent fauna was represented by 9 species of genera *Mus, Apodemus, Microtus, Clethrionomys* with characteristic and constant ecological significance (W = 4.2-53.7%). At forest edge the dominant species were *A. syvaticus* and *A. flavicollis* with more that 70% of small rodent community. In forest belt dominated *A. uralensis* (37.04%), followed by C. glareolus with 18.52%. In wet ecosystems the highest diversity was registered (8 species), dominant being *A. sylvaticus* (33.33%) and *A. agrarius* (31.37%) and in various types of agrocoenoses dominant was *M. spicilegus* with about 40%, followed by *A. sylvaticus* with about 22% (fig. 2).

The proximity of Ichel river, the abundant and diverse vegetation, the rocky relief with many canyons and a rich fauna of rodents and birds create favorable conditions for the existence and prosperity of several species of carnivorous mammals (tab. 1). *M. nivalis* was the rarest species, being observed twice on the rocky slopes near the river meadow [3]. The wild cat *(F. silvestris*) and the otter (*L. lutra*) are rare species, listed in the Red Book of Moldova as vulnerable species [1], and were observed several times in forest and riverine habitats of the area.

Two species of artiodactyl mammals *(C. capreolus, S. scrofa*) were recorded in the area. Both species populate forest ecosystems, but were sporadically observed at forest edge, in for-

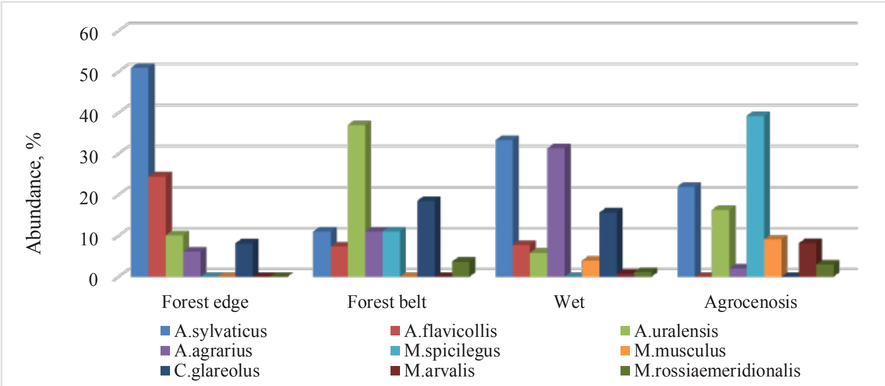

Figure 2. Structure of small rodent communities in various ecosystems of Cricova-Goian area

est shelter belts and in adjacent agricultural ecosystems.

In the localities of the studied area, eight mammal species were registered: *E. roumanicus, C. suaveolens, N. noctula, P. pipistrellus, E. serotinus, M. musculus, R. norvegicus, V. vulpes*.

The area Cricova-Goianul Nou of Ichel river with a high variety of ecosystems – forest, wet and rocky habitats provide favorable conditions for a high number of mammal species, of which 14 are listed in the Red Book of the Republic of Moldova, despite of strong anthropic disturbances.

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STRUCTURE OF SMALL RODENT COMMUNITIES IN ORCHARDS FROM THE CENTRAL PART OF THE REPUBLIC OF MOLDOVA AND BACAU DISTRICT, ROMANIA

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Abstract. The studies were performed in the period 2008-2015 in orchards from the central part of the Republic of Moldova and Bacău County, Romania. In both areas 12 species rodent were registered, of which in central Moldova orchards the species *R. norvegicus* and *P. subterraneus* were not registered, while in Bacau orchards the species *A. uralensis, M. rossiaemeridionalis* and *D. nitedula* weren't recorded. In CM orchards the dominant species was *M. rossiaemeridionalis* with about 30%, followed by *A. sylvaticus* and *A. flavicollis.* In BC orchards the dominant species was *A. flavicollis* with more that 41%, followed by *A. sylvaticus* and *M. arvalis*.The analysis of demographic structure in spring period showed a high proportion of reproductive females in all species, being the highest at *M. rossiaemeridionalis* in CM orchard and in *A. flavicollis* in BC orchard. The share of young individuals constituted up to 40% depending on the species being the highest at *M. rossiaemeridionalis* in CM orchard and in *A. flavicollis* in BC orchard. A significant difference in the area of the individual sectors of *M.rossiaemeridionalis* males and females in the orchard in summer (t = 3.46) and autumn (t = 3.39) was established, as well as for *A. sylvaticus* in the spring and summer period (t = 2.06; 2.03), and for *A.uralensis* in spring and autumn (t = 2.99; 3.44).

Introduction

The rodents are the most spread group of mammals and quickly adapt to environmental changes. Therefore, they are important elements of terrestrial ecosystems, being the dominant vertebrate species in agricultural lands, such as orchards. The orchards, especially abandones ones provide favorable trophic and shelter conditions not only for field rodent, but also for forest rodent species, due to specific vegetation, formed by fruit trees, shrubs and various grass species. The monitoring of rodent communities is of high priority, because about 80% of the republic territory is occupied by agrocenoses [6, 10]. In the central part of the Republic Moldova the agricultural landscape, including orchards, of which many abandoned, occupy large surfaces. The mosaic aspect of the agricultural landscape is particular for some areas of Bacau County, Romania. In both regions important areas are covered with natural or semi-natural forests, that contribute to the rather high diversity of small rodents in adjacent agrocoenoses, among which the orchards are the most favorable for the small rodent fauna. A comparative analysis of small rodent fauna of agricultural ecosystems from central part of the Republic of Moldova and from Bacau County was performed in the past years and revealed the influence of environmental conditions upon the diversity and structure of small rodent communities in several types of agrocenoses [5].

The aim of the study was to reveal the differences between the structure of small rodent communities in orchards from central part of Moldova and Bacau district, Romania.

Materials and methods

The studies were performed in the period 2008-2018 in several orchards from the central part of the Republic of Moldova (CM) and Bacău County (BC). The most of the studied orchards from CM are abandoned, surrounded by other types of agrocoenoses, by pastures and forest belts (fig. 1). In BC the studied orchards were surrounded by other types of agrocoenoses (corn, vineyard) and by natural forest. The rodents were caught with snap traps and live traps, placed in lines at 5-8 m distance. The traps were baited with small bred pieces imbued with sunflower oil.

In order to characterize the biotopic distribution of the species the following indexes were considered: trappability Cc=100n/C, where n – number of individuals, C – number of traps; the frequency F= 100p/P, where P – number of samples, p- samples where the species is present, and the species abundance A = 100n/N, where n – number of individuals of a species in the sample, N – total number of individuals. The ecological significance was calculated (W) using the formula W = F·A/100, where F is frequency of a species and A – abundance index. The species with the significance lower than 1% in the studied biocenoses are considered accidental; 1.1 – 5 % - accessorial; 5.1-10% – characteristic and W > 10% - constant for the given biocenosis.

Figure 1. Abandoned plum and apple orchard limited by pasture and forest belt near Bacioi village, central part of the Republic of Moldova

The spatial structure of the rodent species was studied by the capture-mark-recapture method (CMR) according to standard methodology [11]. The method involves marking a number of individuals in a natural population, returning them to that population, and subsequently recapturing some of them as a basis for estimating the size of the population, the surface of the individual sectors, the migration process. During three study weeks there were processed 1500 trap-nights and marked more than 300 individuals by the method of the phalanx amputation [12].

The area of individual sectors and groups was calculated according to standard methods [2]. The size of the individual sectors was determined for individuals captured at least 3-4 times during a 5-day study. Individuals captured more than 3 times during a census were classified as “residents” and the rest - “migrants”. By the method of exclusion at the border, the sizes of the individual sectors and the movement distance for the “resident” individuals were determined.

Results and discussions

In both areas 12 species were registered: *Apodemus sylvaticus, A. flavicollis, A.uralensis, A. agrarius, Mus musculus, M. spicilegus, Rattus norvegicus, Microtus arvalis, M. rossiaemeridionalis, M. subterraneus, Clethrionomys glareolus and Dryomys nitedula*. In CM orchards the species *R. norvegicus* and *M. subterraneus* were not registered, while in BC orchards the species *A. uralensis, M. rossiaemeridionalis* and *D. nitedula* weren’t recorded. The presence of silvicolous species M. subterraneus in BC orhard can be explained by the proximity of the natural forest ecosystems. The brown rat can be met in cultivated lands situated near villages [1], though the species density was very low in the studied period (only one individual). In the CM the species M. rossiaemeridionalis is one of the dominant species in orchards and A. uralensis is very spread in various types of agricultural ecosystems, especially in adjacent pasture ecosystems [4, 8]. Although the forest dormouse occurs mostly in silvicolous habitat, it was often recorded in orchards with tree and bush vegetation, where it founds favorable conditions similar to the forest biotopes [4].

The species distribution in orchards from both studied areas showed some differences (fig. 2). Thus, in CM orchards the dominant species was *M. rossiaemeridionalis* with about 30%, followed by *A. sylvaticus* and *A. flavicollis*, which constituted about 70% from the rodent community. In BC orchards the dominant species was *A. flavicollis* with more that 41%, followed by *A. sylvaticus* and *M. arvalis*, which constituted over 80% from all rodent species (fig. 2). *A. uralensis* had a rather high percent in rodent community from CM orchard (more than 10%), which confirm the data obtained in other studies [8]. The species *A. agrarius* reached almost 10% in CM orchard and only 4.4% in BC orchard. This fact is probably due to the differences in climatic conditions during the study period, especially in humidity degree, since the species prefers more humid environment. The proportion of *M. spicilegus* is rather close in both areas and usually do not overpass the abundance of 15-18% in orchards [3]. For *M. musculus* the orchard is not a very suitable habitat, because of high density of other rodent species and the proximity of localities, where it finds favorable conditions. Therefore, the species abundance was very low (0.8-1.1%) in both studied areas. The silvicolous species *C. glareolus* is much more abundant in BC (6.6%) than in CM (2.3%) orchards, which is due to the existence of adjacent forest ecosystems in Bacau area.

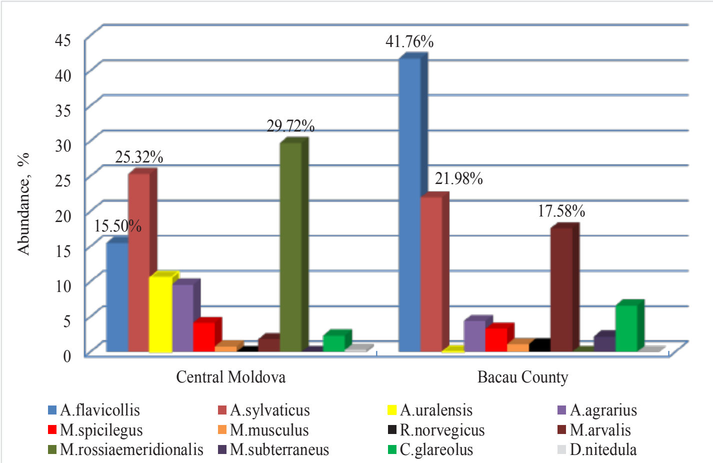

Figure 2. Rodent communities' structure in orchards from Central Moldova and Bacau County

The analysis of demographic structure in spring period (May-June) showed that 80.8% of *M. rossiaemeridionalis* population from orchard in CM are represented by breeding females and 22.8% of them belong to spring generation, which prove a very high reproductive potential. In the same period in BC orchard the reproductive females of *M. arvalis* constituted about half population and juvenile individuals constituted about 38.5%. In *A. sylvaticus* from CM orchard the share of females was of 47.6% and from the BC – 35.7%, which prove a lower reproductive potential that the previous species. In *A. uralensis* the males were dominant with more than 70% and the species was represented only by adult individuals. In *A.agrarius* the female constituted 16.7% in CM orchard, while in BC they constituted more than 90% of the population. The subadult and juvenile individuals constitute about 20% in CM orchard and 16.7% in BC orchard. In A.flavicollis the share of females was of 41.9% in CM orchard and the young individuals constituted 44.4%. In BC orchard the share of females was 42.86% and that of the subadults was about 40%. The *M.spicilegus* reproductive females had a share of 25% and the proportion of young individuals – about 30%, while in BC the species was not registered in spring period.

In the orchard from CM on a surface of one hectare during May-June and in September there were performed capture-mark-release studies in order to reveal the spatial structure of rodent community and its dynamics during the reproduction period. In May the dominant species was *M. rossiaemeridionalis* (50.6%) of which individuals were dispersed on the whole surface. The recapture rate constituted 20.5% and the migrant individuals – 79.5%. The individuals of the species *A. flavicollis* with a share of 20.1% formed 4 intrapopulation groups. The difference in the area of the individual sectors of adult overwintered males (169.4 ± 49.5 m2) and those of the spring generation (97 ± 10.4 m2) was significant ($t=2.5$, $p \leq 0.05$). For females belonging to these generations, no significant difference in the area of the individual sectors was established ($t = 1.5$, $p \leq 1.4$). 54.8% of the individuals were recaptured and the share of migrants was 45.2%, of which 58.1% were males. In *A. sylvaticus* 3 intrapopulational groups were recorded, the species population was formed by several unstructured groups and the individual sectors were little highlighted. *A.uralensis* individuals were marginalized in the upper part of the sector, being closer in the pasture. *A. agrarius* individuals formed two groups. In general, the surface of individual sectors was larger in males than in female, which was established in other studies [4, 7, 9].

In June, the abundance of *M.rossiaemeridionalis* increased by 4.2% compared to May. 75.9% were breeding females, and 42.1% of individuals belong to the summer generation. The recapture rate of the individuals was of 43% and the migrants constituted 57%, which was by 22.5% less than in the previous month of study. The share of *A. flavicollis* increased by 1.5 times and the individuals formed 5 intrapopulational groups. There was a significant reduction in the area of the individual sectors of adult males, their activity decreasing significantly ($t = 2.07$). The recapture rate of the individuals was of 68.9% and the share of migrants of 31.1%. The individuals of *A. sylvaticus* formed four intrapopulational groups.

In the autumn period, a decrease in the abundance of *M.rossiaemeridionalis* species was established in the mark sector (21.4%). The population of this species was formed entirely of young individuals, as well as the population of *M. arvalis* in BC orchard. In both study zones the dominant species was *A.flavicollis* with about 50% in CM orchard and with 53.85% in BC orchard. Its abundance increased about 2.3 times compared to the previous study period. The *A.uralensis* population had a 3.7 time increase in abundance and an overwhelming share of young individuals (95%). The share of marked and recaptured individuals from the previous period was of 28.6%.

