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HELMINTH FAUNA OF AMPHIBIANS (AMPHIBIA), THEIR IMPORTANCE AS VECTORS IN THE FORMATION AND MAINTENANCE OF PARASITIC ZOONOSES

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The defense of the thesis will take place on June 20, 2025, at 14^{00}, within the meeting of the Thesis Public Defence Commission within the State University of Moldova, Institute of Zoology, Chisinau, 1 Academiei str., hall no. 352. (http://www.usm.md)
The doctor habilitatus thesis and the summary can be consulted at the Library of the Moldova State University, the National Library of the Republic of Moldova and on the website of ANACEC: (https://anacec.md/) and on the MSU website (http://usm.md/).

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CONTENT

CONCEPTUAL OUTLINES OF THE RESEARCH

Actuality and importance of the topic addressed.

Currently, although one of the fundamental concerns of contemporary science is the study of disease etiology, which involves understanding host-parasite interactions or relationships, few studies have addressed the joint effects of both host and parasites on the increased risk of outbreaks of parasitic agents, reflecting the growing interest in the biodiversity-disease relationship. The helminthological study in amphibians is important both in determining the helminthic fauna in amphibians and in elucidating the specifics of their circulation in natural and anthropized biotopes and their contact with the host, allowing to establish the parasitological status of some features in the pathogenesis of the formation of outbreaks of parasitic agents and to develop biological measures with epizootic and epidemiological impact to control helminthiasis [13].

In addition to the faunistic importance of research, amphibians are definitive hosts for several classes of helminths, including Cestoda, Monogenea, Trematoda, Secernentea, Adenophorea, Chromaderea and Palaeacanthocephala. In addition, amphibians also serve as intermediate or paratenic hosts for a wide variety of helminths common to fish, birds, mammals and human [10, 12, 21, 23, 24, 17]. In the context of assessing the sources of the spread of parasitosis in the Republic of Moldova, it is necessary to conduct a thorough and comprehensive study of the helminthic fauna in amphibians, and the results obtained will contribute to the identification of their role as bioindicators, in vectorization, formation and maintenance of various groups of parasitic agents, as well as to the development of measures for biological control of parasitic zoonoses.

Description of the situation in the research field and identification of research problems.

Over tens of millions of years, amphibians on the one hand and parasitic agents on the other have developed a variety of relationships. As each of these living components evolved, some of these relationships led to the establishment of the vector, which also involves a wide variety of organisms. Within these complex biological systems, amphibians act as a vector from one invertebrate/vertebrate to another, sometimes even to humans, thus actively participating in the formation and maintenance of outbreaks of parasitic agents, but this aspect both in the Republic of Moldova and in other regions of the world is very little studied.

The necessity of these researches is determined by their role as hosts, as obligatory in the development of parasitic agents, which constitute the favorable environment for the penetration, development and conservation of the evolutionary forms of parasitic agents, having a special role in the contamination of favorable areas for certain parasites, thus participating directly in the formation of parasitic zoonoses, or, on the contrary, contributing to the reduction of a biological risk of development of helminth development. Taking into account that helminthological research has been carried out only on 3 of the 13 amphibian species inhabiting different ecosystems of the Republic of Moldova, the realization of these researches will contribute to deciphering the mechanisms of formation of relationships in the parasite-host system, will establish and evaluate the role of amphibians in vectorization of parasitic agents, because some parasitic zoonotic zoonoses create serious social problems, since they carry the risk of spreading and transmission to humans through the helminth circuit in which amphibians play an important role.

The aim of the study to provide a comprehensive and detailed understanding of the eco-evolutionary peculiarities of the parasite-host system, using amphibians as on example, to assess their role as vectors in the formation and maintenance of outbreaks of parasitic zoonotic diseases, and to develop biological control measures.

Research objectives:

- Identification of the eco-ethological peculiarities of the annual and life cycle of amphibians as hosts of helminths.
- Determination of the diversity of helminth fauna in amphibians from different types of natural and anthropized ecosystems of the Republic of Moldova.
- Assessment of the degree of helminth infestation of amphibians depending on intrinsic and extrinsic factors.
- Determination of adaptation strategies in the parasite-host system in amphibians.
- Assessing the role of amphibians as bioindicators of ecosystems and as hosts for various groups of parasites common to fish, birds, mammals and humans.
- Establishing the importance of amphibians in the formation and maintenance of parasitic outbreaks.
- Development and implementation of biological measures for the control of helminths specific to livestock.

The methodology of the scientific research is based on the theoretical and scientific concepts presented in the works of specialists in the field [3, 4, 10, 13, 32, 33], on the basic principles of the study of ecology and helminthology of caudata and ecaudata amphibians, the evaluation of the main helminthological indices in relation to intrinsic and extrinsic factors [6], as well as the development of innovative biological methods of control of parasites specific to livestock.

Novelty and scientific originality. For the first time in the Republic of Moldova, a systematic and complex approach of amphibians from different types of ecosystems was carried out, finalized with:

- determination of the diversity of the helminth fauna of caudata and ecaudata amphibians in an eco-evolutionary aspect, which allowed to establish the functioning mechanisms in the host-parasite system, in the context of changing climatic factors on the example of amphibians;
- detection of 32 new helminth species for the fauna of the Republic of Moldova;
- evaluation of the degree of spread of helminths in the host population, depending on intrinsic and extrinsic factors, which allowed to establish the cyclic dynamics of parasitic invasion, as well as to identify the critical periods of transmission of parasitic agents from amphibians to other vertebrate groups (fish, reptiles, birds, mammals, humans);
- identification of the parasitological particularities of amphibians as vectors in depth and their importance in the circuit of various parasitic zoonoses common to synanthrope, domestic, wild, pet and human;
- to establish the role of amphibians in the formation and maintenance of outbreaks of dangerous parasitic agents as definitive, intermediate and paratenic hosts;
- development of a biological process for the control of fasciolosis, specific to livestock, with the effect of increasing their productivity, both qualitative and quantitative, by

means of amphibians.

The solved scientific problem consists in elucidating the role of caudata and ecaudata amphibians as true bioindicators of natural and anthropized, aquatic and terrestrial ecosystems populated by them, their importance in vectorization, formation and maintenance of outbreaks of parasitic zoonoses specific to wild animals, domestic, pet and human, in the determination of the formation of relationships in the parasite-host system and in the development of strategic and innovative conceptual methodology for the biological control of ruminant fasciolosis by amphibians.

The theoretical significance. From the theoretical point of view, the scientific results obtained in this work contribute significantly to the completion with new data of the many interdisciplinary aspects in the field of biological, ecological, helminthological sciences and biodiversity conservation in the Republic of Moldova. The determination of the helminthic fauna of amphibians (48 species of which 32 - new species) expands the level of knowledge of the national fauna - as representatives of the animal world. For the first time, the biological records of helminth species found in amphibians have been elaborated and their role as potential definitive, intermediate and paratenic hosts, which, for at least one larval stage, parasitize various animal species, has been determined. At the same time, the scientific results presented in the paper may allow the determination of the formation of relationships in the parasite-host system on the example of amphibians, as well as the establishment of strategies for the functioning of helmintocenosis under conditions of instability of climatic factors.

The applied value of the work consists in the essential contribution in the epizootic evaluation of natural and anthropized, aquatic and terrestrial biotopes populated by amphibians, and as a basis for the development and implementation of biological measures for control and prophylaxis of helminthiasis specific to livestock with impact on the development of the national economy. They will also serve as a basis for state institutions, in the field, to strengthen the legislation of the Republic of Moldova in the field of protection and sustainable utilization of the diversity of the animal world.

Implementation of scientific results. Amphibian helminth preparations complete the collection of the Laboratory of Parasitology and Helmintology of the Institute of Zoology of the SUM and represent a scientific support for researchers, students and teachers. The scientific results are presented in 6 implementation acts, realized within the National Agency for Food Safety, used and implemented in the teaching process at the Faculty of Biology and Chemistry of the State Pedagogical University "Ion Creangă", at the Faculties of Veterinary Medicine of the Technical University of Moldova, the University of Life Sciences of Iasi, Romania and the University of Agronomic Sciences and Veterinary Medicine, Bucharest, Romania, as well as in the public institution - Orhei National Park of the subdivision of the Ministry of Environment of the Republic of Moldova.

Approval of scientific results. The scientific results of the paper have been presented, discussed, reviewed and accepted in various national and international scientific meetings.

Publications on the thesis topic. Based on the results of scientific research, 115 scientific papers are published (20 of mono-authorship), among which: 7 monographs, 3 chapters in monographs, 5 articles in journals included in the Web of Science database, 3 articles in foreign journals in the ANACEC database, 11 articles in category B journals, 5 articles in international collections, 37 articles in national collections, 25 materials of international scientific communications and 18 materials of national scientific communications, 1 invention patent.

The volume and structure of the thesis. The materials of the thesis are presented on 257 pages of basic text divided into: introduction, literature review, results of own investigations presented in 6 chapters, general conclusions and practical recommendations, annotations in Romanian, Russian and English. Bibliography includes 404 bibliographical sources, 168 appendices. The content of the thesis is illustrated with 48 tables and 63 figures.

Keywords: amphibians, hosts, ecology, helminth fauna, taxonomy, vector, parasitic zoonoses, importance, biological control.

1. ECO-EVOLUTIONARY ANALYSIS OF THE ANNUAL AND LIFE CYCLE OF AMPHIBIANS, THE DIVERSITY OF HELMINTHIC FAUNA AND THEIR IMPORTANCE AS VECTORS IN THE FORMATION AND MAINTENANCE OF PARASITIC ZOONOSES OUTBREAKS

This chapter presents a summary analysis of the most current national and international scientific publications on the diversity of the helminth fauna of caudata and ecaudata amphibians, on the principles and eco-evolutionary peculiarities describing the mechanisms of formation and maintenance of host-parasite relationships in the amphibian host-parasite system in order to improve the quality of the environment, animal and human health.

2. MATERIALS AND RESEARCH METHODS

2.1. Study area. Research methods on the biology, ecology and ethology of amphibians throughout their life cycle

The batraco-helminthological investigations were carried out in the period 2013-2024, in the Laboratory of Parasitology and Helminthology of the Institute of Zoology of the USM. The results of scientific researches carried out on 12 amphibian species, including: 2 species of caudata amphibians (*Lissotriton vulgaris, Triturus cristatus*) and 10 species of ecaudata amphibians (*Pelophylax ridibundus, P. lessonae, P. esculentus, Rana dalmatina, R. temporaria, Bufo bufo, Bufotes viridis, Bombina bombina, Hyla arborea, Pelobates fuscus*) are the basis of the thesis. The study area includes 16 districts of the Republic of Moldova: Nisporeni, Calarasi, Telenesti, Soldanesti, Orhei, Straseni, Ungheni and Hincesti in the *Central area*, Glodeni, Singerei, Briceni, Balti and Donduseni districts in the *Northern area* and Cahul, Taraclia and Stefan-Voda districts in the *Southern area of the* country.

The research methodology consisted in the application of standard classical methods to determine the amphibian species mentioned by classical deductive methods [3, 12, 28], as well as to investigate pre-reproductive and post-reproductive migrations, habitat distribution, population frequency and density [4].

2.2. Helminthological research methods in amphibians. Techniques for collecting and determining helminths in amphibians.

In order to carry out helminthological researches, caudate and ecaudate amphibians of two age classes were evaluated: *pre-reproductive*: embryos, larvae (n=3361 ex.), juveniles (n=3436 ex.) and *reproductive*: adults (5711 ex.) of which males (n=2999 ex.) and females (n=2712 ex.).