A significant difference in the area of the individual sectors of *M.rossiaemeridionalis* males and females in the orchard in summer ($t = 3.46$) and autumn ($t = 3.39$) was established, as well as for *A. sylvaticus* in the spring and summer period ($t = 2.06$; 2.03), and for *A.uralensis* in spring and autumn ($t = 2.99$; 3.44). In the orchard the reproductive groups appeared in late spring. The groups in *A.sylvaticus* are more numerous than in *A.uralensis*. In autumn these groups are more numerous than in spring and consisted of 5.2 – 8.3 individuals. In *A.uralensis* in spring-autumn period the males dominated (69-80%), demonstrating the importance of this habitat as a refuge, especially for migrant males.

The studies were performed within the State Program project 20.80009.7007.02 and within collaboration agreement between Institute of Zoology, Republic of Moldova and Museum Complex of Natural Sciences "Ion Borcea", Bacau, Romania.

Conclusions

In both areas 12 species were registered: *Apodemus sylvaticus, A. flavicollis, A.uralensis, A. agrarius, Mus musculus, M. spicilegus, Rattus norvegicus, Microtus arvalis, M. rossiaemeridionalis, M. subterraneus, Clethrionomys glareolus and Dryomys nitedula*. In CM orchards the species *R. norvegicus and M. subterraneus* were not registered, while in BC orchards the species *A. uralensis, M. rossiaemeridionalis and D. nitedula* weren't recorded.

The species distribution in orchards from both studied areas showed some differences. In

CM orchards the dominant species was M. rossiaemeridionalis with about 30%, followed by *A. sylvaticus and A. flavicollis*. In BC orchards the dominant species was *A. flavicollis* with more that 41%, followed by *A. sylvaticus and M. arvalis*.

The analysis of demographic structure in spring period showed a high proportion of reproductive females in all species, being the highest at *M. rossiaemeridionalis* in CM orchard and in *A. flavicollis* in BC orchard. The share of young individuals constituted up to 40% depending on the species being the highest at *M. rossiaemeridionalis* in CM orchard and in *A. flavicollis* in BC orchard.

The recapture rate in the dominant species *M. rossiaemeridionalis* constituted 20.5% and the migrant individuals – 79.5%. The differences between the area of individual sectors of males and females is significant in autumn period and have similar values in spring. The difference in the area of the individual sectors of overwintered males and those of the spring generation in the species *A. flavicollis* in May is significant, while in June there was a considerable reduction in the area of the individual sectors of adult males.

A significant difference in the area of the individual sectors of *M.rossiaemeridionalis* males and females in the orchard in summer (t = 3.46) and autumn (t = 3.39) was established, as well as for *A. sylvaticus* in the spring and summer period (t = 2.06; 2.03), and for *A.uralensis* in spring and autumn (t = 2.99; 3.44).

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TERRESTRIAL VERTEBRATE FAUNA OF THE LANDSCAPE RESERVE „LA CASTEL" FROM THE NORTHERN PART OF THE REPUBLIC OF MOLDOVA

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Abstract. The studies were performed in the period 2015-2021 in various ecosystems of the Landscape Reserve „La Castel" from the northern part of the Republic of Moldova. The amphibian are represented by 11 species of the orders Urodela and Anura. Among reptiles 8 species from two orders have been identified: Testudines with one species and Squamata with 7 species. 11 species of herpethofauna are listed in the Red Book of Moldova. The ornithofauna was represented by over 120 species and is dominated by the silvicolous species. Four bird species are listed in the Red Book of Moldova. The mammal fauna includes 45 species, of which 13 are listed in the Red Book of Moldova. A large maternity colony of Myotis blythii was monitored during the last years. The Reserve „La Castel" represent an important scientific site and the studies will continue.

Introduction

The Landscape reserve „La Castel" is located in Edineț district from the northern part of the Republic of Moldova, at 75 km north-west from Bălţi city within the limits of northern Moldavian plateau. The La Castel gorge is placed above the Racovăț river valley, near the village of Gordinești and is represented by a wide variety of ecosystems of exceptional beauty. On the territory, the emergence of the Toltric calcar with the fossil remains of the fauna was noted and it is a monument of the culture of primitive man. This wonder of nature was formed due to the multimillion years deposits from ancient times. There are only fiew data on fauna from this spectacular area, where the presence of 17 species of herpetofauna, 126 bird species and 26 mammal species are mentioned [1]. In addition, information about some mammal species (shrews, bats, rodents) occurring in the area can be found in several publications [2, 3].

The aim of the work was the study of terrestrial vertebrate diversity in the area, the biotopic distribution of the species and revealing of status of the species occurring in the reserve.

Materials and methods

The studies were performed in the period 2015-2021 in various ecosystems of the Landscape Reserve „La Castel" from the northern part of the Republic of Moldova. The reserve has a surface of 746 ha, is located in the landscape region of plateaus and forest-steppe plateau of northern Moldova and belong to the lower course of the Racovăţ river basin. The area is situated along Racovâţ river valley, is 200 m wide strip with a length of 5 km on the left bank of the river. The main types of ecosystems are the forests, dominated by oak and European hornbeam, steppes, meadows, rocky and river ecosystems. The maximum altitude is 261 m a.s.l., mean altitudes 150-200 m and the slope incline limits vary between 5º-30º [1].

The diversity and distribution of the herpetofauna in the studied ecosystems were estimated by the method of counting routes and fixed plots. The surface of the fixed plots was of 100-2500 m2, the length of the counting routes varied from 0.1 - 3 km. The qualitative and quantitative structure of the bird communities during nesting and migration was elucidated by counting on routes with a length of 2-10 km. In the mammal fauna research the following methods were used: visual observation, trapping and identification from prey bird pellets (small mammals), counting in underground shelters and identification with the ultrasound detector (bats), counting on routes with a length of 1-10 km for large and medium-sized mammals (lagomorphs, carnivores, ungulates).

Results and discussions

The high diversity of biotopes and of the vegetation favored the existence of a rich fauna of terrestrial vertebrate species. The amphibian are represented by 11 species of the orders Urodela and Anura, of which 8 are included in the Red Book of Moldova [4] (tab. 1). Amphibian species have been observed in humid biotopes and near Racovăț river, where they find favorable trophic and reproductive conditions. Among 11 species registered in the area, 8 are included in the Red Book of Moldova [4], and at European level all species are protected, being listed in Annex II (strictly protected animal species) and Annex III (protected animal species) of the Bern Convention (tab. 1).

Table 1. Diversity and distribution of amphibian and reptile species in the ecosystems of the reserve

No	Species	Ecosystem					Status	
		Forest	Forest edge	Riparian	Meadow	Rocky	RBM	Bern Conv.
Amphibia								
1	Triturus cristatus	+	-	+	-	-	VU	Ann. II
2	Triturus vulgaris	+	-	+	-	-	VU	Ann. II
3	Bufo bufo	+	+	+	-	-	VU	Ann. III
4	Bufo viridis	-	+	+	+	+	-	Ann. II
5	Bombina bombina	-	-	+	-	-	VU	Ann. III
6	Rana dalmatina	+	+	+	-	-	VU	Ann. II
7	Rana temporaria	+	-	-	-	-	VU	Ann. II
8	Pelophylax ridibundus	-	-	+	-	-	-	Ann. III
9	Pelophylax esculentus	+	-	+	-	-	-	Ann. III
10	Hyla arborea	+	+	+	+	-	VU	Ann. II
11	Pelobates fuscus	-	+	+	-	+	CR	Ann. II
Reptilia								
12	Emys orbicularis	-	-	+	-	-	EN	Ann. II
13	Anguis fragilis	+	+	+	-	-	-	Ann. III
14	Lacerta viridis	+	+	+	-	+	-	Ann. II

15	Lacerta agilis	-	+	+	+	+	-	Ann. II
16	Natrix natrix	+	+	+	-	+	-	Ann. III
17	Natrix tesselata	-	-	+	-	-	-	Ann. II
18	Coronella austriaca	-	+	-	+	-	EN	Ann. II
19	Vipera berus	+	+	-	-	-	EN	Ann. III

RBM – Red Book of R.Moldova, VU – vulnerable, EN – endangered, CR – critically endangered

Among reptiles 8 species from two orders have been identified: Testudines with one species and Squamata with 7 species (tab. 1). The rare species of snakes C. austriaca and V. berus are forest species and have been observed mainly in the forest glades and at forest edge. In the Red Book of Moldova 3 species of reptiles out of the 8 reported in the area are listed and at European level all species are listed in Annex II and Annex III of the Bern Convention.

The ornithofauna from the area was represented by over 120 species and is dominated by the silvicolous species, due to the presence of natural forest ecosystems, formed by trees of 60-100 years old [1]. Among the forest nesting passerine bird species there can be mentioned *Cuculus canorus, Caprimulgus europaeus, Dendrocopos major, D. minor, D. medius, D. syriacus, Dryocopus martius, Picus canus, Jynx torquilla, Lullula arborea, Anthus trivialis, Oriolus oriolus, Garrulus glandarius, Hippolais icterina, S. borin, Phylloscopus trochilus, Ph. collybita, Ph. sibilatrix, Ficedula hipoleuca, Phoenicurus phoenicurus, Erithacus rubecula, Luscinia luscinia, Turdus merula, T. philomelos, Cyanistes caeruleus, Parus major, Aegitalos caudatus, Sitta europaea, Certhia familiaris, Fringilla coelebs, Coccothraustes coccothraustes, Carduelis chloris, C. carduelis* etc.

At forest edge and in open type biotopes with shrub vegetation the following species were registered: *Phasianus colchicus, Columba palumbus, Streptopelia turtur, Merops apiaster, Upupa epops, Galerida cristata, Alauda arvensis, Hirundo rustica, Delicon urbica, Anthus camplestris, Lanius collurio, L. excubitor, Sturnus vulgaris, Pica pica, Corvus frugilegus, C. corone cornix, C. corax, Sylvia nisoria, S. communis, Phoenicurus ochruros, Passer domesticus, P. montanus, Miliaria calandra, Emberiza citrinella,, E. calandra, E. hortulana, Motacilla alba* etc.

In a cavity of the rocky slope in May-June period, a nest of C. corax was observed and the birds became very anxious when we approached nearby. In the same rocky habitat two pairs of Oenante oenante were registered. Near the river two individuals of Ciconia ciconia were observed, the nest of which was recorded in the nearest Hordinești village (fig. 1). In the riverine biotopes after the nesting period, in July, the species *Ardea alba* and *Ardea cinerea* were registered.

Figure 1. Nest of *Ciconia ciconia* with two baby birds in Hordinești village, June, 2020

The prey bird species observed during the study years were *Milvus migrans*, *Accipiter gentilis, A. nisus, Buteo buteo, Falco tinnunculus, Otus scops, Athene noctua, Strix aluco, Asio otus. A nest of A. noctua* was found in a cavity of the rocky slope above the entrance in the stone mine. Four bird species are listed in the Red Book of Moldova – *A. alba* [EN], *C. ciconia* [VU], *M. migrans* [VU], *D. medius* [VU] [4].

The mammal fauna is rather diverse in the reserve and includes 45 species – one hedgehog species, 5 species of Soricomorpha, 11 bat species, 17 rodent species, one hare species, 8 carnivorous species and 2 artiodactyl species (tab. 2).

The common and eurytopic species such as the hedgehog, the mole, the mole-rat, the wood mouse, the yellow-necked mouse, the fox are rather frequent and widespread in the reserve with a frequency of 43%-100% and characteristic or constant ecological significance (Wa = 7,4% – 38%) in the studied ecosystems. The shrew fauna is represented by 4 species (*Sorex araneus, S. minutus, Crocidura leucodon* and *C. suaveolens)* of which the white-toothed shrew is a vulnerabe species, listed in the Red Book of Moldova [4].

Table 1. Diversity and distribution of amphibian and reptile species in the ecosystems of the reserve

No	Species	Ecosystem					Status	
		Forest	Forest edge	Riparian	Meadow	Rocky	RBM	Bern Conv.
1	Erinaceomorpha							
2	Erinaceus roumanicus	+	+	-	+	+	-	-
3	Soricomorpha							
4	Talpa europaea	+	+	+	+	+	-	Ann. III
5	Sorex araneus	+	+	+	-	-	-	Ann. III
6	Sorex minutus	+	+	+	-	-	-	Ann. III
7	Crocidura leucodon	-	+	+	+	+	VU	Ann. III

8	Crocidura suaveolens	-	+	+	+	+	-	Ann. III
9	Chiroptera							
10	Rhinolophus hipposideros	+	+	-	+	-	EN	Ann. II
11	Myotis blythii	-	+	-	+	+	VU	Ann. II
12	Myotis daubentonii	-	+	+	-	-	VU	Ann. II
13	M. dasycneme	-	-	+	-	-	EN	Ann. II
14	M. mystacinus	-	-	-	-	+	EN	Ann. II
15	Nyctalus noctula	+	+	+	+	+	-	Ann. II
16	Pipistrellus pipistrellus	-	+	-	+	+	-	Ann. III
17	Pipistrellus pygmaeus	+	+	-	+	-	-	Ann. II
18	Eptesicus serotinus	-	+	+	+	+	-	Ann. II
19	Plecotus austriacus	-	+	-	+	+	VU	Ann. II
20	Plecotus auritus	+	+	-	-	-	EN	Ann. II
21	Rodentia							
22	Sciurus vulgaris	+	+	-	-	-	-	Ann. III
23	Spermophilus suslicus	-	-	-	+	-	VU	Ann. II
24	Myoxus glis	+	-	-	-	-	VU	Ann. III
25	Dryomys nitedula	+	+	-	-	-	-	Ann. III
26	Muscardinus avellanarius	+	+	-	-	-	-	Ann. III
27	Nannospalax leucodon	-	+	+	+	+	-	-
28	Ondatra zibethicus	-	-	+	-	-	-	-
29	Arvicola terrestris	-	-	+	-	-	-	-
30	M. spicilegus	-	+	-	+	+	-	-
31	Apodemus sylvaticus	+	+	+	+	+	-	-
32	Apodemus uralensis	-	+	-	+	+	-	-
33	Apodemus flavicollis	+	+	-	-	+	-	-
34	Apodemus agrarius	-	+	+	-	+	-	-
35	Microtus arvalis	-	-	-	+	+	-	-
36	M. rossiaemeridionalis	-	+	+	-	+	-	-
37	Microtus subterraneus	+	-	-	-	-	-	-
38	Clethrionomys glareolus	+	+	-	-	-	-	-
39	Lagomorpha							
40	Lepus europaeus	-	+	-	-	+	-	Ann. III
41	Carnivora							
42	Felis sylvestris	+	+	-	-	-	VU	Ann. II
43	Lutra lutra	-	-	+	-	-	VU	Ann. II
44	Mustela nivalis	+	+	-	-	-	-	Ann. III
45	Mustela putorius	+	+	-	+	+	-	Ann. III
46	Martes martes	+	-	-	-	-	VU	Ann. III

47	Martes foina	+	-	-	-	-	-	Ann. III
48	Meles meles	+	-	-	-	-	-	Ann. III
49	Vulpes vulpes	+	+	-	+	+	-	-
50	Artiodactyla							
51	Capreolus capreolus	+	+	-	-	-	-	Ann. III
52	Sus scofa	+	-	-	-	+	-	-

RBM – Red Book of R.Moldova, VU – vulnerable, EN – endangered, CR – critically endangered

The bat fauna is reprezented by 11 species, of which 7 species (*Rhinolophus hipposideros, M. blythii, M. daubentonii, M. dasycneme, M.mystacinus, Plecotus austriacus, P. auritus*) were registered in Hordinești limestone mines and 4 species (*Nyctalus noctula, Pipistrellus pipistrellus, P. pygmaeus, Eptesicus serotinus*) were reregistered with the ultrasound detector in the evening period.

In the hibernation period in the mines all 7 species of bats were observed, but their diversity varied in different years. Thus, in February 2015 and in April 2018 five species were registered, in April 2021 – four species and in summer period 3-4 species were registered.

In the study years the abundance of the species *M. blythi* was the highest and varied between 88,58% și 98,45%, except 2015, when the species *M. daubentoni* was dominant with 84,62%. For the whole period the most abundant was *M. blythii* with almost 90%, followed by *M. daubentoni* with about 10%, while other species had less than 1%.

In the reproductive period, a large maternity colony of *M. blythii* was found and monitored since 2017 (fig. 2). Each year the number of individuals in colony was growing and this is the largest known colony of this species in the Republic of Moldova. This site is of particular interest and importance for bat conservation.

Figure 2. Maternity colony of *M. blythii* with about 400 pregnant females, may 2019

The arboreal rodents – the dormouse species and the squirrel were recorded in the forest habitats and at forest edge. A colony of *S. siuslicus* was recorded in the pasture situated at the northern limit of the reserve with the coordinates 48.165 N, 28.148 E. The colony number was estimated at about 40-50 individuals.

The semiaquatic species *Arvicola terrestris* and *Ondatra zibethicus* were registered only near the Racovăţ river. The small rodent fauna was represented by 9 species of genera M*us, Apodemus, Microtus, Clethrionomys* with characteristic and constant ecological significance (Wa = 6.8-32%), except *M. subterraneus* which is more rare than other rodent species.

The diversity of biotopes and the abundant trophic resources (rodents, birds) create favorable conditions for the existence and prosperity of many species of carnivorous mammals, which are represented in the area of 8 species (tab. 2). The wild cat (*F. silvestris*), the otter (*L. lutra*) and the wood marten (*M. martes*) are included in the Red Book of Moldova as vulnerable species [4].

The artiodactyl mammals are represented by two species: the roe deer (*C. capreolus*) and the wild boar (*S. scrofa*), which mainly populate forest ecosystems, but can be observed in the adjacent sectors in search for food, especially in the second half of the year.

The picturesque landscape, the specific rocky relief, the variety of natural ecosystems, the abundance and diversity of flora and fauna of the Landscape Reserve "La Castel" represent an important tourist attraction and an area of great significance for scientific research.

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CONTRIBUTIONS TO THE STUDY OF WATER AND SEMI-AQUATIC BIRDS IN THE RAMSAR WETLAND ”LOWER PRUT LAKES”

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Abstract. The Republic of Moldova has few wet areas. One of them was designated in the year 2000 with the status of Wetland of International Importance (Ramsar). The ecosystems of lakes and ponds are home to a multitude of bird species. Aquatic and semi-aquatic species make up a major component of the studied sector and consists of 94 species. Thousands of specimens transit the area during spring and autumn migrations; others retreat in the winter to be safe and to feed; the summer guests arrive in the warm period of the year to breed. Out of the total number of aquatic and semi-aquatic birds, 26 species are protected nationally and internationally. During the study period, rare or accidental species were recorded, such as: Bubulcus ibis, Cygnus columbianus, Branta ruficollis, Grus grus, Sterna caspia, Arenaria interpres, Larus ichthyaetus etc.

Introduction

The wetland between the city Cahul and the village Giurgiulesti covers an area of 19152 ha. The territory includes a segment of the Prut River, Manta and Beleu Lakes, a wide variety of natural and man-made ecosystems, including willow forests, marshy biotopes, wetland habitats, pastures, meadows, etc.

The first data regarding the aquatic and semi-aquatic ornithofauna of the ponds on the left bank of the Prut were collected starting with the 60s of the 20th century. The research on bird fauna intensified after 2000, being published a series of materials about the process of formation of complex colonies of semi-aquatic bird species, about the particularities of the reproduction process of some taxonomic bird groups, about population dynamics and the migratory specificity of aquatic and semi-aquatic birds, the reproductive behavior of some species [3, 4, 5, 11]. In recent years the diversity of the terrestrial vertebrate fauna from the “Prutul de Jos” Reserve, as well as the vulnerability of avifauna in the Lower Prut basin (Republic of Moldova) to climate change were studied [6, 7, 8, 9, 10, 11].

The paper is based on the analysis of older data from field research, own observations and materials published in recent years that contain information about new and accidental species encountered in the Ramsar wetland, “Lakes of the Lower Prut”.

Materials and methods

The research on aquatic and semi-aquatic species of birds was made based on our own observations, carried out between 2017-2021 in the Lower Prut and also with data from specific literature. The method of motion and fixed-point observations was used. The trips on the aquatic surfaces were made by boat. For the identification of the birds were used the 10x50 binoculars and the 15x45 telescope.

Result and discussions

The sector of Lower Prut provides vital conditions for numerous aquatic and semi-aquatic species of birds. Some of them can be met during the spring and autumn migrations; other species arrive for reproduction on summer period. Up to 2019 in this area were mentioned 75 nesting species [4, 11]. According to the latest data from the studied territory, 93 species of birds were identified (tab. 1).

Table 1.Taxonomic structure of aquatic and semi-aquatic avifauna

No.	Order	Share in the studied area			Share of the avifauna of the area from that of the republic
		Species	Share of the order from all aquatic and semi-aquatic avifauna (%)	In Republic of Moldova	
I.	Gaviiformes	1	1,1	2	50,0
II.	Podicipediformes	4	4,3	5	80,0
III.	Pelecaniformes	4	4,3	4	100,0
IV.	Ciconiiformes	13	13,9	13	100,0
V.	Anseriformes	26	27,9	28	92,8
VI.	Gruiformes	9	8,7	10	80,0
VII.	Charadriiformes	37	39,8	50	74,0
Total:		**94**	**100**	**116**	**81,0**

Analyzing the share of the avifauna of the area with that of the Republic of Moldova, we notice that the orders of Charadriiformes (37 species) and Anseriformes (26 species) have the highest degree of participation. The order Gaviiformes is represented by one of the two species encountered in the republic.

The situation of the habitats occupied by the aquatic and semi-aquatic ornithofauna identified in the studied sector, their type of nutrition, biogeographical affiliation, rarity category according to the Red Book of the Republic of Moldova [2] are presented in table 2.

Table 2. The synoptic chart of the aquatic and semi-aquatic species of birds

No	Species/ category of protection	Biogeo-graphic origin	Aviphenologic categories of bird species phenology; trophic grup	Habitat; Observations
Order Gaviiformes				
1.	Gavia stellata	Arct.	Wv; DJF; Ichthyophagous	Encountered on river Prut (Slobozia Mare); lake Manta.
Order Podicipediformes				
2.	Podiceps cristatus	Tp	Sv, Rw; N MAMJJASO; Ichthyophagous	Present on Beleu and Manta lakes, in the ponds in the area, where they also have nesting places; wetlands with marshy vegetation.
3.	Podiceps grisagena	E	Sv; AMJJASO; Ichthyophagous, entomophagous	
4.	Tachibabtus ruficollis	E	Sv, Rw; N J AMJJASO; Zoophagous, entomophagous	
5.	Podiceps nigricollis	E	Sv, Rw; MAMJJASO; Zoophagous	

Order Pelecaniformes				
6.	Pelecanus onocrotalus / En	Mo	Sv; AMJJASO; Ichthyophagous	Present on Beleu and Manta lakes, in the pond in the southern part of Brînza village and in the pond from Colibași village (fig. 1).
7.	Pelecanus crispus / Cr	Mo	Sv; DI AMJJASO; Ichthyophagous	Present on Beleu and Manta lakes.
8.	Phalacrocorax carbo	TP	Sv, Rw; ND MAMJJASO; Ichthyophagous	Present on river Prut, Beleu and Manta lakes.
9.	Phalacrocorax pygmeus / Cr	M	Sv; MAMJJASO; Ichthyophagous	Present in ponds in the localities: Giurgiulești, Slobozia Mare, Văleni, Brînza, Colibași, Manta, Vadul-lui-Isac, Crihana Veche, especially in the sectors where they have a place for nesting, extensive ponds with reeds and willows.
Order Ciconiiformes				
10.	Botaurus stellaris / Vu	Mo	Sv; AMJJASO; Ichthyophagous, zoophagous	Present in the dense reeds of the ponds from Giurgiulești, Slobozia Mare, Văleni, Brînza, Colibași, Manta; Ponds with dense, marshy, and aquatic vegetation.
11.	Ixobrychus minutus	E	Sv; MJJAS; Zoophagous	
12.	Nycticorax nycticorax	M	Sv; AMJJASO; Zoophagous	Encountered in the willows on the banks of river Prut; near Beleu and Manta lakes close to water sources.
13.	Ardeola ralloides / En	M	Sv; MAMJJASO; Zoophagous	Present in the ponds from the localities: Giurgiulești, Slobozia Mare, Văleni, Brânza, Colibași, Manta, Vadul-lui-Isac, Crihana Veche; Large ponds, flooded or swampy lands.
14.	Egretta garzetta	M	Sv; MAMJJASO; Ichtyofagous, zoophagous	Present in extensive ponds, in flooded or swampy lands from the localities: Giurgiulești, Slobozia Mare, Văleni, Brânza, Colibaşi, Manta, Vadul-lui-Isac, Crihana Veche.
15.	Egretta alba/ En	Ch	Sv, Rw; NDJ MAMJ JASO; Ichtyofagous, zoophagous	Present at the shore of the pond on wet meadows, flooded lands, shallow ponds in the localities: Giurgiulești, Slobozia Mare, Văleni, Brânza, Colibaşi, Manta, Vadul-lui-Isac, Crihana Veche
16.	Ardea cinerea	Tp	Sv, Rw; NDJ MAMJJASO; Ichtyofagous, zoophagous	Present in large ponds, not too deep, with thick reeds or emerging vegetation from the localities: Giurgiulești, Slobozia Mare, Văleni, Brânza, Colibaşi, Manta, Vadul-lui-Isac, Crihana Veche.
17.	Ardea purpurea / Vu	M	Sv; MAMJJASO; Zoophagous	Present in the wet ponds and meadows, the flooded lands from the localities: Slobozia Mare, Văleni, Brânza, Colibași, Manta.
18.	Bubulcus ibis	M	Acc; AMJ; Entomophagous	Observed in the pond between Văleni and Brînza villages (fig. 1)

19.	Ciconia ciconia / Vu	EP	Sv, P; FMAMJJASO; Zoophagous	The nests are located in most localities from Cahul district, feeding on wet and swampy lands in the Prut meadow or agricultural fields around them.
20.	Ciconia nigra/ Cr	EP	Sv, P; MAMJJASO; Ichtyofagous, zoophagous	In-flight, solitary specimens are found over the wetland in all the studied sectors; searching for food or in-migration, near Beleu and Manta lakes. It prefers willow forests or the shore of the ponds.
21.	Platalea leucorodia/ Cr	EP	Sv, Rw; AMJJASO; Zoophagous	In number of a few specimens, it is found in the extensive, shallow ponds, with mud and reeds, extended in the localities: Slobozia Mare, Văleni, Brânza and Manta.
22.	Plegadis falcinellus/ Cr	M	Sv; MAMJJASO; Zoophagous	On the pondfront or in swampy areas; shallow ponds, with reeds and willows in the flooded area of the localities: Giurgiulești, Slobozia Mare, Văleni, Brânza, Colibași, Manta, Vadul-lui-Isac, Crihana Veche.
Order Anseriformes				
23.	Cygnus olor/ Vu	E	Mp; NDJFMAMJJASO; Phytophagous, rare zoophagous	The largest number is found on Manta, Beleu and Polderul Brânza lakes; during the reproduction period 1-2 pairs on the ponds from Giurgiulești and Colibași localities; puddles spread with thick reeds on the edges.
24.	Cygnus columbianus	Arc.	Acc; N F; Phytophagous, rare zoophagous	2-4 specimens were recorded on and around Lake Beleu; large areas of water with a vast aquatic vegetation. On Beleu and Manta lakes, large areas of open water with aquatic vegetation.
25.	Cygnus cygnus / Vu	S	Wv; DJF; Phytophagous, zoophagous.	On Beleu and Manta lakes, large areas of water with aquatic vegetation.
26.	Branta ruficollis / Vu	Arc	Wv; DJF; Phytophagous	In very small numbers on Beleu and Manta lakes or on the meadows around them.
27.	Anser anser	Mo	Mp; NDJFMAMJJASO; Phytophagous	A few specimens nest in ponds in Slobozia Mare, Brânza, Manta.
28.	Anser fabalis	Arc.	Wv; DJF; Phytophagous	On Beleu and Manta lakes; on wet meadows, cultivated land in the vicinity of wetlands; in large ponds (for rest).
29.	Anser albifrons	Arc.	Wv; NDJF; Phytophagous	
30.	Anser erythropus/ Vu	Arc.	Wv; DJF; Phytophagous	
31.	Tadorna ferruginea/ Vu	Mo	Sv; MA JASO Phytophagous, zoophagous	In the ponds from the localities: Slobozia Mare, Văleni, Colibași, Manta, Vadul-lui-Isac.
32.	Tadorna tadorna / Vu	Mo	Sv; Rw; N MAMJJASO; Zoophagous, phytophagous	On Beleu and Manta lakes, in waters wealthy in aquatic vegetation.