Methods used in the diagnosis of parasitic agents in amphibians were of two types: *direct* and *indirect* [10, 35]. The internal organs were examined by *wet* and *dry* methods [10]. The morphology of trematodes, nematodes, acanthocephalans and monogeneans was studied on total preparations and on live material under the Novex Holland B series" microscope with objectives 20 - 40 and WF 10X DIN/20MM, LABORLUX D and ZEISS AXIO Imager A 2 eyepiece WF 10X DIN/20MM, LABORLUX D and ZEISS AXIO Imager A 2.

Helminth staining and fixation. For the morpho-structural study of the parasitic agents for their determination, their staining with *Carmine boric (alcoholic) Carmine* was performed [10, 30, 32-34, 36].

2.3. Biological control of parasitic zoonoses

Research for biological control of fasciolosis in ruminants has been carried out using amphibians [26].

2.4. Mathematical and statistical processing of the results obtained

For taxonomic determination of helminth species detected in amphibians (trematodes, nematodes, acanthocephalans, monogeneans, adenophores, chromadoreans) the main species-specific morphometric parameters were calculated using central tendency indicators (mean) and indicators of data dispersion around the mean (variance, standard deviation, standard error, coefficient of variation) [6].

In order to quantify the characteristic of helminth contamination of amphibians and to obtain information on the spread, severity, distribution of helminths in the host population (in amphibians) and to determine the impact on the host, the main helminthological indices of intensity (*II, individuals*) - the minimum and maximum number of parasites of a species and extensivity (*EI, %*) - the percentage of host contamination by a parasite species [29] were evaluated.

In order to quantify seasonal fluctuations in parasite prevalence or intensity, *the Seasonality Index* (*Is)* was calculated. In order to describe and assess the interactions and associations between parasites and different age structures of amphibians, the *Pearson* correlation coefficient (r_{xy}) between the parasite variable and the body mass of amphibian larvae and juveniles, as well as the body mass of adult forms of amphibians was calculated [6].

In order to establish the veracity of the data, mathematical and statistical analysis methods were used using the program package Statistic Workbook 12, Microsoft Excel Worksheet 2021, and the schematic interpretation of the morphology of the parasites was performed using the program CorelDROW Graphics Suite X4.

3. ECO-EVOLUTIONARY PARTICULARITIES OF THE ANNUAL LIFE CYCLE OF CAUDATA AND ECAUDATA AMPHIBIANS AS A SOURCE OF EXISTENCE AND EVOLUTION OF HELMINTHS IN THE CONDITIONS OF MOLDOVA

3.1. Phases of the annual life cycle of amphibians: hibernation, reproductive migrations and reproduction

Amphibians, in the course of a long evolutionary process, have evolved particular strategies for survival and utilization of their two characteristic environments - aquatic and terrestrial -, which manifest themselves differently during the annual and life cycle. Thus, the results reflect a detailed description of the annual cycle of caudata and ecaudata amphibians, applying as a basic research methodology - synecological analysis, which allowed us to highlight also the most susceptible life stages of amphibians for helminth infection.

Hibernation. As the diurnal temperature drops below +7^{0}C (October-November), the amphibian species assessed go into hibernation, first the adults, then (after 15-20 days) the young. The first species to hibernate are thermophilic species (*Hyla arborea, Bombina bombina, Bufotes viridis*), followed by other species. Amphibians, after all, overwinter in terrestrial habitats (with the exception of the *Pelophylax eesculenta* complex - *Pelophylax lessonae*, *Pelophylax ridibundus, Pelophylax esculentus* - which overwinter in water). The hibernation period is long and varies depending on the species: from about 140 days (*Rana temporaria* and *R. dalmatina*) to about 160 days (*Hyla arborea, Bombina bombina*). Adult males usually emerge from hibernation first, followed by females after 10-15 days. This is a phenomenon that has developed and consolidated over the course of evolution, due to the fact that the males are the first to move in spring to the aquatic pools (species of amphibians that hibernate on land) or (*Pelophylax* complex) to the mating stations of the same aquatic pool where they overwinter - which do not coincide with their wintering grounds.

During the field surveys, carried out between October and November, an original phenomenon of movement of adult individuals (*Bubo bufo, Hyla arborea*) by means of *partial pre-reproductive migrations*, determined by the ecological optimum, was recorded.

Breeding migrations. For each species, this phase of the annual life cycle depends on certain species-specific characteristics and their degree of ecological plasticity. From this point of view, *mass migrations* are present in only a few species of native fauna: *Bufo bufo* and, partially, in *Rana temporaria*. Thus, in *Bufo bufo* during March-April, but no more than 3-5 days in a row, three categories of individuals were detected: already formed pairs, solitary males and solitary females (Figure 3.1).

The other amphibian species belong to the thermophilic category, which is why they come out of hibernation later and their rate of migration to the breeding pools differs greatly from that of northern species in that they come out of hibernation later than the other species, do not migrate en masse, but move solitarily to the breeding pools, as the actual breeding period is also longer (up to 1.5 months).

According to long-term field research it has been established that amphibians use some and the same aquatic pools for breeding, which leads to a high survival risk of these populations with so-called "hoomyng" behavior, compared to their breeding pools.

Depending on the types of breeding pools, the amphibian species assessed fall into two broad ecological categories:

a) species using constant/ perennial pools;

b) species that use temporary aquatic pools.

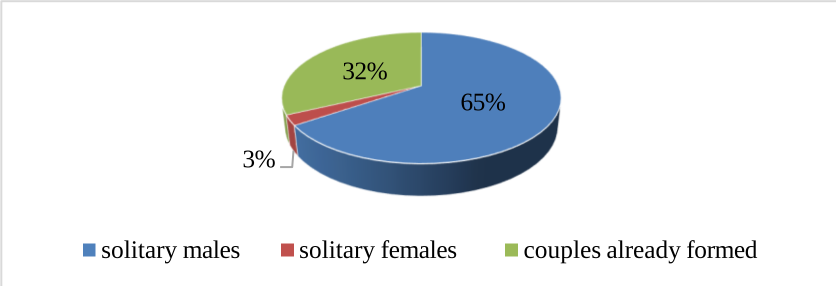

Fig. 3.1. Categories of individuals *Bufo bufo* heading to breeding pools

The first ecological category includes the species *Bufo bufo*, *Rana temporaria, Pelophylax ridibundus* and, partially, *Hyla arborea, Triturus cristatus, Rana dalmatina,* which are ***k-strategi*** species with a relatively constant and quite numerous population density.

The second ecological category includes the species *Rana dalmatina, Pelobates fuscus, Lissotriton vulgaris, Bombina bombina, B. variegata, Bufotes viridis* being ***r-strategi*** species with a high population only in favorable years.

3.2. Mating, oviposition, embryonic and larval development

Acupulation and oviposition. The assessed amphibians are characterized by ***amplexus*** (Ecaudate) and ***tandem*** (Caudate) acouplature. Depending on the specifics of oviposition, the 12 species of amphibians assessed are divided into: *species with simultaneous oviposition* and *species with gradual oviposition*. In the first case, the eggs consisting of about 3-14 thousand eggs (*Bufo bufo*, *Bufotes viridis*, *Rana dalmatina*), and in the second case, the eggs are laid in several smaller intact clutches (*Hyla arborea*) or even one (*Triturus cristatus*).

According to the obtained results, we have established that the process of oviposition over a relatively longer period of time has an obvious ecological and evolutionary explanation. Thus, firstly, it is due to the arrival of females in reproductive pools in turn and represents an efficient adaptation to protect eggs from predators (dragonfly larvae, dragonfly larvae, puff-hoppers, etc.) or even from individuals of other amphibian species (*Triturus cristatus*). On the other hand, the gradual laying of eggs leads to differentiation of larvae according to the rate of individual development, resulting in the emergence of larval populations of different ages, so that this process of differentiation leads to a fundamental decrease in trophic competition.

Embryonic and larval development. The embryonic development of amphibians is short and varies from one population to another, ranging from 14 days (*Triturus cristatus*, *Lissotriton vulgaris*) to 19 days (*Rana dalmatina*, *Bufo bufo*, *Bufotes viridis*) with three characteristic stages: *early embryonic stage* (10 hours old), *late embryonic stage* (11 days old), *and final embryonic stage* (15 days old).

According to scientific data on the embryonic development of amphibians, we have found that embryos, thanks to their specific adaptations during evolution, do not enter into intraspecific competition or, at least, show a minimal degree of this.

Larval development - according to the duration, rhythm and specificity of realization - varies from species to species, which, as a rule, goes through 5-6 characteristic stages. According to the duration of the larval development period, the amphibian species evaluated are divided into: *species with short larval development* (*Bufo bufo,* partially, *Bufotes viridis*), *species with medium larval development* (*Rana temporaria, R. dalmatina, Bombina bombina, B. variegata*, *Pelophylax esculenta* complex) and *species with long larval development* (*Triturus cristatus, Lissotriton vulgaris, Pelobates fuscus*).

3.3. Reproductive success and parental input

After analyzing the annual reproductive success of amphibians, we determined that the species *Triturus cristatus* has the highest relative value of this biotic parameter, which is extremely important for the future survival of the species, which is 5.3%. The species *Hyla arborea*, *Bufo bufo* and *Bufotes viridis* have a medium reproductive success, equal, respectively, to 2.5%, 1.8% and 1.0%, and the species *Rana dalmatina* is characterized by the lowest reproductive success - 0.9%.

According to parental input, the amphibians assessed are divided into *a)* species with high parental input, where the size of juveniles is 45% of the body size of mature individuals (*Triturus cristatus*); *b)* species with medium parental input, where the size of juveniles is 25- 29% of the size of parents (*Rana dalmatina, Hyla arborea*), *c)* species with low parental input, where the size of juveniles is 15-17% of the size of parents (*Bufo bufo, Bufotes viridis*).

Therefore, it is absolutely indispensable to study in detail the annual life cycle of amphibian species that are recognized and hunted as helminth host species, because it constitutes a sound scientific conceptual basis for elucidating the mechanism by which the an example.

4. HELMINTH FAUNA OF CAUDATA AND ECAUDATA AMPHIBIANS IN THE ECOSYSTEMS OF THE REPUBLIC OF MOLDOVA. TAXONOMY AND BIOLOGICAL CYCLES

On the territory of the Republic of Moldova the diversity of helminthic fauna in domestic (cattle, sheep, goats, birds, etc.) and wild (wolves, foxes, micromammals, deer, birds, etc.) vertebrate animals registers more than 200 species of parasitic agents, some of which have been recorded in humans [2, 7, 16, 22, 27]. Of these, 48 species have been recorded in caudate and ecaudate amphibians throughout the country.

In the context of the assessment of the state of ecosystems and the establishment of the sources of the spread of parasites in domestic and wild animals and humans on the territory of the Republic of Moldova, we have conducted a comprehensive batraco-helminthological study of all species of caudate and ecaudate amphibians from various terrestrial and aquatic habitats, natural and anthropized habitats of the Republic, which will significantly contribute to the identification of their role as definitive, intermediate and paratenic hosts, as vectors, but also in the formation and maintenance of outbreaks of dangerous parasitic agents.

Exposure and description of data on the taxonomic approach to the structure of the helminth fauna in amphibians with detailed description of all species at all taxonomic levels (species, genus, subfamily, family, superfamily, subordinate, order, class, and phylum) with detailed description of the etiology of each species of parasitic agents, their life cycles, geographic distribution, and the ecology of invasion complexes in relation to the host, under conditions of instability and constantly changing climatic factors, will contribute directly to the understanding of the mechanism of formation and maintenance of host-parasite relationships in the host-parasite system, using amphibians as an example.