33.	Anas strepera	Tp	Sv; AMJJASO; Phytophagous, rare zoophagous	Present on the Beleu, Colibași, and Manta lakes; ponds with dense aquatic vegetation.
34.	Anas penelope	S	P; N MA SO; Phytophagous	Present on the Beleu and Manta lakes; ponds with water luster and aquatic macrophytes.
35.	Anas acuta	S	Wv; DJF; Zoophagous, phytophagous	Present on Beleu and Manta lakes.
36.	Anas platyrhynchos	Tp	Mp; NDJFMAMJJASO; Phytophagous	In the ponds from the localities: Slobozia Mare, Văleni, Colibași, Manta, Vadul-lui-Isac
37.	Anas crecca	Tp	Sv, Wv; NDJFMAMJJASO; Phytophagous, rare zoophagous	In the ponds from the localities: Slobozia Mare, Văleni, Brânza, Colibași, Manta, Vadul-lui-Isac
38.	Anas querquedula	Tp	Sv; MAMJJASO; Zoophagous	In the ponds from the localities: Slobozia Mare, Văleni, Colibași, Manta, Vadul-lui-Isac
39.	Anas clypeata	Tp	Sv, P; MAMJJASO; Zoophagous	In the ponds from the localities: Slobozia Mare, Văleni, Colibași, Manta, Vadul-lui-Isac
40.	Netta rufina/Vu	M	P; NO; Phytophagous	Present on the Beleu and Manta lakes.
41.	Aythya marila	A	Wv; NDJ MO; Zoophagous, phytophagous	Present on the Beleu and Manta lakes.
42.	Aythya fuligula	S	Wv; NDJFMAMJJASO; Phytophagous, zoophagous	Present on the Beleu and Manta lakes.
43.	Aythya ferina	E	Sv,Wv; NDJFMAMJJASO; Phytophagous, zoophagous	Present on the Beleu and Manta lakes; In the ponds from Brânza and Colibași with water luster and aquatic macrophytes (fig. 2).
44.	Aythya nyrocca/ Cr	WP	Sv, Wv; NDJFMAMJJASO; Phytophagous	In the ponds and places wealthy in reeds and willows from the localities: Giurgiulești, Slobozia Mare, Văleni, Brânza, Colibași, Manta, Vadul-lui-Isac, Crihana Veche.
45.	Mergellus albellus	S	Wv; DI; Ichtyofagous, zoophagous	Present on the Beleu and Manta lakes;
46.	Mergus merganser	Tp	Wv; DJF; Ichtyofagous, zoophagous	Present on the Beleu and Manta lakes;
47.	Mergus serrator	S	Wv; DJ; Ichtyofagous	Present on the Beleu and Manta lakes;
48.	Bucephala clangula	S	Wv; F; Phytophagous, zoophagous	Present on the Beleu and Manta lakes, in puddles.

Order Gruiformes				
49.	Grus grus	Tp	Acc; SO; Phytophagous, zoophagous.	In flight over Lake Beleu.
51.	Porzana parva/ Vu	Wp	Sv; MJJA; Phytophagous, zoophagous	Wet meadows, rich in vegetation.
52.	Porzana porzana / Vu	Wp	Sv; AMJJASO; Phytophagous, zoophagous	
53.	Porzana pusilla/ Vu	Wp	Sv; AMJJASO; Phytophagous, zoophagous	
54.	Crex crex/ En	E	Sv; MJJ; Phytophagous, zoophagous	
55.	Galinulla chloropus	Wp	Sv; MAMJJASO; Phytophagous, zoophagous	It is present in the wetland near water sources, stagnant water basins, wealthy in vegetation.
56.	Fulica atra	Tp	Sed.; NDJFMAMJJASO; Phytophagous, zoophagous	On the aquatic surfaces from all the localities from the Prut meadow; ponds with dense aquatic vegetation and water eyes.
57.	Rallus aquaticus	E	Mp; NDJ MAMJJASO Phytophagous, zoophagous	
Order Charadriiformes				
58.	Himantopus himantopus/Vu	Mo	Sv; AMJJASO; Zoophagous	At the pondfront in the localities: Slobozia Mare, Văleni, Brânza, Colibași, Manta, Vadul-lui-Isac, Crihana Veche. Ponds not too deep, muddy or sandy shores (gig. 2). Wet meadows, shores, open arable or grassy lands; swamps with low vegetation.
59.	Recurvirostra avosetta/ Vu	Mo	Sv, Rw; N MAMJJAS; Zoophagous	
60.	Haematopus ostralegus/ Vu	Mo	Sv; N MAMJJAS; Zoophagous	
61.	Charadrius dubius	Mo	Sv, P; AMJJASO; Zoophagous	
62.	Charadrius hiaticula	Mo	P; N A SO; Zoophagous	
63.	Vanellus vanellus	Mo	Sv; NMAMJJASO; Zoophagous, ichtiofagous	
64.	Stercorarius parasiticus	Arc.	P; SO; Zoophagous, ichtiofagous	Rarely near Lake Beleu and Manta.
65.	Calidris alba	Arc	Wv; DJF; Zoophagous	At the water's edge in the ponds from the localities: Slobozia Mare, Văleni, Brânza, Colibași, Manta, Vadul-lui-Isac, Crihana Veche.
66.	Calidris minuta	Arc.	P; AM SO; Zoophagous	
67.	Calidris terninckii	Arc.	P; ND AM SO; Zoophagous	
68.	Calidris ferruginea	Arc.	P; AM SO; Zoophagous	
69.	Calidris alpinia	Arc.	P; AM SO; Zoophagous	
70.	Limicola falcinellus	Arc.	P; ND AM SO; Zoophagous	
71.	Philomachus pugnax	S	P; AMJJAS, Zoophagous	
72.	Lymnocryptes minimus	S	P; NDJ MASO; Zoophagous	
73.	Gallinago gallinago	E	Sv;AMJJAS; Zoophagous,ichtiofagous	

74.	Limosa limosa	Mo	Sv, P; MAMIIAS; Phytophagous, zoophagous	At the pondfront, stretches of water with marshy vegetation; slow flowing waters, lakes in most localities;
75.	Limosa lapponica	S	P; S; Phytophagous, zoophagous	
76.	Numenius arquata	E	Sv; MAMJJAS; Zoophagous	
77.	Tringa erythropous	S	P; MA AS; Zoophagous	
78.	Tringa totanus	Mo	P; MAMJJAS; Zoophagous	
79.	Tringa nebularia	S	P; MA SO; Zoophagous	
80.	Tringa glareola	S	P; MA SO; Zoophagous	
81.	Tringa hypoleuca	S	P; MA SO; Zoophagous	
82.	Tringa ochropus	S	P; RI; MA SO; Zoophagous	
83.	Sterna caspia	Mo	P; MA SO; Zoophagous	
84.	Phalaropus lobatus	Mo	P; MA SO; Zoophagous	In the ecosystem of Lake Beleu; Stretched puddles with shallow water.
85.	Arenaria interpres	Arc.	P; MA SO; Zoophagous	In the coastal area of Lake Manta
86.	Larus ridibundus	Tp	Sed.; NDJFMAMJJASO;Zoophagous	In flight, on the waterfront or on the aquarium in most localities; Prefers sandy shores.
87.	Larus cachinnans	Tp	Sed.;NDJFMAMJJASO; Zoophagous	Encountered in the studied sector on Beleu and Manta lakes; in puddles with stretches of water and marshy vegetation, slowly flowing waters.
88.	Larus fuscus	Arc.	Wv; IFM; Phytophagous, zoophagous	
89.	Larus canus	S	Wv; F; Zoophagous, ichtyofagous	
90.	Larus ichthyaetus	M	Sv; M; Phytophagous, zoophagous	On Lake Beleu and in its neighboring pond.
91.	Sterna hirundo	E	Sv; AMJJASO; Zoophagous, ichtyofagous	In the ponds of the vast majority of localities in the studied area, in greater numbers near Beleu and Manta lakes.
92.	Chlidonias hybridus	M	Sv; MAMJJASO; Zoophagous, ichtyofagous	
93.	Chlidonias niger	E	Sv; AMJJAS; Zoophagous, ichtyofagous	
94.	Chlidonias leucopterus	E	Sv; AMJJA; Zoophagous, ichtyofagous	


Figure 1. ***Pelecanus onocrotalus*** **and** ***Bubulcus ibis*** **– rare species**


Figure 2. ***Aythya ferina*** **and** ***Recurvirostra avosetta*** **– vulnerable species**

Conclusions

The Ramsar wetland "Lakes of the Lower Prut" has an important role for 94 aquatic and semi-aquatic species of birds. Out of these, 26 are under national and international protection, with varying degrees of danger: 14 – vulnerable species; 6 - critically endangered species; 4 - endangered species. The studied area is used by thousands of birds during the autumn and spring migrations, also in the summer when they find favorable conditions for reproduction. According to the types of nutrition are distributed: 1 - ichthyophagous/entomophagous; 1- entomophagous; 1 – zoophagous/ entomophagous; 7 – ichthyophagous; 10 – phytophagous; 16 – ichthyophagous/ zoophagous; 25 – phytophagous/ zoophagous; 33 – zoophagous.

From a phenological point of view, 46 species are short-sighted visitors; 21 are passage species; 16- winter visitors; 4 - partially migratory; 3 - sedentary; 3 - meet accidentally; some species fall into several phenological categories. The following species have an irregular presence in the area: *Bubulcus ibis, Cygnus columbianus, Grus grus, Sterna caspia, Arenaria interpres, Larus ichthyaetus,* etc.

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THE EVOLUTION OF CASTORIDS (MAMMALIA, RODENTIA) IN THE REPUBLIC OF MOLDOVA

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Abstract. Beavers first appear in Asia, where fossil remains date back to the Eocene (33-36 million years ago). The most widespread Pleistocene giant beaver fossils were Siberian beavers – Trogontherium cuvieri and the North American beaver – Castoroides ohioensis. According to recent research, the oldest beavers in the Republic of Moldova are known from the deposits of Sarmatian (11.5 million years) – Steneofiber aff. depereti Mayeri, Chalicomys jaegeri (Kaup), Palaeomys castoroides Kaup, Trogontherium minutum minutum Franzet et Storch., T. minutum rhenanum Franzet et Storch. and Monosaulax cainarensis Lungu. In the Meotian (8.7-5.0 million years) only two species were recorded – Trogontherium minutum rhenanum Franzet et Storch. and Castor aff. praefiber Deperet. et Lungu.

Introduction

The only and skilled architect-rodent is the beaver, being one of the largest rodents by weight and length, in the northern part of the Globe, reaching over 30 kilograms in weight and body length up to one meter. Its merit is due to its intense work on the amazing, strange and skilful constructions of dams (plateaus) built of branches, trunks and mud, as a result of which whole rows of trees disappear on the waterfront.

Material and methods

Paleontological research has been conducted on beaver materials from the time period 11.5 million years ago to the present. The paleontological materials collected by the author and the collections of the Paleontological Museum of the Institute of Zoology (Museum of Fossil Wildlife Complexes from the Republic of Moldova) were used.

Resuls and discussions

On the territory of the Republic of Moldova the oldest discoveries of skeletal reminiscences of beavers were recorded at the age of about 11.5 million years ago from the middle Sarmatian, lower phase of the Upper Pleistocene, “Moldavian” faunistic complex. From the archaic alluvial deposits from Tătăreşti, Cahul district are known to the following species of fossil beavers: *Monosaulax savinovi* Lutchev (a P4 premolar tooth with a length of 5.7 mm and a width of 4.1 mm) [5]; *Monosaulax cf. munutus* (Meyer) from the alluvium from Etulia, UTA Gagauzia – a P4 premolar, 6.8 mm long and 7.9 mm wide [18]. From Musaitu, Taraclia district comes the species *Trogontheruim aff. minus* New., from which the following original pieces: a fragment of the damaged cranial box and the upper jaws with the teeth, P4-M2 on both branches (the length of the dental row on the occlusal surface is 13.8 mm dex. and 13.6 mm sin.; a premolar – P4 dex. with a length of 6.0 mm and a width of 6.2 mm sin.; a second fragment of the left branch of the upper jaw with teeth P4-M2 (the length of the dental row being 16.2 mm, the length of the premolar P4 being 6.5 mm, and the width – 6,8 mm, the dimensions of the upper molars having: M1 – length 8.9 mm, width 4.9 mm, and M2 – length 9.0 mm and width 5.2 mm) [16; 4].

From the alluvial deposits of the Upper Pleistocene, the late phase (17,000-11,000 years ago) which is attributed to the Haprovean Faunistic Complex, were collected: from Etulia, UTA Gagauzia, from the ravines, natural monument, upper layer, ninth terrace of the Prut river – a fragment of the upper jaw of beaver *Trogontherium aff. minus* New with teeth: the premolar P4 and the molar M1 slightly damaged, the length of the premolar P4 being 7.4 mm, and the width – 9.0 mm.

From the pebble quarries, from Salcia, Anenii Noi district, fossils of Trogontherium cf. cuvieri were collected: from the lower layer a lower beaver jaw of Trogontherium cf. cuvieri Fisch. (straight branch, without anterior part and ascending branch, with teeth M1-M3; an incisor fragment (original piece) with a length of 8 cm, the anterior-posterior diameter (vertical) following the cutting surface being 8.0 mm, the lateral diameter in the same place being 10.0 mm. Fossils of *Trogontherium cf. cuvieri* Fischer – a lower jaw, straight branch, without anterior and ascending portions with teeth P4-M3, was also discovered in the pebble quarry from Vadul-lui-Vodă, the ninth terrace of the Dniester [8; 15].