4.1. Etiology, taxonomy and life cycles of monogeneans

Monogeneans are parasitic agents with the simplest life cycle of all parasitic flatworms. They have no intermediate hosts and are parasites of amphibians and fish. Although they are hermaphrodites, the male reproductive organs come into function before the female. A ciliated larva called oncomiracidia, with numerous posterior hooks, emerges from the eggs. This is the life stage responsible for host-to-host transmission.

The study of the helminth fauna in caudata (*Pelophylax ridibundus, P. lessonae, P. esculentus, Rana dalmatina, R. temporaria, Pelobates fuscus, Hyla arborea, Bombina bombina, Bufo bufo, Bufotes viridis*) and ecaudata (*Triturus cristatus, Lissotriton vulgaris*) amphibians revealed the presence of a single monogenean species.

In this subchapter the species *Polystoma integerrimum* Froelich, 1791 is described with reference to its taxonomic classification, synonyms, hosts, its localization in the host organism, its distribution, etiology, for which the original schematic layout of the species is also given with the description of the structure and morphometric parameters (body length and width, average hook length, length and width of eggs), which were subjected to mathematical and statistical analysis by evaluating indicators of central tendency (mean) and indicators of dispersion of data around the mean (variance, standard deviation, standard error, coefficient of variation), life cycle, volume, habitat from which the material was collected, and the literature consulted (Figure 4.1).

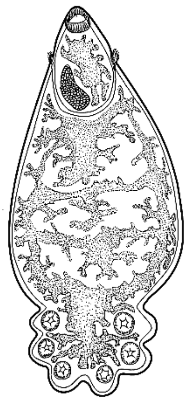

Fig. 4.1.
P. integerrimum.
The general aspect.
Orginal.

4.2. Etiology, taxonomy and life cycles of trematodes

The helminthological researches carried out on amphibian species from the whole territory of the Republic of Moldova show that they are infested with 30 species of digenean trematodes: *Opisthioglyphe ranae* Fröhlich, 1791, *Haematoloechus variegatus* Rudolphi, 1819, *Cephalogonimus retusus* Dujardin, 1845, *Gorgodera varsoviensis* Ssinitzin, 1905, *Pleurogenes claviger* Rud., 1819, *Candidotrema loossi*, Africa, 1930, *Pleurogenoides medians* Olsson, 1876, *Prosotocus confusus* Looss, 1894, *Diplodiscus subclavatus* Pallas, 1760, *Codonocephalus urniger* Rudolphi, 1819, *Gorgoderina* *vitelliloba* (Olsson, 1876) Ssinitzin, 1905, *Haplometra cylindracea* Zeder, 1800, *Macrodera longicollis* Abild, 1788, *Paralepoderma cloacicola* Luhe, 1909, *Paralepoderma brumpti* Buttner, 1951, *Plagiorchis elegans* Rudolphi, 1802, *Metaleptophallus gracillimus* Luhe, 1990, *Telorchis stossichi* Goldberger, 1911, *Holostephanus volgensis* Sudarikov, 1962, *Pharyngostomum cordatum* (Diesing, 1850) Ciurea, 1922, *Tylodelphys excavata*, Rudolphi, 1803, *Neodidiplostomum spathoides* Dubois, 1931, *Neodiplostomum major* Dubinina, 1950, *Neodiplostomum corvinum* Dubinina et Kulakova, 1960, *Strigea falconis* Szidat, 1928, *Strigea sphaerula*, Rudolphi, 1803, *Parastrigea robusta* szidat 1928, *Clinostomum complanatum* Rudolphi, 1819, *Opisthodiscus diplodiscoides* Cohn, 1904, *Isthmiophora melis* Schrank, 1788. Of the 30 trematode species established in amphibians, 20 are new species for the fauna of the Republic of Moldova (Figure 4.2 - 4.21).

Fig. 4.2.
G. vitelliloba.
The general aspect. Orginal.

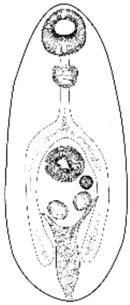

Fig. 4.3.
***H. cylindracea.* The general aspect. Orginal.**

Fig. 4.4.
M. longicollis.
The general aspect. Orginal.

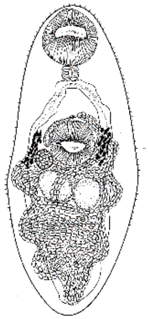

Fig. 4.5.
***P. cloacicola.* The general aspect. Orginal.**

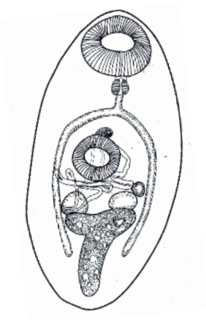

Fig. 4.6.
P. brumpti.
The general aspect. Orginal.

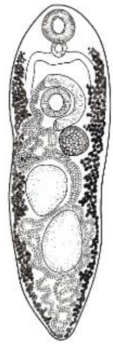

Fig. 4.7.
***P. elegans*.**
The general aspect. Orginal.

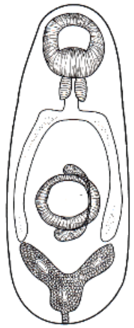

Fig. 4.8.
***M. gracillimus.* The general aspect. Orginal.**

Fig. 4.9.
T. stossichi.
The general aspect. Orginal.

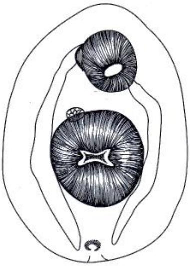

Fig. 4.10.
H. volgensis.
The general aspect. Orginal.

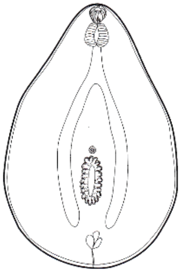

Fig. 4.11.
P. cordatum.
The general aspect. Orginal.

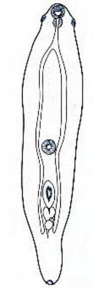

Fig. 4.12.
T. excavata.
The general aspect. Orginal.

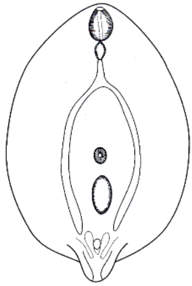

Fig. 4.13.
N. spathoides.
The general aspect. Orginal.

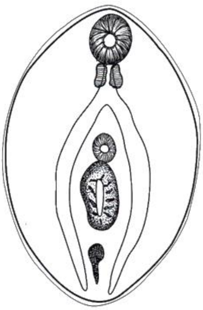

Fig. 4.14.
N. major.
The general aspect. Orginal.

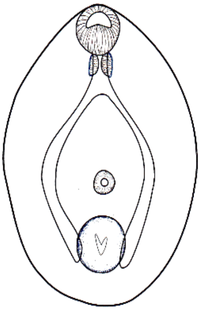

Fig. 4.15.
***N. corvinum*.**
The general aspect. Orginal.

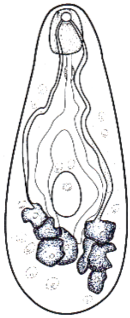

Fig. 4.16.
S. falconis.
The general aspect. Orginal.

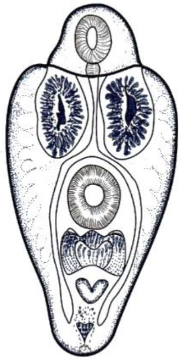

Fig. 4.17.
S. sphaerula.
The general aspect. Orginal.

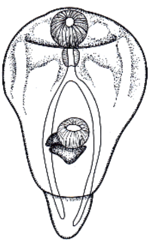

Fig. 4.18.
P. robusta.
The general aspect. Orginal.

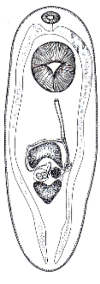

Fig. 4.19.
C. complanatum.
The general aspect. Orginal.

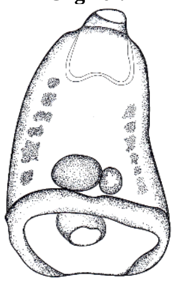

Fig. 4.20.
O. diplodiscoides.
The general aspect. Orginal.

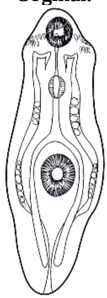

Fig. 4.21.
I. melis.
The general aspect. Orginal.

4.3. Etiology, taxonomy and life cycles of nematodes

Amphibian nematodes comprise five main groups: Enoplida, Oxyurida, Strongylida, Ascaridida and Spirurida. The systematics, geographic distribution, and host diversity indicate parasitic relationships that existed long before the emergence and evolution of tetrapods. Some groups of parasitic agents' amphibian-specific, such as Enoplida, have been acquired from other vertebrate host groups, which is a common and current theme in the evolution of new nematode taxa. The majority of amphibian nematodes evolved primarily in these hosts, probably in the early Cenozoic. Ascaridids in amphibians evolved in the mid-Mesozoic, and are currently the most primitive representatives (Cosmocercoidea) found in anurans [1].

The helminthological researches carried out on caudata and ecaudata amphibians from the entire territory of the Republic of Moldova reveal their infestation with 12 species of nematodes: *Cosmocerca ornata* Dujardin, 1845; *Oswaldocruzia filiformis* Goeze, 1782; *Oswaldocruzia duboisi* Ben Slimane, Durette-Desset & Chabaud, 1993; *Icosiella neglecta* Diesing, 1851; *Spirocerca lupi*, larva Rudolphi, 1809; *Spiroxys contorta*, larva Rudolphi, 1819; *Physocephalus sexalatus*, larva Molin, 1860; *Ascarops strongylina*, larva Rudolphi, 1819; *Agamospirura sp. II*, larva; *Hystrichis tricolor* Dujardin, 1845, larva; *Toxocara canis* Werner, 1782, L3 larva; *Rhabdias bufonis* Schrank, 1788. Of the 12 nematode species established in amphibians, 9 are new to the fauna of the Republic of Moldova (Figure 4.22 - 4.29).

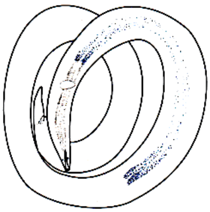

Fig. 4.22.
S. lupi.
The general aspect.
Orginal.

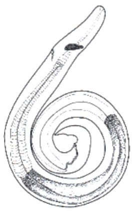

Fig. 4.23.
P. sexalatus.
The general aspect.
Orginal.

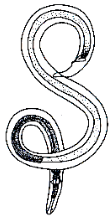

Fig. 4.24.
A.strongylina.
The general aspect.
Orginal.

Fig. 4.25.
S. contorta.
The general aspect.
Orginal.

Fig. 4.26.
T. canis.
The general aspect.
Orginal.

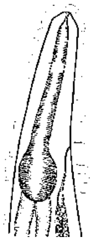

Fig. 4.27.
R. bufonis.
The general aspect.
Orginal.

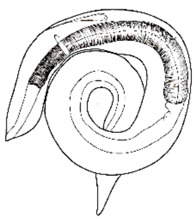

Fig. 4.28.
Agamospirura sp. II.
The general aspect.
Orginal.

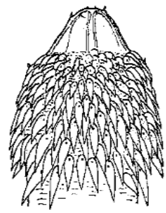

Fig. 4.29.
H. tricolor.
The general aspect.
Orginal.

Therefore, the importance of describing the diversity of the nematode fauna in amphibians, by identifying the main morphometric parameters with the elaboration of biological records and detailed description of their life cycle, makes it possible to understand the specifics of their movement in natural and anthropized biotopes, but also their contact with the host, which allows to assess the degree of knowledge of a particular group of hosts (definitive, intermediate, complimentary and reservoir) in a given area, the nature of the distribution of this group of parasitic agents depending on the hosts and territory, the seasonal dynamics of the formation of parasitic zoonoses, as well as the general parasitological situation, the pathogenesis of the formation of outbreaks of parasitic agents and the development of measures with epizootic and epidemiological impact.

4.4. Etiology, taxonomy and life cycles of acanthocephalans

Although there are some widely distributed species of acanthocephalans across the globe reported from various vertebrate groups, amphibians are hosts to a small number of species of acanthocephalans, for which amphibians most commonly serve as intermediate or paratenic hosts [19].

Among the species of acanthocephalans that have been reported in the caudata and ecaudata amphibians of the natural and anthropized ecosystems of the Republic of Moldova, the presence of 5 species was established: *Acanthocephalus ranae* Schrank, 1788; *Acanthocephalus lucii* O.F. Müller, 1776; *Pseudoacanthocephalus bufonis* Shipley, 1903; *Sphaerirostris teres*, larva Rudolphi, 1819; *Centrorhyncus aluconis* Müller, 1780. Of the 5 species of acanthocephalans established in amphibians, 3 are new species for the fauna of the Republic of Moldova (Figure 4.30 - 4.32).

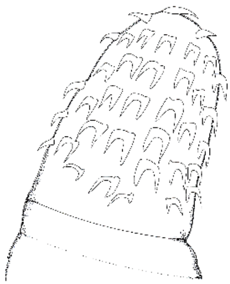

Fig. 4.30.
***A.lucii*.**
The general aspect. Orginal.

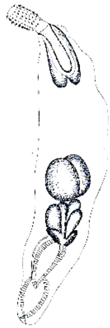

Fig. 4.31.
P. bufonis.
The general aspect. Orginal.

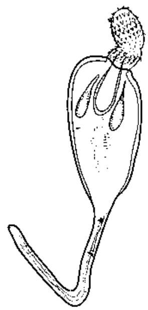

Fig. 4.32.
C. aluconis.
The general aspect. Orginal.

According to the study of the life cycle of helminth species, the role of amphibians as definitive hosts for 41.7% of species, intermediate hosts - for 41.7% of species and paratenic hosts for 16.6% of species was determined.

At the same time, it was found that the helminths detected in amphibians according to the evolutionary cycle are monogenean species in 14.58% of cases, digenean - in 16.67% of cases, trigenic - in 60.4% of cases and tetragenic - in 8.3% of cases.

4.5. Organic specificity of helminth elements (eggs, larvae, adults) depending on the host species

The issue of parasite specificity is of concern to many parasitological researchers and is very important not only for resolving problems related to the evolution and phylogeny of both hosts and parasites, but also for practical problems related to the control of different zoonotic parasitic agents [25].

The helminthological surveys carried out on caudata and ecaudata amphibians (*Triturus cristatus, Lissotriton vulgaris, Pelophylax ridibundus, P. lessonae, P. escuelntus, Rana dalmatina, R. temporaria, Hyla arborea, Pelobates fuscus, Bufo bufo, Bufotes viridis, Bombina bombina*) during the period 2013-2024 shows that the detected helminths have a very varied organic specificity so that they are detected in stomach, small intestine, large intestine, lungs, liver, gonads, body cavity, limb muscles, submandibular muscles, abdominal muscles, heart, brain, spinal cord, bladder, etc.

As a result of the helminthological investigations of the species of caudata and ecaudata amphibians depending on the organic specificity of the invasion, it was established that the parasitic agents, in their biological cilium, manifest certain habitat preferences in relation to the age structure of their hosts.

Thus, in adult forms of amphibians out of the 47 species of helminths detected 44.7% of the species have organic specificity in a single organ, 38.3% of the species have organic specificity in 2 organs, 10.6% - 3 organs, 4.3% - 4 organs, and 2.1% of the species inhabit 6 amphibian organs. In amphibian juveniles of the 33 helminth species detected, 72.8% of species have specificity in only one organ, 18.2% - 2 organs, 18.2% - 2 organs, 3.0% - 3 organs, 3.0% - 3 organs, 3.0% - 4 organs, and 3.0% of helminth species were established in 8 organs (Figure 4.33).

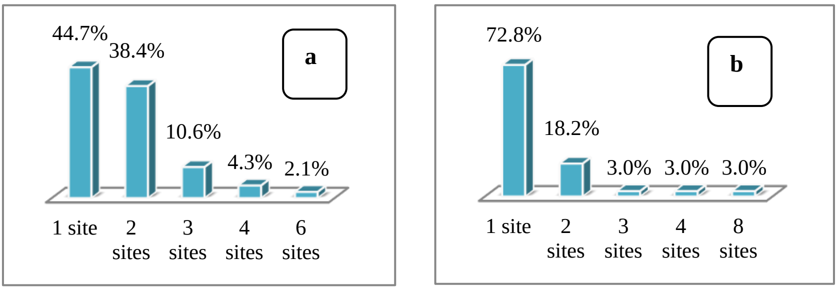

Fig. 4.33. Plurality of organic specificity of helminth helminths in amphibians: a - adult forms of amphibians, b - amphibian juveniles.

In amphibian larvae, all 12 helminth species were localized to only one site.

Therefore, according to the scientific results obtained on the organic specificity of helminths it has been established that the habitational variety of invasion is not only conditioned by the host and the host environment, but is largely due to the rapidity with which it develops, or the faster the sexual maturation, the lower the organic specificity of invasion. Where sexual maturation of the parasitic agent is slower or of longer duration then the organic specificity of invasion is also higher in the amphibian organism.

At the same time, it was determined that the localization of parasitic agents in various organs and organ systems of amphibians of different age structures represents a threat to them, which demonstrates the necessity of studying the helminth fauna in the ecology of amphibians as potential vectors and in their conservation as limiting factors.

5. FACTORS INFLUENCING THE HELMINTH FAUNA AND THE SPECIFICITY OF MANIFESTATION IN THE HOST POPULATION DEPENDING ON INTRINSIC AND EXTRINSIC FACTORS

The distribution and dynamics of parasite occurrence in a given environment, over time and in different hosts, as well as the factors regulating the relationship between host and parasite at the individual or population level, is a rather complex study with in-depth consideration of various biological and ecological aspects of organisms. Despite significant scientific advances, parasites are still widespread in all productive animal species, causing significant losses of meat, milk, wool, eggs and other animal products [7, 13].

5.1. The degree of helminth infestation of caudata and ecaudata amphibians in the ecosystems of the Republic of Moldova

In order to determine the structure of the helminth fauna in caudata and ecaudata amphibians, their infestation with 48 helminth species was detected.

According to the data obtained on the host species *Pelophylax ridibundus*, it was determined that it was infested with 43 helminth species, including 26 species of trematodes, 12 species of nematodes and 5 species of acanthoccephalans.

Quantitative analysis of parasitological indices in *P. ridibundus* species shows an increased variability in the degree of helminth infestation throughout the life cycle. Thus, infestation with the trematode species *O. ranae* was established in 23.77 ± 4.11% of cases (*II* - 28.93 ex.), *H. variegatus* in 13.70 ± 0.18% of cases (*II* - 3.87 ex.), *C. retusus* in 1.03 ± 0.65% of cases (*II* - 13.25 ex.), *C. retusus* in 1.03 ± 0.65% of cases (*II* - 13.25 ex.), H. *variegatus* in 13.70 ± 0.18% of cases (*II* - 3.87 ex.), *C.* spp.), *G. varsoviensis* in 3.10 ± 0.07% of cases (*II* - 2.17 ex.), *G. viteliloba* in 0.78 ± 0.38% of cases (*II* - 10.00 ex.), *P. claviger* in 0.78 ± 0.22% of cases (*II* - 6.00 ex.), *C. loossi* in 2.07 ± 0.85% of cases (*II* – 14.75 ex.), *P. medians* in 6.98 ± 1.00% of cases (*II* – 14.63 ex.), *P. confusus* in 13.95 ± 0.76% of cases (*II* – 15.87 ex.), *D. subclavatus* in 34.88 ± 0.58% of cases (*II* – 7.60 ex.), *O. diplodiscoides* in 0.26% of cases (*II* – 2.00 ex.), *C. urniger*, mtc. in 8.01 ± 1.01% of cases (*II* – 17.00 ex.), *P. robusta*, mtc. in 1.29 ± 1.87% of cases (*II* – 30.80 ex.), *S. sphaerula*, mtc. in 3.36 ± 1.59% of cases (*II* – 36.77 ex.), *H. cylindracea* in 7.49 ± 1.13% of cases (*II* – 26.21 ex.), *P. elegans* in 6.72 ± 1.37% of cases (*II* – 27.35 ex.), *N. major*, mtc. in 1.55 ± 2.15% of cases (*II* – 32.33 ex.), *N. corvinum*, mtc. in 0.26% of cases (*II* – 18.00 ex.), *N. spathoides*, mtc. in 0.52 ± 0.72% of cases (*II* – 24.00 ex.), *H. volgensis*, mtc. in 0.52 ± 0.93% of cases (*II* – 15.00 ex.), *P. brumpti*, mtc. in 9.04 ± 3.07% of cases (*II* – 40.34 ex.), *S. falconis*, mtc. in 1.29 ± 3.31% of cases (*II* – 64.60 ex.), *T. excavata*, mtc. in 3.88 ± 2.59% of cases (*II* – 64.40 ex.), *I. melis*, mtc. in 3.88 ± 1.88% of the cases (*II* - 52.40 ex.), *C. complanatum*, mtc. in 4.91 ± 0.31% of the cases (*II* - 5.37 ex.), and infestation with the trematode species *P. cordatum,* mtc. was recorded in 3.10 ± 0.94% of the cases (*II* - 14.00 ex.).

The quantitative analysis of parasitological indices in the species *P. ridibundus* denotes infestation with the nematode species *C. ornata* in 33.07 ± 0.51% of cases (*II* – 10.39 ex.), *O. filiformis* in 12.92 ± 0.20% of cases (*II* – 5.18 ex.), *O. duboisi* in 3.88 ± 2.13% of cases (*II* – 18.40 ex.), *I. neglecta* in 11.89 ± 0.27% of cases (*II* – 6.65 ex.), *S. lupi*, larva in 7.49 ± 2.96% of cases (*II* – 39.03 ex.), *S. contorta*, larva in 1.81 ± 4.89% of cases (*II* – 42.14 ex.), *P. sexalatus*, larva in 3.36 ± 2.54% of cases (*II* – 34.69 ex.), *A. strongylina*, larva in 7.24 ± 6.61% of cases (*II* – 123.11 ex.), *Agamospirura sp. II*, larva in 1.29 ± 0.44% of cases (*II* – 9.20 ex.), *H. tricolor*, larva III in 1.89 ± 0.18% of cases (*II* – 3.57 ex.), *T. canis*, larva in 2.84 ± 12.50% of cases (*II* – 152.00 ex.), and infestation with the nematode species *R. bufonis* was recorded in 1.29 ± 0.02% of cases (*II* – 3.20 ex.).