In the upper layer of alluvium from the Salcia quarry, Anenii Noi district, Lower Pleistocene, Odesan Faunistic Complex (Psecupsian), 2.58-1.8 million years, a lower jaw, straight branch, was discovered by the beaver *Trogontherium cuvieri Fisch*. The ascending part of this part is damaged, the dentition is complete (P4 - M3), almost entire incisor (slightly damaged posterior end), its length is 148 mm, the antero-posterior diameter behind the cutting surface is 13.0 mm, the medial-lateral diameter is 10.5 mm.

From the alluvial deposits of the VIII terrace of the Prut, from the ravines from Cismichioi, ATU Gagauzia, which dates back to the age of the Lower Pleistocene and in which a variety of rodent fauna was discovered, only Castoridae gen. sp. and a P4 premolar of beaver *Trogontherium sp*., with length 7.3 mm and width – 7,8 mm.

In the pebble of the 7th terrace of the Dniester, the Tamanian faunal complex from Copanca village, Causeni district a lower jaw was discovered, the left branch of *Trogontherium cuvieri* Fisch. The ascending (anterior) part is damaged, the teeth are present P4 - M3 and the anterior portion of the incisor well developed – posteriorly following the cutting surface being 13.5 mm and the lateral diameter 11.0 mm.

V. A. Topacevskii after a tooth fragment from the alluvium from Tiraspol (Colcot's Rock) established its presence of Castor aff. fiber) [13]. Other beaver fossils from Middle Pleistocene deposits in the Republic of Moldova have not been found.

In the Upper Pleistocene, the beaver species Castor fiber L. appears, Upper Paleolithic (42,000-10,000 years ago), which continued into the Holocene (10,000 – present). Skeletal remains (incomplete jaws, isolated teeth, limb bones), which have been discovered at several resorts of prehistoric people, of various archaeological cultures, show that this beaver was hunted, especially for its beautiful fur, flesh and castorine – a dark, fragrant oily liquid, secreted from the gland under the tail, used in folk medicine as a good and precious remedy.

The following Paleolithic resorts are known from the Upper Pleistocene, where beaver bones were found:

Duruitoarea Veche, Lower Paleolithic, Axel culture – 44/7 (35,000-10,000 years ago) here and below the counter – skeletal remains, to the denominator – the number of individuals) [9; 3]; the late Paleolithic, the Stone Age ends with the Mesolithic period (10,000-7,000 years ago).

Buteşti, cave, Mustie culture – 1 [13]; Duruitoarea Veche, Madlen culture – 16/4 [9; 2]; Brânzeni I, Upper Paleolithic, Szelet culture (Brânzeni) – 8/3 [9; 2]; Brânzeni I, Mesolit – 2/1 [17; 9]; Trinca I, Mesolit – 2/1 [11].

Most localities from the Republic of Moldova, where skeletal remains of river beaver were found, belong to the Middle Holocene (42,000-8,200 years ago), being recorded at Neolithic (years) and Eneolithic archeological resorts; to the Neolithic resorts; Bugo-Nistrean culture, near the city of Soroca (Soroca I, Soroca II, Soroca III, Soroca V) were discovered 22 skeletal remains from 8 individuals [1, 10].

Insignificant skeletal reminiscences of beaver were also collected from other Neolithic resorts: Floreşti, band-linear pottery culture – 4/2 [19; 20], Selişte, Orhei district, culture Criş – 3/1, [10]; Sacareuca, Teleneşti district, Criş culture – 1; Floreşti I, Boian culture – 24/12 [20].

Most beaver fossil finds from the Middle Holocene date to Eneolithic archeological sites, Tripolie culture (IV-III millennia BC e. n.), listed below:

Early Trypillia culture: Floreşti, 36 skeletal remains from 17 individuals [20] Rogojeni I, Floreşti district – 4/2 [10]; Floreşti III – 4/2 (unical pieces); Alexandrovca, Floreşti district – 2/1 [10]; Putineşti I, Floreşti district – 2/1 [10]; Ruseştii Noi, Ialoveni district – 10/3 [14]; Solonceni I, Rezina district – 5/2 [7; 10]; Holercani I, Dubăsari district – 1 [20];

Middle Trypillia culture: Solonceni II, Rezina district – 2/2 [20; 10]; Gura Căinarului, Floreşti district – 1 (unical pieces);

Late Trypillia culture: Costeşti II, Râşcani district – 1 [10]; Costeşti IV, Râşcani district – 63/29 [10]; Brânzeni III, Edineţ district – 8/3 [21; 10]; Brânzeni IV, Edineţ district – 1, [8]; Iablona I, Glodeni district – 3/1 (unical pieces);

Trypillia culture: Mereşovca-Cetăţuie, Ocniţa district – 8/2 [12]. Lonely beaver graves were found at the resorts Gumelniţa (III-II millennia BC e. n.) from the southwest of the Republic of Moldova; Vulcăneşti II, UTA Găgăuzia – 3/1 [10] and Taraclia, Taraclia district – 1 (unical pieces), in the lats years the species appeared of the Lower Prut meadow [6].

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Conclusions

The first beaver fossils on the territory of the Republic of Moldova were found about 11.5 million years ago (Middle Sarmatian). Later, during the Pliocene and Quaternary (Late Pleistocene, four million years ago), the wooded banks of rivers waters run from Moldova lived species of beavers: *Monosaulax savinovi* Lutchev, *Monosaulax cf. minutus* (Meyer), T*rogonthe-*

rium aff. minus New., *Trogontherium cf. cuvieri Fisch and Trogontherium cuvieri* Fisch. In the Upper Pleistocene, Upper Paleolithic, which continued into the Holocene (42,000-10,000 years ago), the river beaver species *Castor fiber L.* appears. It's been gone for centuries (the middle of the second millennium of our era), then appeared in the last years in the meadow of the Lower Prut.

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SEASONAL AND MULTIANNUAL DYNAMICS OF SEDENTARY SPECIES POPULATIONS OF HUNTING INTEREST

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Abstract. The aim of the research was to study the number dynamics of the populations of dominant sedentary species of hunting interest as a theoretical context in arguing sustainable measures for the management of economic interest fauna. The studies between 2000-2021 shows that after a period of depression in the populations of sedentary species of hunting interest in 2004-2012 there is an increase from 2016-2017 till present of reproductive stocks in all studied species on average by 95%. The losses in the cold period of the year varied depending on the climatic conditions of the cold period of the year, as well as the age structure at the end of the reproductive period: for the hare between 14% and 30%, with an average of 23%; in grey partridge from 56% to 71% and in pheasant they fluctuate between 48% and 56% of the autumn number, being lower in warm winters (37%). Analyzing the dynamics of annual increases in sedentary small game species in different climatic conditions, it was found that arid conditions during nesting and offspring growth, decrease annual increases by 143% in pheasants, 122% in hares, and only 74% in partridges, which is a species less dependent on aridizations in the vegetative period.

Introduction

The number of game species, which fully capitalize on the support capacity of the hunting ground with an ecologically balanced demographic structure, without impact on the ecosystem is determined as the optimal number. The optimal number of each species varies cyclically in the conditions of keeping unchanged the regime offered by the hunting fund. The amplitude of the variation of the number of individuals is determined, on the one hand by the biological parameters of the species (reproduction age, annual reproductive potential, longevity, etc.), and on the other hand by the degree of ecological comfort within ecosystems, mainly trophic and disturbance, including predation pressure [3,5]. The mechanism of biological cycles takes place against the background of the trophic factor and the influence of climatic factors [6, 7]. Abundant food, accompanied by favorable climatic conditions (mild winters) causes resistant individuals, high fertility (increased number of juveniles, possibly several annual lots), favorable conditions for raising new-born individuals, increased annual growth, low juvenile mortality, leading to a population in qualitative and quantitative ascent, until it reaches the maximum density that the land can support [2, 4]. The task of hunting management is to ensure the density of game species as close as possible to the optimal one, counteracting by biotechnical measures any oscillation of the real density, which brings the populations of the species to extreme limits. The problems of managing the non-forested hunting funds are related to the ecology of agricultural ecosystems and solving them will raise the hunting productivity by building an agrosystem on ecological principles [4]. The study of the population dynamics of the species of economic importance will serve as scientific support in their sustainable management.

Materials and methods

In order to achieve the objectives, research methods were used related to the characteristic of structural ecological indices: the method of routes, the method of sampling, the method of direct observations; the method of detecting the traces of vital activity; density index method; the method of visualizing the images captured with the help of photo or video cameras, the method of evaluations in open field with the use of night vision devises. The studies were carried out in districts from the southern, northern and central parts of the republic, sample plots were selected in ecotone areas of different ecosystems (forest, aquatic and paludous, meadows, rocky sites, forest belts, localities), and in the adjacent habitats - agrocenoses, forest ecosystems with different degrees of heterogeneity and anthropic activity. For the characteristic of the biotopic distribution of the species, the frequency index was used F = 100p/P, where P - the number of samples, p- the samples in which the species is present; and the dominance of the species D = 100n/N, where n - the number of individuals of a species in the sample, N - the total number of individuals from all species, both indices are expressed as a percentage. In order to highlight the position of the species or taxonomic group in the biocenosis, the ecological significance (WA) was calculated according to the formula Wa = Fa·Da/100, where Fa - group frequency and Da - abundance index. Taxonomic species or groups with a significance of up to 1% in the analyzed cenoses are considered accidental; 1.1 - 5% - accessorial; 5.1-10% - characteristic and Wa > 10% - constant for the characterized biocenosis.

The statistical and factorial analysis, graphical interpretation of the results was performed using the Microsoft Excel and Statistics programs.

Results and discussions

The study of the dynamics of autumn number and reproductive stocks in the European hare (*Lepus europaeus*) population – the main game species in open lands, during the population cycle (2012 - 2021) allowed to establish a year to year increase in spring number with variations between 2.8 and 42%, as well as in autumn number from - 4.5 to 34% (fig. 1; tab. 1).

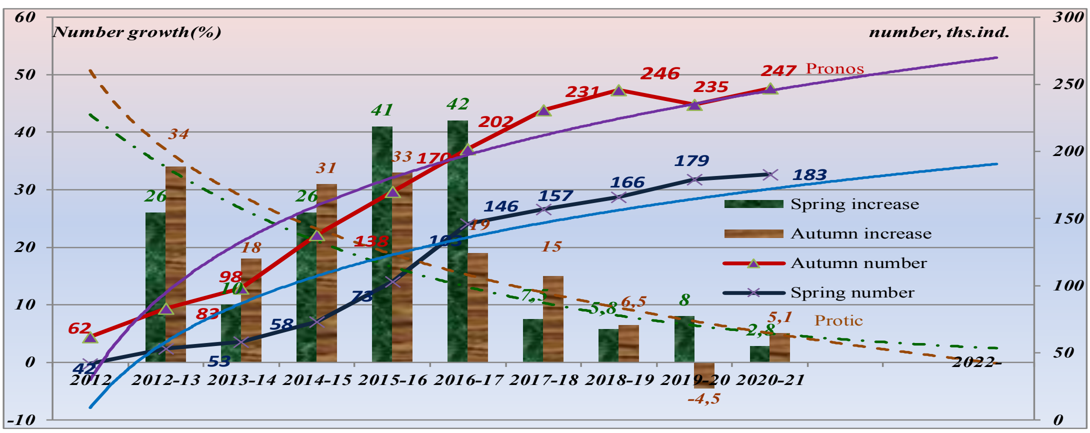

Figure 1. Dynamics of spring and autumn number annual increase during the populational cycle of 2012-2021 and development trends for the next years in European hare populations

The number increase in spring and autumn is much higher (29 and 27% respectively) at the initial stages of the population cycle and decrease considerably (4.8 and 5.5% respectively), with negative values (-4.5%) in autumn 2020 (with drastic arid conditions), when the population reaches maximum values, approaching the support capacities of agricultural ecosystems for the hare. The trend lines of the numbers and the inter-annual numerical increase in the hare, during the population cycle, allowed to predict in the following years a decrease of both the spring and the autumn increase resulting in a slower growth and even stopping it in the following years.

Table 1. Dynamics of populational parameters during the population cycle of 2012-2021 in hare in agrarian ecosystems of the R. Moldova

Population parameter	Period									
	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021
Spring number (thousand ind.)	42	53	58	73	103	146	157	166	179	183
Autumn number (thousand ind.)	62	83	98	128	170	202	231	246	235	247(progn.)
Annual reproductive growth (%)	**48**	**57**	**69**	**75**	**59**	**38**	**48**	**48**	**31**	**35(progn.)**
Spring increase* (%)		26	10	26	41	42	8	7,5	8	2,8
Autumn increase* (%)		34	18	31	33	19	15	6,5	-4,5	5,1(progn.)
Winter loss (%)		15	30	29	19	14	22	28	28	23

Losses in the cold period of the year varied, depending on the climatic conditions of the cold period and the age structure at the end of the reproductive period, between 14% and 30%, with an average of 23%. The most considerable winter losses (29,30%) were observed in the winters of 2014-2015 when the annual reproductive growth was maximum (69.75%) provided by the summer-autumn generations. In the autumn populations these years young specimens dominated and most of them do not survive until the beginning of the hunting season (December). Significant losses (17.5% of the autumn population) in October-December of the years 2019-20 were caused by the unfavorable climatic conditions, which also activated the parasitic and viral impact. As a result of the examination of the coprological samples it was found that the extent of the invasion in the hare (*Lepus europaeus*) is with *Trichocephslus leporis* is of 14,2%, *Fasciola hepatica* – 8,2%, *Dicrocoelium lanceolatum*-18,6%, *Strongyloides papillosus*-21,4%, with oocysts of *Eimeria stiedai* de 42,2% and *Eimeria perforans* de 34,6%. Climatic conditions in the reproductive period strongly determine the annual increase and the age structure of this increase, estimated on average over the cycle at 51%. At the same time, winter conditions, the biotopic structure of the hunting fund with trophic conditions, shelter, parasitic pressure, the presence of predators [3, 5] and poaching [1] are the determining factors of the annual increases, of the demographic structure of the autumn population and of the reproductive stock.

Analyzing the multiannual dynamics of the reproductive number of the partridge population (Perdix perdix) in the agrarian ecosystems of the republic during 2000-2021 it was found that during the years 2004-2010, due to a series of ecological and anthropic conditions, the species number began to decrease, reaching the minimum levels (12.8 thousand birds) with densities between 2 and 4 pairs per 1000 ha (fig.2). After the numerical increase (79%) of 2017, the reproductive stock of partridge is maintained at 43-55 thousand pairs with a slight upward trend in the next years.