Quantitative analysis of parasitological indices in the host species *P. ridibundus* denotes infestation with the acanthocephalan species *A. ranae* in 4.91 ± 0.09% of cases (*II* – 2.21 ex.), *A. lucii* in 4.65 ± 0.09% of cases (*II* – 3.11 ex.), *P. bufonis* in 1.55 ± 0.10% of cases (*II* – 2.00 ex.), *S. teres*, larva in 0.78 ± 0.09% of cases (*II* – 2.00 ex.), and infestation with the species *C. aluconis* was recorded in 0.52 ± 0.14% of cases (*II* – 3.00 ex.).

The host species *Pelophylax lessonae* is characterized by infestation with 42 helminth species, including 26 trematode species, 11 nematode species and 3 acanthocephalan species. According to the quantitative analysis of parasitological indices of the species *Phelophylax lessonae*, infestation with the trematode species *O. ranae* in 17.65 ± 1.03% of cases (*II* - 11.95 ex.), *H. variegatus* in 15.97 ± 0.09% of cases (*II* - 2.29 ex.), *G. varsoviensis* in 3.78 ± 0,20% of cases (*II* - 3,56 ex.), *G. viteliloba* in 4.20 ± 0.03% of cases (*II* – 1.50 ex.), *P. claviger* in 1.68 ± 0.32% of cases (*II* – 6.50 ex.), *C. loossi* in 0.42% of cases (*II* – 3.00 ex.), C. *loossi* in 0.42% of cases (*II* – 3.00 ex.), *P. medians* in 5.04 ± 0.44% of cases (*II* – 9.75 ex.), *P. confusus* in 8.40 ± 1.28% of cases (*II* – 15.86 ex.), *D. subclavatus* in 33.19 ± 0.43% of cases (*II* – 7.82 ex.), *O. diplodiscoides* in 5.88 ± 0.87% of cases (*II* – 22.36 ex.), *C. urniger,* mtc. in 8.40 ± 0.18% of cases (*II* - 5.00 ex.), *P. robusta,* mtc. in 5.04 ± 3.82% of cases (*II* - 63.58 ex.), *S. sphaerula,* mtc. in 9.66 ± 0.21% of cases (*II* - 14.91 ex.), S. *sphaerula,* mtc. in 9.66 ± 0.21% of cases (*II* - 14.91 ex.), *H. cylindracea* in 1.26 ± 0.56% of cases (*II* - 22.00 ex.), *P. elegans* in 6.72 ± 0.57% of cases (*II* - 7.31 ex.), *N. corvinum,* mtc. in 3.78 ± 0.11% of cases (*II* - 3.11 ex.), *N. spathoides,* mtc. in 2.52 ± 0.55% of cases (*II* – 10.50 ex.), *H. volgensis,* mtc. in 2.10 ± 0.94% of cases (*II* – 21.20 ex.), *P. brumpti,* mtc. in 6.72 ± 3.48% of cases (*II* – 69.75 ex.), *S. falconis,* mtc. in 5.04 ± 1.94% of cases (*II* – 73.17 ex.), *T. excavata,* mtc. in 2.10 ± 0.58% of cases (*II* - 26.60 ex.), *T. stissichi,* mtc. in 3.78 ± 1.22% of cases (*II* - 39.33 ex.), *I. melis*, mtc. in 0.42% of cases (*II* - 14.00 ex.), *M. lomgicolis*, mtc. in 2.10 ± 0.18% of cases (*II* - 5.60 ex.), *C. complanatum,* mtc. in 9.24 ± 0.28% of cases (*II* - 7.86 ex.), and infestation with the trematode species *P. cordatum,* mtc. was recorded in 2.10 ± 2.02% of cases (*II* - 30.00 ex.).

The quantitative analysis of parasitological indices in the species *P. lessonae* demonstrates its infestation with the nematode species *C. ornata* in 30.67 ± 0.91% of cases (*II* – 11.05 ex.), *O. filiformis* in 13.45 ± 0.26% of cases (*II* – 5.28 ex.), *O. duboisi* in 3.78 ± 5.42% of cases (*II* – 31.22 ex.), *I. neglecta* in 5.46 ± 0.30% of cases (*II* – 7.15 ex.), *S. lupi*, larva in 2.94 ± 1.85% of cases (*II* – 29.57 ex.), *S. contorta*, larva in 1.68 ± 2.60% of cases (*II* – 56.50 ex.), *P. sexalatus*, larva in 5.88 ± 2.59% of cases (*II* – 29.43 ex.), *A.*

strongylina, larva in 5.46 ± 3.56% of cases (*II* – 97.92 ex.), *Agamospirura sp. II,* larva in 1.68 ± 0.03% of cases (*II* – 2.75 ex.), *T. canis*, larva in 2.52 ± 0.81% of cases (*II* – 85.83 ex.), and infestation with the nematode species *R. bufonis* was recorded in 2.97 ± 0.26% of cases (*II* – 3.14 ex.).

Quantitative analysis of the parasitological indices of the same host species shows infestation with the acanthocephalan species *A. ranae* in 6.30 ± 0.09% of cases (*II* - 2.73 ex.), *A. lucii* in 0.84 ± 0.23% of cases (*II* - 3.50 ex.), and infestation with the larvae of the species *S. teres* in 10.00% of cases (*II* - 1.00 ex.).

Helminthological surveys of *Pelophylax kl. esculentus* have demonstrated infestation with 30 helminth species, including 19 species of trematodes, 9 species of nematodes and 2 species of acanthocephalans.

According to the quantitative analysis of parasitological indices in the species *P. esculentus,* infestation with the trematode species *O. ranae* was established in 22.33 ± 0.27% of cases (*II* - 4.88 ex.), *H. variegatus* in 8.00 ± 0.23% of cases (*II* - 4.21 ex.), *C. retusus* in 9.00 ± 0.23% of cases (*II* - 5.15 ex.), *G. varsoviensis* în 6.00 ± 0.05% din cazuri (*II* – 1.50 ex.), *G. viteliloba* in 0,33% of cases (*II* – 1.00 ex.), *P. claviger* in 5.67 ± 0,26% of cases (*II* – 4.18 ex.), *C. loossi* in 0.33% of cases (*II* - 4.00 ex.), *P. medians* in 5.67 ± 0.44% of cases (*II* - 22.18 ex.), *P. confusus* in 10.33 ± 0.31% of cases (*II* - 6.10 ex.), *D. subclavatus* in 15.67 ± 0.30% of cases (*II* - 7.02 ex.), *O. diplodiscoides* in 0.33% of cases (*II* - 9.00 ex.), *C. urniger,* mtc. in 8.67 ± 0.24% of cases (*II* - 7.69 ex.), C. *urniger,* mtc. in 8.67 ± 0.24% of cases (*II* - 7.69 ex.), *O.*), *P. robusta,* mtc. in 8.00 ± 0.16% of cases (*II* - 4.88 ex.), *H. cylindracea* in 6.33 ± 0.01% of cases (*II* - 1.05 ex.), *N. corvinum*, mtc. in 0.33% of cases (*II* - 4.00 ex.), *N. spathoides,* mtc. in 0.67 ± 0.49% of cases (*II* - 18.00 ex.), *H. volgensis,* mtc. in 0.33% of cases (*II* - 18.00 ex.), *P. brumpti,* mtc. in 3.33 ± 4.73% of the cases (*II* - 34.40 ex.), and *T. excavata*, mtc. infestation was recorded in 7.00 ± 0.79% of cases (*II* - 67.76 ex.).

Quantitative analysis of parasitological indices in the host species *P. esculentus* shows infestation with nematode species *C. ornata* in 33.00 ± 0.62% of cases (*II* - 10.40 ex.), *O. filiformis* in 21.00 ± 0.26% of cases (*II* - 7.46 ex.), *O. duboisi* in 3.00 ± 0.08% of cases (*II* - 1.78 ex.), *O.*), *I. neglecta* in 18,00 ± 0,19% of cases (*II* - 4,11 ex.), *S. lupi,* larva in 0,33% of cases (*II* - 2,00 ex.), *P. sexalatus,* larva in 13,67 ± 0,82% of cases (*II* - 49,71 ex.), *A. strongylina,* larva in 1,33 ± 0,61% of cases (*II* - 16,00 ex.), *Agamospirura sp. II,* larvae in 0.33% of the cases (*II* - 22,00 ex.), and infestation with *T. canis,* larvae was recorded in 11.33 ± 1.93% of the cases (*II* - 134,00 ex.). According to the helminthologic evaluation, the infestation of the host with the acanthocephalan species *A. ranae* was detected in 12.33 ± 0.15% of cases (*II* - 3.19 ex.), and with the species *A. lucii* in 0.33% of cases (*II* - 2.00 ex.).

In contrast to the green frog group, agile frog and common frog are characterized by a lower diversity of their helminth fauna. In order to determine the structure of the helminth fauna of *Rana dalmatina,* it was determined that it is infested with 22 species of helminths, of which 13 species of trematodes, 7 species of nematodes, one species of acanthocephalans and one species of monogeneans.

Assessment of parasitological indices in *Rana dalmatina* shows infestation with the trematode species *O. ranae* in 21.14 ± 0.49% of cases (*II* - 6.17 ex.), *H. variegatus* in 23.21 ± 0.06% of cases (*II* - 1.23 ex.), *C. retusus* in 17.86 ± 0.19% of cases (*II* - 4.50 ex.), *G. varsoviensis* in 10.71 ± 0.06% of cases (*II* - 1.17 ex.), *G. viteliloba* in 14.29 ± 0.19% of cases (*II* - 2.50 ex.), *P. claviger* in 14.29 ± 0.34% of cases (*II* - 4.25 ex.), *P. medians* in 12.50 ± 0.66% of cases (*II* – 6.57 ex.), *D. subclavatus* in 10.71 ± 0.06% of cases (*II* –2.17 ex.), *P. robusta,* mtc. in 16.07 ± 0.81% of cases (*II* – 36.33 ex.), *S. sphaerula* in 12.50 ± 1.85% of cases (*II* – 39.29 ex.), *H. cylindracea* in 8.93 ± 0.73% of cases (*II* - 10.60 ex.), *T. excavata,* mtc. in 7.14 ± 0.43% of cases (*II* - 20.00 ex.), and infestation with *T. stossichi,* mtc. was recorded in 14.29 ± 0.72% of cases (*II* - 9.63 ex.). Quantitative evaluation of parasitological indices in the host species *R. dalmatina* showed infestation with the nematode species *C. ornata* in 30.36 ± 0.79% of cases (*II* - 8.35 ex.), *O. filiformis* in 26.79 ± 0.49% of cases (*II* - 4.07 ex.), *O. duboisi* in 14.29 ± 0.10% of cases (*II* - 1.63 ex.), *O. duboisi* in 14.29 ± 0.10% of cases (*II* - 1.63 ex.), O. *filiformis* in 26.79 ± 0.49% of cases (*II* - 4.07 ex.), *O. duboisi* in 14.29 ± 0.10% of cases (*II* - 1.63 ex.), *S. contorta* in 10.71 ± 0.37% of the cases (*II* - 8.67 ex.), *P. sexalatus,* larva in 12.50 ± 9.28% of cases (*II* - 71.00 ex.), *A. strongylina,* larva in 12.50 ± 0.20% of cases (*II* - 3.29 ex.), and *R. bufonis* infestation was recorded in 17.86 ± 0.08% of cases (*II* - 2.10 ex.). Quantitative analysis of parasitological indices showed host infestation with the acanthocephalan species *A. ranae* in 12.50 ± 0.13% of cases (*II* - 1.71 ex.) and with the monogenean species *P. integerrimum* in 19.64% of cases (*II* - 1.00 ex.).

The structure of the helminth fauna of *Rana dalmatina* is characterized by the presence of 18 species of helminths, including 10 species of trematodes, 6 species of nematodes, one species of acanthocephalans and one species of monogeneans.

According to the assessment of the degree of invasiveness of the species, it was established that it was infested with the trematode species *O. ranae* in 23.58 ± 0.44% of cases (*II* - 6.52 ex.), *C. retusus* was recorded in 9.43 ± 0.10% of cases (*II* - 1.09 ex.), *G. varsoviensis* in 11.32 ± 3.09% of cases (*II* - 10.58 ex.), *G. viteliloba* in 10.38 ± 0.05% of cases (*II* - 1.27 ex.), *P. claviger* in 7,55 ± 0,28% of cases (*II* - 6,88 ex.), *P. medians* in 6,60 ± 0.57% of cases (*II* – 10.14 ex.), *P. confusus* in 9.43 ± 0.13% of cases (*II* - 3,40 ex.), *D. subclavatus* in 8.49 ± 0.17% of cases (*II* - 3.00 ex.), *H. cylindracea* in 20.75 ± 0.50% of cases (*II* - 20.60 ex.), and infestation with *P. elegans* in 18.87 ± 0.36% of cases (*II* - 7.45 ex.). Quantitative analysis of parasitological indices of the host species *R. temporaria* shows infestation with the nematode species *C. ornata* in 10.38 ± 0.08% of cases (*II* - 2.27 ex.), *O. filiformis* in 27.36 ± 0.16% of cases (*II* – 3.00 ex.), *O. duboisi* in 13.21 ± 0.43% of cases (*II* – 9.21 ex.), *A. strongylina,* larva in 24.53 ± 0.71% of cases (*II* – 23.12 ex.), *A. strongylina,* larva in 24.53 ± 0.71% of cases (*II* – 23.12 ex.), *Agamospirura,spII* larvae in 16.98 ± 1.63% of cases (*II* - 23.12 ex.) and infestation with *R. bufonis* species was recorded in 20.75 ± 0.52% of cases (*II* - 12.00 ex.), with the acanthocephalas species *A. ranae* in 17.92 ± 0.27% of the cases (*II* - 4.95 ex.), and infestation with the monogenean species *P. integerrimum* was determined in 13.21 ± 0.05% of cases (*II* - 1.36 ex.).

The diversity of the helminth fauna of *Bufo bufo* is characterized by the presence of 17 species of helminths, including 3 species of trematodes, 9 species of nematodes, 4 species of acantocephalans and one species of monogeneans. Thus, quantitative analysis of parasitological indices in the host species *B. bufo* revealed its infestation with the trematode species *O. ranae* in 0.32% of cases (*II* - 1.00 ex.), *D. subclavatus* in 0.65% of cases (*II* - 4.00 ex.), and *P. robusta,* mtc. in 0.32% of cases (*II* - 24.00 ex.). According to the evaluation of the helminth infestation of the given species, it was determined that the species was infested with the nematode *C. ornata* in 93.83 ± 0.78% of cases (*II* – 9.47 ex.), cu specia *O. filiformis* in 73.05 ± 0.28% of cases (*II* – 6.78 ex.), *O. duboisi* în 29.55 ± 0,26% of cases (*II* – 6.40 ex.), *I. neglecta* in 4.87% of cases (*II* – 1.00 ex.), *S. lupi,* larva in 1.30 ± 0.14% of cases (*II* – 4.00 ex.), *P. sexalatus,* larva in 0.65 ± 0.97% of cases (*II* – 28.00 ex.), *A. strongylina,* larva in 4.55 ± 6.82% of cases (*II* – 43.43 ex.), *Agamospirura sp. II,* larva in 1.95 ± 0.33% of cases (*II* - 10,67 ex.), *R. bufonis* in 65.58 ± 0.54% of cases (*II* – 10.16 ex.), with the acanthocephala species *A. ranae* in 0.61% of cases (*II* – 3.00 ex.), *P. bufonis* in 1.62% of cases (*II* – 1.00 ex.), *P. bufonis* in 1.62% of cases (*II* – 1.00 ex.), *S. teres,* larva in 1.30% of cases (*II* - 1.00 ex.), *C. aluconis* in 0.32% of cases (*II* - 3.00 ex.), and infestation with the monogenean species *P. integerrimum* in 8.44 ± 0.03% of cases (*II* - 1.54 ex.).

The diversity of the helminth fauna of *Bufotes viridis* species is characterized by the presence of 18 species of helminths, including 5 species of trematodes, 9 species of nematodes, 3 species of acanthocephalans and one species of monogeneans. According to the quantitative analysis of the parasitological indices of the host denotes its infestation with the trematode species *O. ranae* in 3.94 ± 0.04% of cases (*II* - 2.00 ex.), *D. subclavatus* in 0.30% of cases (*II* - 4.00 ex.), *P. robusta,* mtc. in 0.30% of cases (*II* – 44.00 ex.), *S. sphaerula* in 16.36 ± 0.06% of cases (*II* – 2.59 ex.), *H. cylindracea* in 24.55 ± 1.31% of cases (*II* – 25.04 ex.), with the nematode species *C. ornata* in 72.73 ± 1.22% of cases (*II* – 12.16 ex.), *O. filiformis* in 19.09 ± 0.51% of cases (*II* – 7.44 ex.), *O. duboisi* in 3.94 ± 0.26% of cases (*II* – 4.16 ex.), *S. lupi,* larva in 25.45 ± 1.18% of cases (*II* – 60.00 ex., *P. sexalatus,* larva in 17.88 ± 0.76% of cases (*II* – 53.00 ex.), *S. contorta,* larva in 0.30% of cases (*II* – 11.00 ex.), *A. strongylina,* larva in 11,82 ± 5,95% of cases (*II* - 146,54 ex.), *Agamospirura sp. II,* larvae in 0.91 ± 1.87% of.cases (*II* - 57.67 ex.), and infestation with the nematode species *R. bufonis* in 47.27 ± 1.37% of cases (*II* - 17.20 ex.). Evaluation of parasitological indices in the species *B. viridis* showed infestation with the acanthocephalan species *A. ranae* in 2.42% of cases (*II* - 1.00 ex.), *S. teres,* larva in 0.30% of cases (*II* - 1.00 ex.), *P. bufonis* in 3.64% of cases (*II* - 1.00 ex.), and infestation with the monogenean species *P. integerrimum* in 10.30 ± 0.04% of cases (*II* - 1.68 ex.).

The structure of the helminth fauna of the species *Pelobates fuscus* is characterized by the presence of 13 species of helminths, of which 9 species of trematodes and 4 species of nematodes. According to the quantitative analysis of its parasitological indices, the infestation with the trematode species *O. ranae* was established in 20.71 ± 0.10% of cases (*II* - 3.37 ex.), *H. variegatus* in 18.18 ± 0.01% of cases (*II* – 1.03 ex.), *G. viteliloba* in 0.33% of cases (*II* – 1.00 ex.), *P. claviger* in 19.19 ± 0.03% of cases (*II* – 1.08 ex.), *P. medians* in 20.71 ± 0.22% of cases (*II* – 6.10 ex.), *P. confusus* in 20.20 ± 0.22% of cases (*II* – 7.88 ex.), *D. subclavatus* in 19.70 ± 0.09% of cases (*II* – 2.54 ex.), *S. sphaerula, mtc*. in 20.20 ± 0.33% of cases (*II* – 8.75 ex.), *N. spathoides,* mtc. in 11.62 ± 4.56% of cases (*II* - 92.26 ex.), and infestation with *P. brumpti,* mtc. in 8.59 ± 0.41% of cases (*II* - 11.18 ex.). Quantitative analysis of parasitological indices in the host species shows infestation with the nematode species *C. ornata* in 26.26 ± 0.08% of cases (*II* - 2.83 ex.), *O. filiformis* in 16.67 ± 0.06% of cases (*II* - 2.02 ex.), *A. strongylina,* larva in 14.14 ± 0.06% of cases (*II* - 2.14 ex.), and infestation with *R. bufonis* species was established in 21.72 ± 0.18% of cases (*II* - 7.51 ex.).

The host species *Bombina bombina bombina* is characterized by the presence of 20 helminth species, including 15 species of trematodes, 3 species of nematodes, one species of acanthocephalans and one species of monogeneans. According to the quantitative analysis of the parasitological indices of this host species, infestation with the trematode species *O. ranae* was established in 15.69 ± 0.09% of cases (*II* - 4.07 ex.), *H. variegatus* in 19.44 ± 0.09% of cases (*II* - 2.60 ex.), *G. viteliloba* in 6.79 ± 0.03% of cases (*II* - 1.45 ex.), *P. claviger* in 17.80 ± 0.09% of cases (*II* - 5.08 ex.), *P. medians* in 15.69 ± 0.11% of cases (*II* - 7.34 ex.), *P. confusus* in 18.27 ± 0.17% of cases (*II* - 6.60 ex.), *D. subclavatus* in 19.44 ± 0.23% of cases (*II* - 7.08 ex.), *C. urniger*, mtc. in 11.24 ± 0.26% of cases (*II* – 13.98 ex.), *S. sphaerula*, mtc. in 4.45 ± 0.20% of cases (*II* – 20.37 ex.), *H. cylindracea* in 7.03 ± 0.25% of cases (*II* – 17.00 ex.), *H. volgensis,* mtc. in 6.32 ± 0.08% of cases (*II* – 4.15 ex.), *P. brumpti,* mtc. in 36.77 ± 0.94% of cases (*II* – 28.76 ex.), *P. cloacicola,* mtc. in 3.98 ± 0.09% of cases (*II* – 9.29 ex.), *Strigea falconis,* mtc. in 11.48 ± 0.19% of cases (*II* – 9.24 ex.), *T. excavata,* mtc. in 3.75 ± 1.95% of cases (*II* – 181.56 ex.), with the nematode species *C. ornata* in 44.73 ± 0.09% of t cases (*II* – 5.03 ex.), *A. strongylina,* larva in 4.22 ± 0.16% of cases (*II* – 12.50 ex.), *R. bufonis* in 7.96 ± 0.02% of the cases (*II* – 1.38 ex.), R. *bufonis* in 7.96 ± 0.02% of cases (*II* – 1.38 ex.), with the acanthocephala species *A. ranae* in 28.10 ± 0.04% of cases (*II* - 1.62 ex.), and infestation with the monogenean species *P. integerrimum* was recorded in 5.62% of cases (*II* - 1.00 ex.).

Hyla arborea, being the only arboreal species of amphibians in the country, is characterized by its infestation with 13 species of helminths, including 7 species of trematodes, 5 species of nematodes and one species of acanthocephalans. The quantitative evaluation of parasitological indices shows that it is infested with the trematode species *O. ranae* in 28.42 ± 0.15% of cases (*II* - 2.14 ex.), *P. claviger* in 20.14 ± 0.10% of cases (*II* - 2.98 ex.), *P. medians* in 25.90 ± 0.27% of cases (*II* - 6.93 ex.), *D. subclavatus* in 20.86 ± 0.06% of cases (*II* - 1.67 ex.), *S. sphaerula,* mtc. in 17.99 ± 0.18% of cases (*II* - 8.20 ex.), S. *sphaerula,* mtc. in 17.99 ± 0.18% of cases (*II* - 8.20 ex.), *N. major,* mtc. in 15.47 ± 0.44% of cases (*II* – 6.65 ex.), *S. falconis,* mtc. in 14.75 ± 1.80% of cases (*II* – 75.68 ex.), *C. ornata* in 30.22 ± 0.25% of cases (*II* – 9.29 ex.), *O. filiformis* in 23.74 ± 0.28% of cases (*II* - 8.61 ex.), *O. duboisi* in 20.14 ± 0.07% of cases (*II* - 2.32 ex.), *I. neglecta* in 21.58 ± 0.11% of cases (*II* - 4.47 ex.), *Agamospirura sp. II* larvae in 18.35 ± 0.17% of cases (*II* - 5.43 ex.), and the acanthocephalan species *A. ranae* was recorded in 23.74 ± 0.02% of cases (*II* - 1.08 ex.).