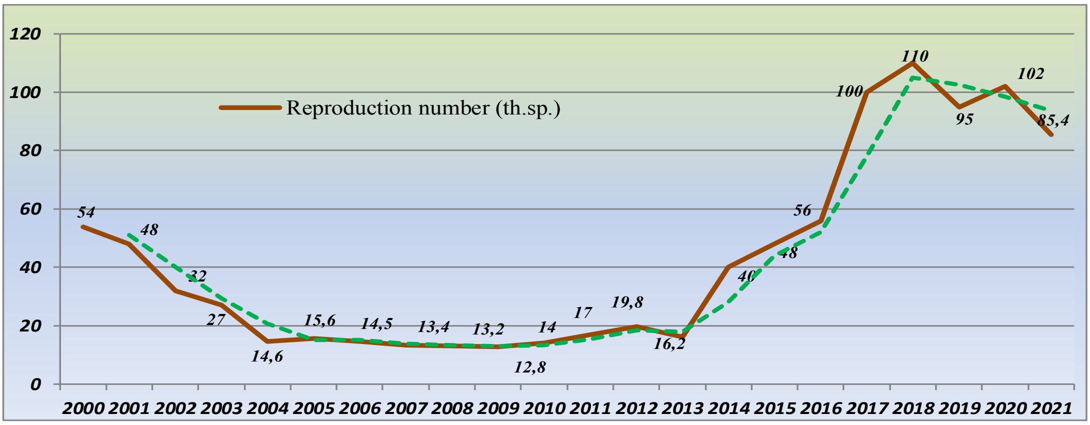

Figure 2. Multiannual dynamics (2000-2021) and the trend of reproductive number in partridge population in the following years

The analysis of the annual dynamics of the partridge number in the last four years showed that the annual increase can reach 270% of the breeding population with an average of 172%, and the most significant losses start in autumn (October - November) with 45% from the number at the beginning of October. During the hunting season for hare (November-December) the partridge population loses other 16.8% (fig. 3).

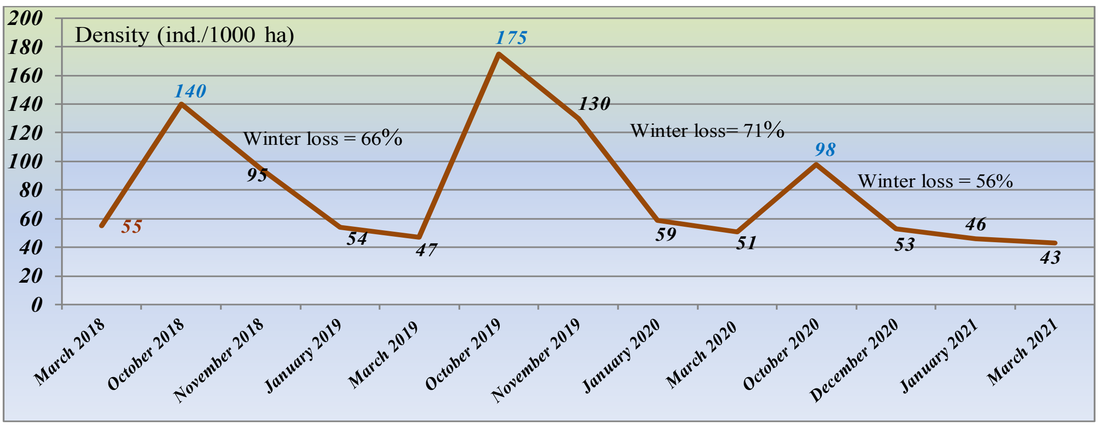

Figure 3. Annual dynamics of partridge density (ind./1000 ha) in agricultural ecosystems during 2018-2021

In winter, the specimens remain more resistant and viable and the losses for the period January-February constitute on average only 9.5% of the winter population. Thus, the partridge population in the cold period of the year usually suffers losses that can exceed 71% of the estimated number in autumn.

The distribution of sedentary species of small game (hare and partridge) during the winter prove that the hare is a constantly encountered species (WA = 12.4-23.8%) in multiannual crops (vineyards, orchards), fallow grounds and autumn plowed lands, being characteristic (WA = 5.8%) on pastures and hayfields. The partridge has constant ecological significance in fallow ground (WA = 16.2%), in pastures and hayfields (WA = 12.1%); it is characteristic (WA = 9.3%) in multiannual crops and accessorial plowed lands (WA = 4.7%).

Evaluating the dynamics of the pheasant population (Phasianus colchicus) during the last 6 years can be observed an increase in the dynamics of the species acclimatization process due to the protection measures in the cold period of the year, adequate extraction quotas and annual increase, and permanent completion of the population with pheasants from farms (fig. 4). The decrease in the dynamics of the annual autumn growth also shows that the pheasant is close to reach the maximum possible number for the current support capacity of the pheasant populated biotopes.

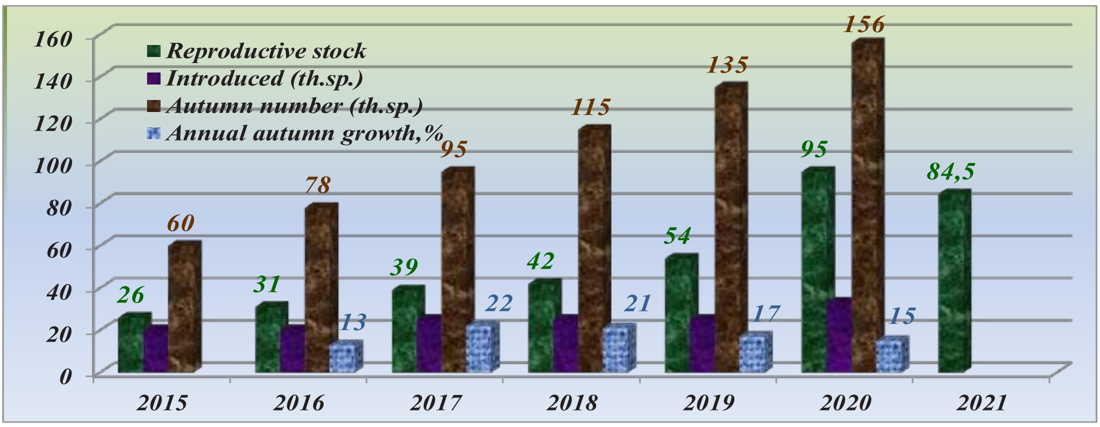

Figure 4. Multiannual dynamics of the population parameters of the pheasant in the acclimatization process

The annual increases, during the evaluated period, due to the climatic conditions during the reproductive period, ranged between 37% (under stressful arid conditions) and 103-126% under favorable conditions. The losses in the cold period of the year fluctuate between 48% and 56% of the autumn number, being much lower in warm winters (37%).

The dynamics analysis of annual increases in sedentary small game species in different climatic conditions showed that arid conditions, during nesting and offspring growth, decrease the annual increases by 143% in pheasant, 122% in hare, and by only 74% in partridges, being a species less dependent on aridizations during the vegetative period (fig.5).

Conclusions

After a period of depression in the hare population, there is an upward increase from year to year in both spring and autumn population number and these increase are much higher (29% and 27% correspondingly) at the initial stages of the population cycle and decrease considerably (4.8% and 5.5% respectively), when the population reaches maximum values, approaching the support capacities of ecosystems.

The partridge after the numerical depression in 2004-2010 registered a numberic increase (79%) in 2017, after which the reproductive stock of the species remained at 43-55 thousand pairs with a slight upward trend in the next years.

The pheasant population demonstrates an increase in the dynamics of the acclimatization process, when the annual increases ranged between 37% (under stressful arid conditions) and 103-126% under favorable conditions.

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SEASONAL AND MULTIANNUAL DYNAMICS OF RODENT SPECIES NUMBER UNDER THE INFLUENCE OF CLIMATE FACTORS AND ANTHROPIC MODIFICATIONS

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Abstract. The research was carried out in agricultural experiment stations in the central area of the Republic of Moldova during a period of 35 years. It was determined that cyclic oscillations with an interval of 3-4 years are not typical of Microtus arvalis, as can be seen in other parts of the area. Intense anthropogenic influence determines the type of numerical dynamics. A certain periodicity is observed in the species Mus spicilegus, but during the last 30 years the dynamics has become acyclic. In the populations of the species Apodemus sylvaticus there was a more pronounced periodicity of the oscillation of the herd, once every two years.

Introduction

Population dynamics is one of the basic problems of animal ecology, it has a great practical importance for society. Of particular interest is the oscillation of rodent numbers, which manifests itself in three temporal aspects: seasonal changes in the number, process, which takes place with the onset of reproduction and up to mortality during the generative period of one year; annual dynamics - the variation of the number of populations during different years and the multiannual dynamics, when comparing the quantitative indices during a consecutive series of years. Small rodent species have a short lifespan and breed for several generations.

The annual differences in the number of rodent populations are recorded, comparing the indices from different years, but from the same seasons. Most often the size of the herd is compared in spring, before the beginning of the breeding season or for the annual peak phase - at the end of the reproduction period. In this case, the factors that influence the existence of the population in different years (winter and spring-summer conditions) are analyzed. Therefore, the determining factors of the environmental capacity are investigated.

The multiannual dynamics of the small rodent population has been recorded since ancient times, creating difficulties for the human population in the supply of food. The peak phases were particularly pronounced for *Microtus arvalis, Microtus rossiaemeridionalis, Clethrionomys glareolus, Mus spicilegus, Apodemus sylaticus* etc. Their appearance in large numbers, and then their disappearance are processes which were regularly recorded and were of interest for elucidating the causes of these phenomena.

Analyzing the variation of the population, the emphasis is more on the study of the variation of reproductive intensity, but not on the causes of mortality. Only high fertility causes an increase in population. The reduction of the mortality level, the reproduction continuing to take place, have as a consequence the increase of the population density. In this case, the intrapopulation mechanisms of the inverse link act, and the reproductive potential decreases.

In order to solve the problem of rodent herd dynamics, it is necessary to divide the population by age groups. The nature of the periodicity of microtine population dynamics remains an enigma to this day. Several hypotheses and postulates have been formulated regarding the nature of population fluctuations, which, however, do not fully elucidate this problem. Great importance is given to the mortality factor in the dynamics of the number of the population [11]. Mortality was analyzed in light of the nutrition factor [10]. The importance of the climatic factor was demonstrated by Kalabuhov N.I. [5]. The "predator-prey" relationship was elucidated by Sludschii A.A. [12]. At the same time, a dependence of numerical dynamics on intrapopulation factors was established. Thus, Kalabuhov N.I. identified in individuals certain qualitative changes of an adaptive nature [5]. Formozov A.N. researched the reproductive potential of rodents [13]. One of the basic ecological theories – self-regulatory dynamics of livestock was substantiated by D. Chity [1]. External factors of mortality have been elucidated, causing the decline of populations: abiotics (climate, floods) and biocenotics (nutrition, predators, diseases) modifiers (environmental), dependent or independent of population density. This fact denotes the complex nature of the influence of ecological factors on microtine populations in space and time. Some ecologists consider that these populations oscillate cyclically for 3-4 years [2], others - that they have non-periodic numerical eruptions [9].

Elucidating the problem of population dynamics, a correlation of the intensity of reproduction with the phase of oscillation of the number of population was determined [7]. It is necessary to mention that the argument is the reproduction, and the consequence – the phase. Research priorities focus on the role of reproduction. It is necessary to determine the age groups (winterers, individuals in the current year, generations), which contribute to the increase of the population, which is of great importance for the elaboration of the prognosis, which is paramount in practical terms.

The problem of population dynamics is divided into two components: minor and major wave cycling [6]. The first type, of short duration, represents the result of the increase of the herd in case of a normal reproduction of the population in the conditions of favorable ecological conditions. The population goes through the peak phase over 3-5 years after the depression. The reduction of ecological capacity, the increase of mortality, as a result of unfavorable weather conditions or lack of food, move the peak phase. In this case, there is an interval of 7-8 years between these phases. So, at the base of the minor wave cycling is the high potential of reproduction in geometric progression. The study of population dynamics, at the same time as its fundamental importance, allows to formulate recommendations regarding the regulation of the number of animal individuals, their protection and rational use.

Materials and methods

The scientific material was collected from the agricultural experiment stations in the central area of the Republic of Moldova during 35 years. Field research methods related to the characteristic of structural ecological indices were used: the method of paths, the method of squares [8]. The determination of the specific composition and abundance of rodent species was performed by the methods of relative appreciation of the numerical herd – night traps, route counts, test plots, trophic activity, etc. [9], and the absolute numerical evaluation – by using traps (four lines of 25 traps with an interval of 20 m) on sample sectors with an area of 1 ha over a period of 5 days [10]. The following parameters were recorded in the captured animals: species, sex, age, physiological and reproductive status.

Results and discussions

As a result of changes in agriculture, there have been radical transformations in wildlife complexes in general and small mammal communities in particular. Often the species of this group are pests of agricultural crops. Essential changes have also taken place in the dynamics of the number of small mammals [3]. During the years of mass reproduction and rapid growth of the *M. arvalis* herd, it causes considerable damage to agriculture and, especially, to alfalfa, clover, wheat, etc. Along with this species, others such as *M.spicilegus*, *A.sylvaticus, A.uralensis, A. agrarius*, etc can cause damage too. Therefore, it is necessary to maintain an admissible density of populations of these species in concrete living conditions [4].

The climatic conditions during the study period were varied. The mildest winter was during the growing season of small mammals, when no negative temperatures were recorded. At this stage the summer was arid, with a moderate amount of precipitation. The harshest winter was for a smaller number of studied species than the multiannual average. The depression phase coincided with a snowless quite warm winter, and the drought created unfavorable conditions for reproduction.

There was a seasonal, an annual, but also a multiannual oscillation in the number of species. For the species, indicated above, an almost synchronous annual variation of the numerical number is typical. Particular attention was paid to the oscillation of the number of the main species for the respective phases of growth, maximum and depression. A significant difference in the average annual catch coefficient was established, calculated for the growth phase, maximum and depression. Thus, the significant difference of the parameter nominated in the comparative plan for the maximum phase and the depression phase for a probability $P < 0.05$ is $t = 5.6$, and for the maximum phase and the growth phase - $t = 8.9$. This indicates that cyclic oscillation of the number is typical of small mammals. But the length of the full oscillation cycle for each species is different.