The structure of the helminth fauna of the species *Triturus cristatus* is characterized by the presence of 10 species of helminths, of which 6 species of trematodes and 4 species of nematodes. Quantitative evaluation of parasitological indices of the host revealed infestation with the trematode species *O. ranae* in 46.06 ± 0.41% of cases (*II* - 6.51 ex.), *P. claviger* in 16.09 ± 0.12% of cases (*II* - 5.04 ex.), *P. medians* in 16.72 ± 0.11% of cases (*II* - 7.23 ex.), *P. confusus* in 14.51 ± 0.05% of cases (*II* - 2.22 ex.), *D. subclavatus* in 28.71 ± 0.10% of cases (*II* - 7.97 ex.), *P. brumpti,* mtc. in 17.99 ± 0.20% of cases (*II* - 8.20 ex.), *C. ornata* in 55.87 ± 0.43% of cases (*II* - 5.86 ex.), *O. filiformis* in 8.83 ± 0.12% of the cases (*II* - 5.14 ex.), *O. duboisi* in 9.78 ± 0.04% of the cases (*II* - 1.77 ex.), and infestation with *S. contorta*, larvae in 30.60 ± 2.31% of the cases (*II* - 48.39 ex.).

The diversity of the helminth fauna of the species *Lissotriton vulgaris* is characterized by the presence of 12 species of helminths, of which 7 species of trematodes and 5 species of nematodes. Quantitative analysis of parasitological indices in the host species shows infestation with the trematode species *O. ranae* in 52.14 ± 0.19% of cases (*II* - 5.63 ex.), *P. claviger* in 8.56 ± 0.14% of cases (*II* - 6.91 ex.), *P. medians* in 11.28 ± 0.19% of cases (*II* – 8.97 ex.), *D. subclavatus* in 29.57 ± 0.36% of cases (*II* – 7.70 ex.), *S. sphaerula,* mtc. in 12.84 ± 0.06% of cases (*II* – 3.24 ex.), *H. volgensis,* mtc. in 7.00 ± 0.08% of cases (*II* – 4.44 ex.), *C. ornata* in 24.90 ± 0.18% of cases (*II* – 4.20 ex.), *O. filiformis* in 12.45 ± 0.10% of cases (*II* – 3.41 ex.), *O. duboisi* was recorded in 7.39 ± 0.10% of cases (*II* - 2.95 ex.), *S. contorta*, larvae in 16.73 ± 2.13% of the cases (*II* - 25.70 ex.), and infestation with *Agamospirura sp. II*, larvae was determined in 6.23 ± 0.08% of cases (*II* - 4.94 ex.).

The evaluation of the structure of the helminth fauna in amphibians and the degree of helminth infestation allowed us to conclude that infestation is achieved depending on host-specific biological and ecological factors determined by the host's ethological peculiarities during an annual and life cycle: hibernation, reproduction, metamorphosis, leaving summer sites, initiation of hibernation phase and the degree of synanthropization of each host species.

If, in terms of the diversity of the helminth fauna, there is a divergence in the number of species of parasites detected in the amphibian species evaluated, then the degree of helminth infestation of amphibians ranges from 28.14% of cases in infested *R. dalmatina* specimens to 76.33% of cases in infested *P. ridibundus* specimens (Figure 5.1).

Thus, the behavior of amphibians and their complex predator-prey trophic interactions at different periods of their annual and life cycle allow for a specific formation of their parasitic fauna. Therefore, the formation of amphibian species-specific helminthic fauna and the degree of helminth invasiveness differing from one host to another reflect the adaptability of amphibians to the conditions of their environment and their physiological changes that represent a mechanism of formation of relationships in the parasite-host system.

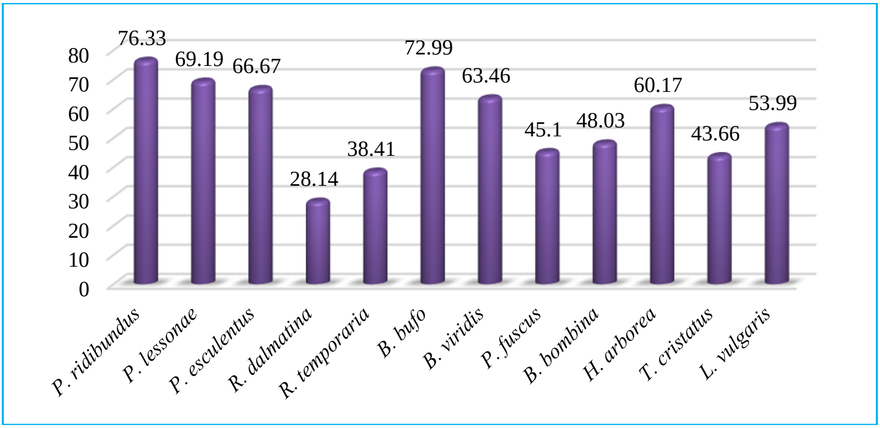

Fig. 5.1. Degree of helminth infestation of amphibians

5.2. Mono- and polyinvasions of caudata and ecaudata amphibians in ecosystems of Moldova

With the aim of deciphering the mechanisms involved in the study of disease etiology and understanding the interaction or relationships in the host-parasite system in amphibians, as well as addressing the common effects of both host and parasites on the increased risk of outbreaks of parasitic agents, and in the light of the increased interest in the biodiversity-disease relationship, the degree of helminth infestation of amphibians in mono- and polyinvasive aspects during their life cycle was assessed.

According to the helmintological investigations carried out, *P. ridibundus* is infested in monoinvasive aspect in 31.0% of cases and in polyinvasive aspect in 69.0% of cases, *P. lessonae* is infested in monoinvasive aspect in 27.7% of cases and in polyinvasive aspect in 72.3% of cases, and *P. esculentus* is infested in monoinvasive aspect in 25.3% of cases and in polyinvasive aspect in 74.7% of cases. In brown ranids, in the host species *R. dalmatina*, monoinvasive infestation was determined in 17.9% of cases and polyinvasive infestation in 82.1% of cases, and in *R. temporaria*, monoinvasive infestation in 24.5% of cases and polyinvasive infestation in 75.5% of cases. In the amphibians of the Bufonidae family it was found that in the species *B. bufo* monoinvasive infestation was recorded in 4.2% of cases and polyinvasive in 95.8% of the cases, and in the host species *B. viridis* monoinvasive in 8.5% of cases and polyinvasive in 91.5% of cases. In the amphibian species *H. arborea* infestation in monoinvasive aspect was determined in 16.5% of cases and in polyinvasive aspect in 83.5% of cases, *P. fuscus*, the infestation in the form of monoinvasion is 24.2% of cases and in the form of polyinvasions was recorded in 75.8% of cases, in *B. bombina*, the infestation in the form of monoinvasion was established in 26.2% of cases and in the form of polyinvasion was recorded in 73.8% of cases (Figure 5.2).

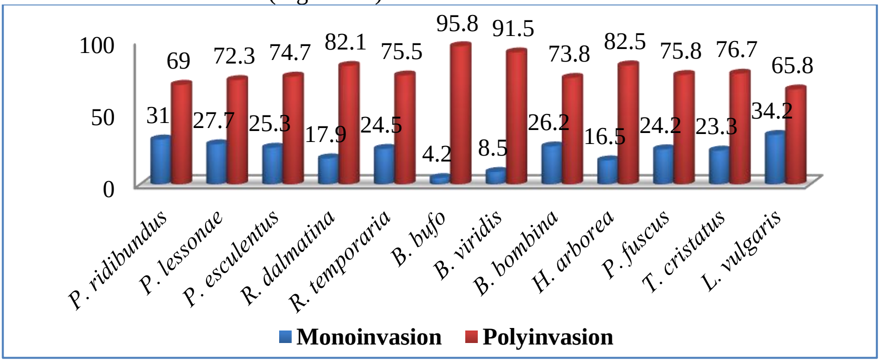

Fig. 5.2. Structure of helminthic fauna in amphibians in terms of mono- and polyinvasion

In *T. cristatus*, the monoinvasive infestation was determined in 23.3% of cases and the polyinvasive infestation was recorded in 76.7% of cases, while in *L. vulgaris*, the monoinvasive infestation was identified in 34.2% of cases and the polyinvasive infestation was recorded in 65.8% of cases (Figure 5.2).

In order to assess the complex evaluation of the helminth fauna of amphibians, the degree of helminth infestation of amphibians in mono- and polyinvasive aspect in relation to the host genus was assessed. Therefore, according to the obtained data, it was established that both male and female species of amphibian species are predominantly infested in polyinvasive aspect (Figure 5.3, 5.4).

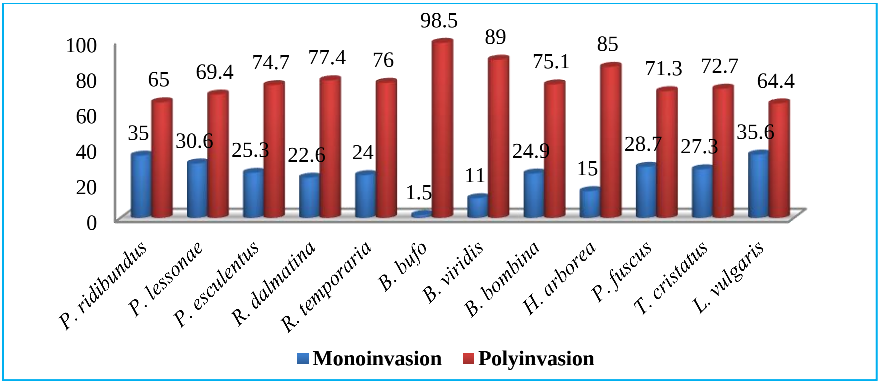

Fig. 5.3. Structure of the helminthic fauna in male amphibians in terms of mono- and polyinvasion

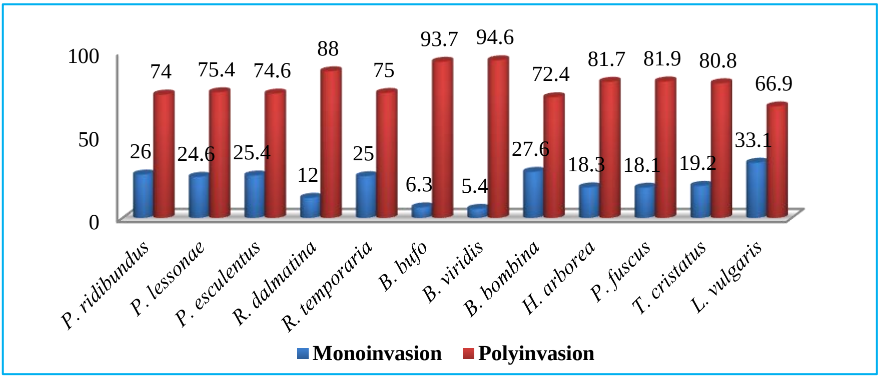

Fig. 5.4. Structure of the helminthic fauna in female amphibians in terms of mono- and polyinvasion

Thus, the helminthological results obtained on mono- and polyinvasive infestation of amphibians allowed us to establish that polyinvasive infestation predominates in all 12 amphibian species evaluated, both in each host species and in dependence on host genus.