In the *M. arvalis* species, the maximum phase is followed by a decreasing phase with a deep depression and a gradual exit from it. There will be a moderate relative growth over the next 3 years. *A. sylvaticus,* unlike *M.arvalis*, is exposed to smaller multiannual oscillations. After the year of the peak phase, the decrease phase occurs, but a deep depression is not reached, which favors in the following year an increase in the number of this species. The number of M. spicilegus species is also exposed to oscillations, but their multiannual amplitude is smaller, registering an increase in the years, when the other species has a lower number. The dynamics of the dominant species was established during the months of the respective phases (fig.1).

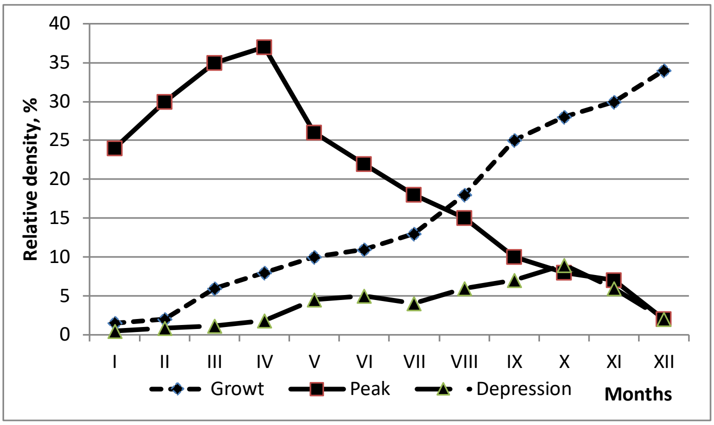

Figure 1. Seasonal dynamics of the dominant small rodent species in different phases of the population cycle

In the spring of the year of the peak phase the number is higher compared to the other phases, which contributed to its subsequent growth. Unlike other species, the number of microtines during the growth and peak phase increases from 60-70 colonies per hectare in July to 400-500 and more in late autumn. The demographic structure of the populations of the studied species represents one of the ecological parameters, which influences the oscillation of their number.

Figure 2 reflects the multiannual dynamics of the general population processes of rodent species in communities over a period of over 40 years. There is a pronounced cyclicity of the relative density of these species, the highest values of this parameter being recorded in the early and late 80s of the past century, when multiannual forage grass crops occupied large areas and were the main feeding stations for microtine. Subsequently, with the decrease of the areas of these crops, the nutrition base for the plantivore rodent species was reduced, which also influenced the decrease in their number.

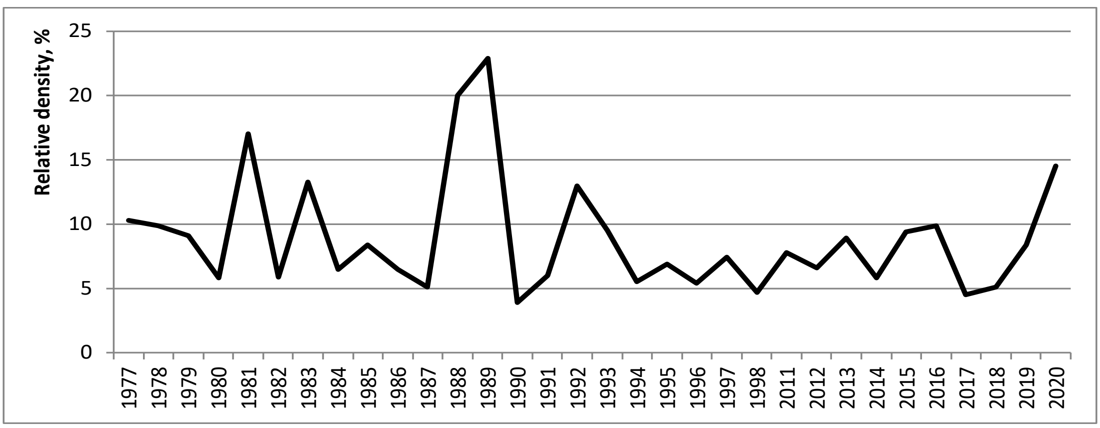

Figure 2. Multiannual dynamics of relative density of rodent species

Figure 3 shows the multiannual dynamics of the relative density of the dominant rodent species in agrocenoses. Research has shown that the dynamics of rodent populations in agrocenosis tends to be periodic, but anthropogenic and climatic factors disrupt this process.

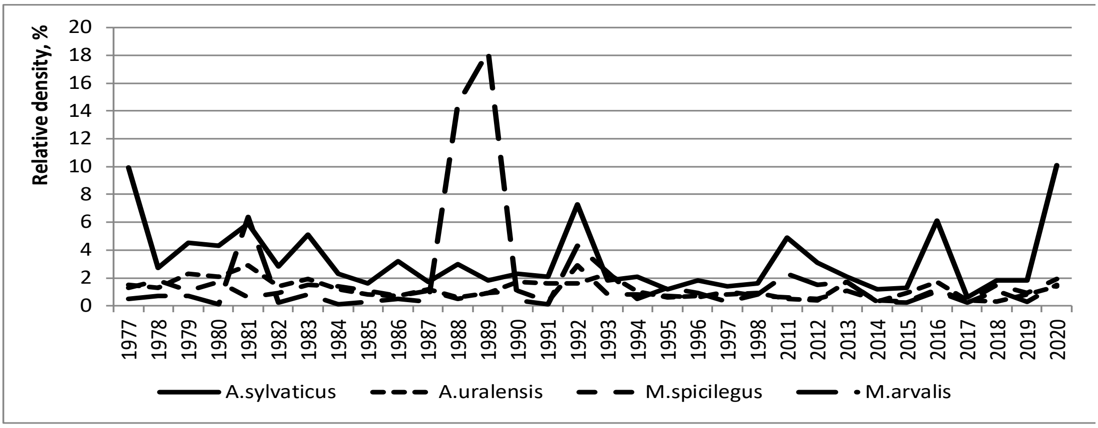

Figure 3. Multiannual dynamics of relative density of dominant rodent species in agrocenoses

Thus, for example, in *Microtus arvalis*, cyclical oscillations with an interval of 3-4 years are not typical, as can be seen in other parts of the area [2]. Possibly, the intense anthropic influence determines the type of numerical dynamics. A certain periodicity is also observed in the species *M. spicilegus*, but in the last 30 years its dynamics becomes acyclic. In the populations of the species *A. sylvaticus* there was a more pronounced periodicity, once every two years, of the number oscillation [3].

The multiannual cycle of microtines is divided into the following phases, which reflect the numerical strength, but also their survival. The minor undulating cycle includes the phase of depression, which is preceded by the decrease, the individuals of the population are caught more frequently in the refuge stations - the forest belts, where they survive, the living conditions during this period being unfavorable. Their density on the field of perennial grasses, adjacent to the refuge sectors, in the summer months is minimal (1-2 colonies / ha). At this stage the reproduction is manifested, but it is quite weak. In summer, the survival of the summer generation is 13%, and that of the spring generation – 22%. Usually, the onset of microtine populations is stimulated by unfavorable conditions, but this factor is not mandatory.

Low temperatures, rains, early frosts, unstable snow cover, frost, etc. contribute to rodent mortality. Under these conditions, at a high density, microtines suffer of food insufficiency. Usually, after the waves with a small amplitude, the reduction of the number takes place, more or less gradually, against the background of the decrease of the seasonal maximums, which is explained by the depletion of food resources. But after the significant peak phases the decrease is catastrophic and the density curve suddenly decreases.

The phase of increasing of rodent number follows the first phase after the improvement of living conditions in the refuge stations and outside them, this being a premise of increasing the viability of the population in these stations, increasing the density of groups, which stimulates dispersion. Outside the refuge sectors, the favorable conditions ensure the survival and intensity of the reproduction of individuals, which have dispersed.

The following factors contribute to the increase in the number of population: food supply in winter conditions, satisfactory food quality, large harvests, an optimal combination of rainfall and solar heat, which ensures the growth of crop and spontaneous plants, poor crops harvesting, reserve pastures sectors and agricultural fields, non-compliance with agrotechnical measures - shallow plowing and sowing of grass on stubble, weeding of fields and shrubs, an early spring without rapid melting, early start of plant growth, a late and warm autumn, which contributes to a second vegetation, optimal weather conditions for wintering, thick snow cover, which provides protection for microtines, but also preserves vegetation.

In this phase on the perennial grass fields the groups are made up of 2-3 colonies, which do not overlap. In late summer - early autumn the density on perennial grass fields increases to 80-100 colonies per hectare, and further – to 200-300 colonies per hectare. Survival increases from 33.7% in September to 58.6% in October, and the number of females of the late summer generation, which reproduce (67.4%), exceeds that of spring (35.1%). As a result, the groups disperse on the neighboring grass fields, where a density of 10-15 colonies per hectare was recorded.

The population is going through the peak phase. This is also a phase of preparation for the subsequent decline, which consists in stopping reproduction due to the physiological limit, but also stress.

Often the peak phase can take 1-2 years. The multi-annual peak phase, against the background of the large population wave, is registered under the influence of the land mosaicity.

Conclusions

It was established that the significant difference of the annual average capture coefficient in comparative plan for the maximum phase and the depression phase is $t = 5.6$, $P < 0.05$ and for the maximum phase and the growth phase – $t = 8.9$. This demonstrates that cyclic oscillation of the number is typical of small rodents.

It was determined that for *Microtus arvalis*, cyclic oscillations with an interval of 3-4 years are not typical, as can be seen in other parts of the area. Intense anthropogenic influence determines the type of numerical dynamics. A certain periodicity is observed in the species *M. spicilegus*, but in the last 30 years the dynamics becomes acyclic. In the populations of *A. sylvaticus* there was a more pronounced periodicity of the number oscillation – once every two years.

During the growth phase, in autumn the density of Microtus arvalis species on perennial grass fields increases up to 200-300 colonies per hectare. Survival increases from 33.7% in September to 58.6% in October, and the number of females of the late summer generation, which reproduce (67.4%), exceeds that of spring (35.1%).

The work was carried out within the State Program project 20.80009.7007.02.

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USING IN SILICO RFLP METHOD FOR THE STUDY OF MC1R GENE ALLELES IN THE SPECIES SUS SCROFA

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Abstract. In the present study we aimed to use a bioinformatics algorithm that predict RFLP fragmentation of swine MC1R alleles simulating the sequence digestion with over 700 restriction enzymes. The results show several restriction enzymes that have the potential to be used for genotyping of Sus scrofa individuals and for differentiation between hybrids and pure line wild boars (WB)/domestic pigs (DP). Genotyping studies of wild boars and domestic pigs in Moldova populations would allow the determination of introgression/backcrossing degree and would contribute to the elaboration of adequate conservation measures

Introduction

The *MC1R* is a gene that regulates melanin synthesis and in some animals plays a central role in the pigmentation process. It contains instructions for producing the protein *Melanocortin 1 Receptor* which is located in the melanocyte membrane. Sequencing analyzes showed that species *Sus scrofa* has several alleles of this gene. The multiple allelism of the *MC1R* gene can be observed phenotypically through the diversity of skin pigmentation in pigs. The wild-type allele named E^{+} has been identified in wild boars, E^{D1} allele – in domestic breeds Large Black and Meishan, E^{PD2} in Hampshire, Large White and Pietrain breeds [1,5], while recessive allele e is associated with the breed Duroc (Fig.1).

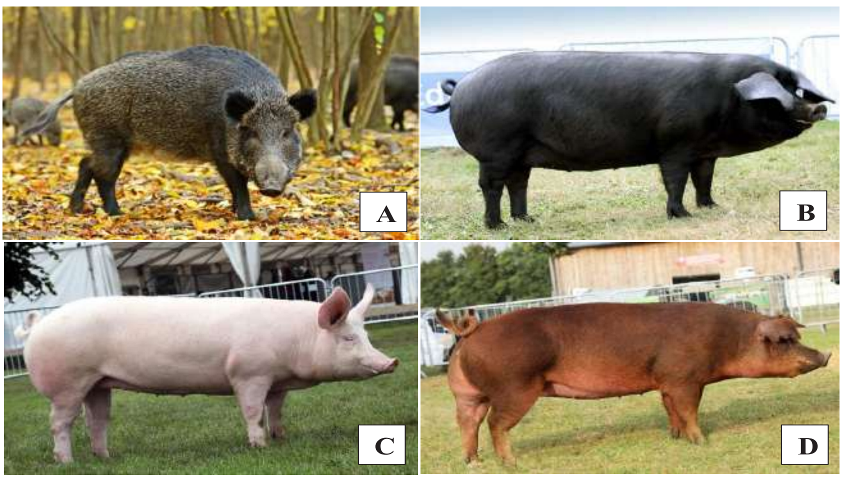

Figure 1. Phenotypic skin color traits in the species *Sus scrofa* with different *MC1R* alleles (A - Wild Boar [3], B - Large Black [4], C - Large White [4], D – Duroc [4])

The wild boar is the ancestor of the domestic pig and can successfully crossbreed with it. This can happen unintentionally in areas of open or semi-open swine farms or by intentional crossing. WB populations have been increasing worldwide, and in many countries it is considered a forest and agricultural pest [10]. A recent hypothesis suggests that introgressive hybridization between wild boar and domestic pig increases WB invasiveness [2]. Also, there are concerns that hybrids between wild and domestic animals may affect the genetic integrity and wild adaptive potential of WB. Currently, the degree to which WB and DP hybridization occurs in the Republic of Moldova is unclear.

Sequencing of the *MC1R* swine gene would be an extremely effective way for pig genotyping, however, even today it is not very accessible and remains an expensive molecular method. This makes necessary the use of cheaper alternatives for identification of pig hybrids. One of these alternatives would be the discrimination between sequences of interest by using *Restriction Fragment Length Polymorphism (RFLP)*. In the present study we aimed to use a bioinformatics algorithm that predict *RFLP* fragmentation of swine *MC1R* alleles simulating the sequence digestion with over 700 restriction enzymes.

Materials and methods

In the species *Sus scrofa*, the *MC1R* gene has 963 base pairs and wild-type allele (E+) is fully expressed in pure line wild boars. Other alleles differ by changes in the following positions [1,5]:

1. c.370G>A - Hampshire, Large White and Pietrain breeds (EPD2)
2. c.283G>A, c.305T>C and c.363T>C - Large Black and Meishan (ED1)
3. c.491C>T and c.727G>A - Duroc breed (e)

The wild-type *MC1R* sequence has been extracted in *FASTA* format from *NCBI* [6], and alleles alignment has been performed in *R language* [9] using *DECIPHER* [11] and *Biostrings* [7] packages (Fig.2).

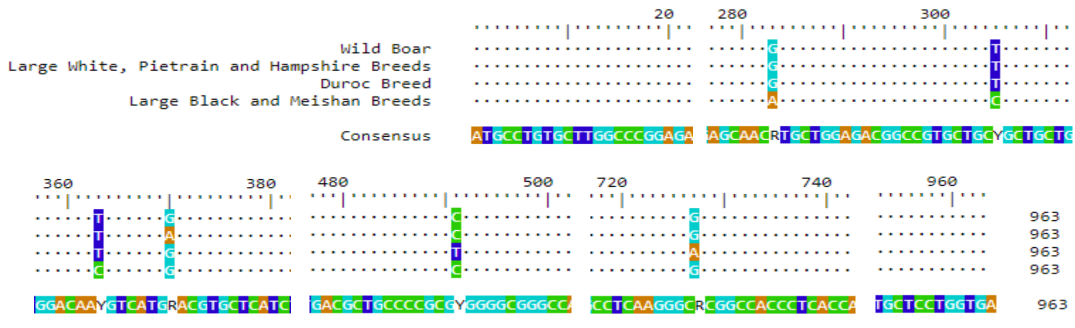

Figure 2. Swine MC1R alleles alignment

Simulation and visualization of *Restriction Fragment Length Polymorphism (RFLP)* patterns from *DNA* sequences were accomplished utilizing *seqRFLP* package [8] in R. This package includes functions for handling *DNA* sequences, especially for simulating *RFLP* patterns based on selected restriction enzymes and *DNA* sequences.

Results and discussions

In silico digestion of the studied sequences allowed the identification of several restriction enzymes that have the potential to be used for genotyping of Sus scrofa individuals and for differentiation between hybrids and pure line WB/DP. In Fig.3 is shown the simulated electrophoregram obtained after virtual digestion of studied allelic sequences with the next restriction enzymes: *AjiI, BspHI, BstUI, NspI, FnuDII, MhlI, PcsI, TspDTI*.

The enzymes shown in fig. 3 were selected following individual in silico analyzes of over 700 candidates and they can discriminate between sequences as follows:

1. *AjiI, BspHI and TspDTI* – distinguish among E^{PD2} and other alleles
2. *BstUI, FnuDII and MhlI* – distinguish among e and other alleles
3. *NspI and PcsI* – distinguish among E^{D1} and other alleles

Theoretically, it is expected that in the Republic of Moldova the predominant allele in pig farms is E^{PD2}, therefore the enzymes from the first category that could discriminate between this domestic allele and E+ wild-type are of increased interest. *AjiI (BmgBI)* candidate recognizes *CAC^GTC* sequences while *BspHI* cuts at *T^CATGA* sites, both enzymes having an optimum temperature of 37°C. Although *TspDTI* discriminates by producing two electrophoretic bands in E+ allele and three in E^{PD2}, it is more difficult to be found, that's why the first two candidates seem to be more practical. The pure lines of Large Black, Meishan and Duroc breeds are probably less common in Moldova, however, the enzymes from categories 2 and 3 present an interest for determination of possible heterozygotes, carriers of E^{D1} and e alleles. Other restriction enzymes that may be useful for differentiating *MC1R* variants are *BdaI, BstNSI, BtrI, CciI, FatI, PagI, RcaI, SduI, XceI*.

Figure 3. Simulated electrophoregram obtained after virtual restriction enzymes (AjiI, BspHI, BstUI, NspI, FnuDII, MhlI, PcsI, TspDTI) digestion of Sus scrofa allelic sequences

In the current study we used *MC1R* gene polymorphism for identification of swine genotyping possibilities with *in silico RFLP* method. Good results for differentiation between hybrids and pure lines could be achieved only by correct enzymes selection and by their combination. The combination would increase the chance of a correct result and would allow the identification of heterozygous individuals. Genotyping studies of wild boars and domestic pigs in Moldova populations would allow the determination of introgression/backcrossing degree and would contribute to the elaboration of adequate conservation measures. We recommend the wet laboratory testing of the above mentioned enzymes and the *in vitro* evaluation of their genotyping potential.

The study has been conducted within the State Program project 20.80009.7007.02.

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THE STATE OF MOLDAVIAN VIPER (VIPERA URSINII MOLDAVICA) IN THE CONTEXT OF THE ACTION PLAN FOR THEIR CONSERVATION (ANNEXED TO THE BERN CONVENTION)

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Abstract: Based on the generalization of data from previous publications and own results, regarding the distribution of Meadow Viper (Vipera ursini) in the Dniester - Prut interfluve, the current species state in the context of the Action Plan for conservation, annexed to the Bern Convention, is characterized. The need to complex study of local habitat status and to take measures for their conservation is discussed. The influence of some anthropogenic and climatic factors upon the habitats and the problems of their conservation in the current conditions of agrolandscape are analyzed.

Introduction

The meadow viper, *Vipera ursinii*, has a relict, post-glacial distribution and occurs as a series of isolated populations in limited areas of southern and central Europe. Five currently described subspecies (*Vipera ursinii ursinii, Vipera u. macrops, Vipera u.graeca, Vipera u.rakosiensis, Vipera u. moldavica*) are in severe decline. This species is now considered by herpetologists to be one of the most endangered snakes in Europe. Hungarian (*Vipera u.rakosiensis*) and Moldavian (*Vipera u. moldavica*) meadow vipers are now on the verge of extinction and, with only 13 and 4 known populations respectively, are amongst the most endangered of all vertebrate taxa in the European area. Therefore these species have been included in the conservation action plan. Species Action Plan has been commissioned by the Standing Committee of the Bern Convention in order to assess the extent of the problems faced by the meadow viper in Europe and to make appropriate recommendations for specific actions to address these problems. Taking into account the Moldova's commitments to the Berne Convention we mention that the study on situation of this species is quite actual and necessary. The originality of the data consists in the revision of the populations described about 50 years ago.

Material and methods

The paper presents the results of multiannual estimates made during field research on the spread of steppe viper in the Prut-Dniester area. Estimates were made by the route method during periods of maximum activity of the reptiles. The information obtained as a result of personal communications with the local population was also taken into account. As a result of the comparative analysis of the multiannual data obtained during this period and those of the publications of the previous authors, changes were identified, which took place in the herpetofaunistic complex of the last decades. The character of these changes was examined in faunal and archaeological aspect.

Rezults and discussion

The subspecies *Vipera ursini moldavica* was described [2] based on specimens collected from the right bank of the Prut river (Valea lui David, Iaşi County). The phylogenetic analysis of the representatives from different parts of the area showed that the specimens are distinguished by some morphological and genetic features, which allowed the authors to describe them as a separate subspecies and a population that has been genetically isolated for a long time in the region between the Carpathians and the Prut. On the right bank of Prut river several more local populations of this subspecies have been recorded, but they are on the verge of extinction and require urgent conservation measures [1, 3].

The existence of steppe viper populations in the Republic of Moldova described in the 1970's of the XX century [5] until now has not been confirmed, but the presence of the species is possible on some steppe sectors, which have been preserved in the Middle Prut Plain. This assumption is based on the fact that in 2003 a population of blotched snake (*Elaphe sauromates,* Palass 1814) was discovered here, which was also preserved in the landscape resrve "Suta de Movile" and the natural reserve "Pădurea Domnească" [9].

Possibly, this population is a "relict" from the former rich herpetofauna that once existed in the Bălţi steppe. It is isolated from the populations of southern Moldova at a distance of 300 km and represents the northern limit of the species area. Also, in the left Prut river basin (steppe area of Bălţi and Făleşti) there are many steppe sectors identical in terms of natural appearance and the degree of anthropogenic impact with those on the right Prut basin, populated by vipers. This fact increases the probability of the existence of *V.ursinii moldavica* in the Dniester-Prut interfluve.

The results of the study of the current spread of amphibian and reptile species in the Dniester-Prut interfluve show that the historical formation of the herpetofaunistic complex was determined by the landscape differentiation of this geographical area. The steppe areas of Balti and Bugeac are separated by the central forest area „Codrii", which is in fact an extension of the Central Moldavian Plateau. This differentiation largely determined the specific composition and spatial distribution of the species [7, 8]. The formation of the serpent fauna took place both on account of the forest forms (*E. longissima, C. austriaca, V. berus*) coming from the Carpathians, and of the steppe ones (*C. jugularis, E. sauromates, V. ursini*), which have spread from the Ponto-Caspian region, entering the Plain of the Middle Prut through its corridors and possibly through the corridors of Răut.

Being historically isolated in the Prut Plain, the populations of these species probably differ genetically, which allows us to consider the existence of the subspecies *V.u.moldavica* possible as well in the Republic of Moldova.

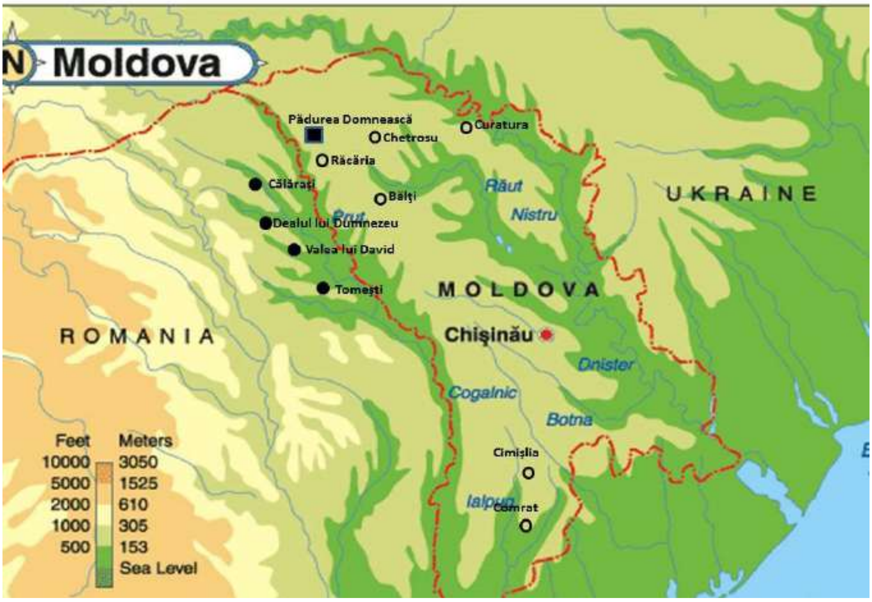

Figure 1 The populations of *V. ursini* described in the 1970 by Tofan (empty circle), those of existing *V.u.moldavica* (black circle) and *E. sauromates* (black square).

In Moldova, the steppe viper is considered an endangered species. The last data that confirm its existence refer to the population near Ciucur-Mingir locality from Cimişlia district (1970). Until 1960-70 it was considered a widespread species [6]. In other source information of 11 specimens collected in Moldova can be found [4]. These data show that in the last century the steppe viper was widespread in the Prut-Dniester area. Its area occupies the southern steppe area and the Bălţi steppe in the north of the country isolated from the Codri Plateau.

In the last 10-15 years, during field studies conducted in various ecosystems, including the steppe areas, the species was not found (but there were no special projects to search and assess the state of this species). Taking into account that in Moldova the local populations of some rare species (for example, *E. sauromates*) are still preserved in small areas, as well as the presence of steppe viper in the Middle Prut Plain in Romania, we assume that *V. ursinii* could have survived on the Dniester-Prut area as small populations. This requires special field research aimed at finding this particular species. In Bulgaria, where this species was considered extinct, it was recently found.

The decline of the species was determined by the drastic reduction of natural steppes by about 90%. The steppe areas of Balti and Bugeac almost entirely have been transformed into agricultural fields and the remaining sectors are small, isolated and have a degraded appearance due to excessive grazing.

In the point 2.7 of the Action Plan for the Conservation of the Meadow Viper (*Vipera ursinii*) in Europe it is indicated that research has been carried out in the Republic of Moldova on the spread of steppe vipers. However, it is necessary to mention that until the last years, not enough data have been accumulated to confirm the extinction of the species. Special field studies was conducted (Korsos Z., Ujvari B, Tsurcan V.) but not all potential sectors were covered. In point 4.2.6. it is recommended to carry out urgent research in the Republic of Moldova regarding the subspecies *V. u. moldavica*. That is why it is necessary to carry out a project aimed at elucidating the current state and developing recommendations for the detection, protection and conservation of the subspecies in existing habitats. In case of identification of local populations, the measures and recommendations provided in p. 3.2 will be taken. In point 4.6.1. it is recommended to practice in particular for Hungary, Romania and the Republic of Moldova the captive reproduction and reintroduction in nature of the steppe viper. This method will be used to recover depressed populations and repopulate potential habitats, where this species was once widespread.

Conclusions

The existence of steppe viper populations in the Republic of Moldova described in the 1970's of the XX century until now has not been confirmed, but the presence of the species is possible on some steppe sectors, which have been preserved in the Middle Prut Plain (landscape reserve "Suta de Movile" and natural reserve "Pădurea Domnească"), as well as in Bugeac steppe areas. Considering that in Moldova, the local populations of some rare species (for example, *E. sauromates*) are still preserved in small areas, as well as the steppe viper in the Middle Prut Plain in Romania, we assume that *V. ursinii* could have survived on the Dniester-Prut area as small population. The decline of the species was determined by the drastic reduction of natural steppes by about 90%. The steppe areas of Balti and Bugeac, almost entirely, have been transformed into agricultural fields and the remaining sectors are small, isolated and have a degraded appearance due to excessive grazing. That is why it is necessary to carry out a project aimed at elucidating the current state and developing recommendations for the detection, protection and conservation of the subspecies in existing habitats.

The work was carried out within the State Program project 20.80009.7007.02

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SUSTAINABLE USE AND PROTECTION
OF ANIMAL WORLD IN THE CONTEXT OF
CLIMATE CHANGE

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