In order to determine the structure of the helminth fauna in amphibeines, it was found that they are infested concomitantly with various helminth species, and this process is the result of their interrelations with biological diversity. Thus, in the host species *P.*

ridibundus the process of polyparasitism was manifested in various associations: with 2 species - 23.3% of cases, with 3 species - 26.7% of cases, with 4 species - 14.5% of cases, with 5 species - 11.1% of cases, with 6 species - 7% of cases, with 7 species - 6.3% of cases, with 8 species - 5.9% of cases, with 9 species - 2.6% of cases, with 10 species - 1.9% of cases, and with 11 species - 0.7% of cases. In the host species *P. lessonae*, the polyparasitic process was identified in associations with 2 species - 34.9% of cases, 3 species - 22.1% of cases, 4 species - 15.7% of cases, 5 species - 10.5% of cases, 6 species - 7% of cases, 7 species - 4.1% of cases, 8 species - 2.9% of cases, and 9 species - 2.9% of cases. In the host species *P. esculentus*, polyparasitism occurred in associations with 2 species - 42.1% of cases, 3 species - 21.5% of cases, 4 species - 17.1% of cases, 5 species - 6.1% of cases, 6 species - 7.9% of cases, 7 species - 3.1% of cases, 8 species - 1.4% of cases, 9 species - 0.4% of cases, and 10 species - 0.4% of cases.In the host species *R. dalmatina* the polyparasitic process was detected in associations with 2 species - 45.7% of cases, with 3 species - 37% of cases, with 4 species - 13% of cases, and with 5 species - 4.3% of cases. In the host species *R. temporaria*, polyparasitism occurred in associations with 2 species - 30.4% of cases, with 3 species - 32.9% of cases, with 4 species - 22.8% of cases, with 5 species - 11.4% of cases, and with 7 species - 2.5% of cases.

In the host species *B. bufo* polyparasitism was manifested in associations with 2 species - 35.4% of cases, with 3 species - 33.3% of cases, with 4 species - 27.2% of cases, and with 6 species - 4.1% of cases. In the host species *B. viridis*, polyparasitism was recorded in associations with 2 species - 29.5% of cases, 3 species - 29.1% of cases, 4 species - 25.2% of cases, 5 species - 9.6% of cases, 6 species - 2.3% of cases, 7 species - 2.6% of cases, and 8 species - 1.7% of cases.

In the host species *B. bombina*, polyparasitism occurred in associations with 2 species - 29.7% of cases, with 3 species - 23.0% of cases, with 4 species - 9.5% of cases, with 5 species - 17.7% of cases, with 6 species - 6.9% of cases, with 7 species - 6.0% of cases, with 8 species - 5.7% of cases, and with 9 species - 1.5% of cases. In the host species *H. aborea* the polyparasitic process was determined in associations with 2 species - 29% of cases, with 3 species - 40.7% of cases, with 4 species - 24.2% of cases, and with 5 species - 6.1% of cases. In the host species *P. fuscus*, polyparasitism occurred in associations with 2 species - 39.7% of the cases, with 3 species - 39.7% of the cases, with 4 species - 12.3% of the cases, with 5 species - 7.6% of the cases, and with 6 species - 0.7% of the cases.

In the host species *T. cristatus* the polyparasitism process was identified in associations with 2 species - 43% of cases, with 3 species - 32.6% of cases, with 4 species - 18.6% of cases, with 5 species - 5% of cases, and with 6 species - 0.8% of cases. In the host species *L. vulgaris* the polyparasitic process was manifested in associations with 2 species - 67.9% of cases, with 3 species - 27.3% of cases, with 4 species - 4.2% of cases, and with 5 species - 0.6% of cases.

At the same time, the evaluation of the obtained data allowed us to conclude that the possibility of simultaneous infestation of a single host (amphibians) with several species of parasitic agents reflects the trophic relationships in the ecosystem (prey-predator) specific to amphibians, but also their polyinfection process demonstrates the increased degree of amphibians in vectorization of common parasitic agents of wild, domestic, pet and human animals.

5.3. The spread of parasitic agents in the host population by age

The study of the age dependence of the helminth fauna is a major problem in ecological parasitology [9, 13].

In amphibians, assessing how infestation dynamics differ with host age is important to accurately predict infestation patterns that can help mitigate amphibian population declines or extirpations associated with wildlife and domestic wildlife disease.

In order to assess age-dependent helminth infestation of amphibians, specimens of two age classes were helminthologically investigated: pre-reproductive (embryos, larvae, juveniles) and reproductive (adults).

The examination of embryos of amphibian species of the genera *Rana, Pelophylax, Bufo, Bufotes, Hyla, Pelobates, Triturus, Lissotriton* and *Bombina* did not show the presence of invasive elements.

Therefore, according to the helminthological data obtained, it was established that the larvae of *P. esculentus* species are infested with 7 species of helminths, and the quantitative analysis of parasitological indices allowed to establish the infestation with *O. ranae* in 15.56 ± 0.03% of cases (*II* - 4.29 ex.), *C. retusus* in 24.44 ± 0.34% of cases (*II* - 6.40 ex.), *P. robusta* in 11,11 ± 0,35% of cases (*II* - 6,40 ex.), *S. sphaerula* in 13,33 ± 0,50% of cases (*II* - 11,00 ex.), *H. cylindracea* in 24,77 ± 7,31% of cases (*II* - 31,27 ex.), *S. falconis* in 13.33 ± 0.62% of the cases (*II - 8.50* ex.), and *I. melis,* mtc. in 17.78 ± 1.36% of the cases (*II* - 19.50 ex.).

P. lessonae larvae were infested with 3 species of helminths, as *O. ranae* in 10.87 ± 0.07% of cases (*II* - 1.20 ex.), *H. cylindracea* in 6.25% of cases (*II* - 1.00 ex.), and *I. melis,* mtc. in 8.70% of cases (*II* - 1.00 ex.).

In the hybrid species of the green frog complex, *P. esculentus*, the presence of 3 species of parasitic agents was also established, in which the infestation with the trematode species *O. ranae* in 31.11% of cases (*II* - 1.00 ex.), *P. robusta* in 35.56% of cases (*II* - 1.00 ex.), and the infestation with the species *H. cylindracea* was recorded in 31.11% of cases (*II* - 1.00 ex.).

In the larvae of *R. dalmatina*, 6 trematode species were identified, including digenean and one monogenean species. According to the evaluation of helminthological indices, the larvae were infected with *O. ranae* in 15.09 ±0.05% of cases (*II* - 1.13 ex.), *G. vitelliloba* in 15.09% of cases (*II* - 1.00 ex.), *S. sphaerula* in 15.09 ± 0.05% of cases (*II* - 1.13 ex.), *H. cylindracea* in 28.30 ± 0.06% of cases (*II* - 1.27 ex.), *T. stossichi* in 13.21% of cases (*II* - 1.00 ex.), and *P. integerrimum* infestation was recorded in 13.21 ± 0.05% of cases (*II* - 1.14 ex.).

In the larvae of amphibian larvae of the family Bufonidae the presence of 3 helminth species was determined. According to the quantitative analysis of the helminthological indices of invasion, it was determined that the larvae *of B. bufo* species were infested with 2 species of helminths, of which *O. ranae* species in 64.89 ± 0.17% of cases (*II* - 1.44 ex.) and *P. robusta* in 68.92 ± 0.03% of cases (*II* - 1.06 ex.). Quantitative analysis of the helminthological indices in the larvae *of B. viridis* species showed infestation with *O. ranae* in 8.47% of cases (1.00 ex.), *P. robusta* in 37.29% of cases (*II* - 1.00 ex.), and *S. sphaerula* in 62.79% of cases (*II* - 1.00 ex.).

In the larvae of the amphibian species *Hyla arborea* and *Bombina bombina* the presence of 3 helminth species each was established. Thus, in the larvae of the species *H. arborea* infestation with the trematode species *O. ranae* was recorded in 25.93% of cases

(*II* - 1.00 ex.), *S. falconis,* mtc. *S. sphaerula* in 62.79% of cases (*II* - 1.00 ex.) each. In the larvae *of B. bombina*, infestation with the trematode species *O. ranae* was established in 24.59% of cases (*II* - 1.00 ex.), *S. falconis,* mtc. and *S. sphaerula* were recorded in 27.87% of cases (*II* - 1.00 ex.) and 47.54% of cases, respectively.

As the juveniles leave the aquatic pools in which they have developed, they adapt to a different living environment with a different faunal diversity, and as a result their trophic rations change and become more varied.

In order to assess the diversity of the helminth fauna in juvenile amphibian (postmetamorphic phase - prereproductive phase) caudata and ecaudata, a richer structure of their helminth fauna was recorded, ranging from 4 helminth species in juvenile *T. cristatus* to 24 helminth species in juveniles *P. ridibundus*.

Thus, in the juveniles of *P. ridibundus* the diversity of the helminth fauna is represented by 15 species of trematodes, 6 species of nematodes and 3 species of acanthocephalans whose helminthological indices of invasion are highly variable. According to their quantitative analysis, *O. ranae* infestation was established in 24.07 ± 4.00% of cases (*II* - 27.77 ex.), *H. variegatus* in 12.96 ± 0.25% of cases (*II* - 4.36 ex.), *C. retusus* in 0,935% of cases (*II* - 6,00 ex.), *G. viteliloba* in 15,74 ± 0,25% of cases (*II* - 3,94 ex.), *P. confusus* in 3,70 ± 0,46% of cases (*II* - 7,25 ex.), *D. subclavatus* in 12.96 ± 0.15% of cases (*II* – 3.64 ex.), *D. subclavatus* in 12.96 ± 0.15% of cases (*II* – 3.64 ex.), *C. urniger,* mtc. in 2.78 ± 0.06% of cases (*II* – 1.67 ex.), *S. sphaerula,* mtc. in 8.33 ± 0.29% of cases (*II* – 12.33 ex.), *H. cylindracea* in 10.19 ± 0.70% of cases (*II* – 20.18 ex.), *H. volgensis,* mtc. in 0.93% of cases (*II* - 12.00 ex.), *P. brumpti,* mtc. in 13.89 ± 1.78% of cases (*II* - 14.33 ex.), *S. falconis,* mtc. in 18.52 ± 0.88% of cases (*II* - 20.35 ex.), *S. falconis,* mtc. in 18.52 ± 0.88% of cases (*II* - 20.35 ex.), *T. excavata,* mtc. in 8.33 ± 2.92% of cases (*II* - 32.78 ex.), and infestation with *I. melis,* mtc. was recorded in 12.96 ± 0.60% of cases (*II* - 20.00 ex.). Infestation of juveniles with the nematode species *C.ornata* was determined in 20.37 ± 0.27% of *cases (II* – 5.09 ex.), *O. filiformis* in 1.85% of cases (*II* – 4.00 ex.), *I. neglecta* in 2.78 ± 0.11% of cases (*II* – 1.67 ex*.), S. lupi, larva in 7.41* ± 1.52% of cases (*II* – 15.50 ex.), *A. strongylina,* larva 0.93% of cases (II – 14.00 ex.), *T.canis,* larva in 9.26 ± 0.44% of cases (*II* – 11.40 ex.), and with the acantocephalan species *A. ranae* in 2.78 ± 0.17% of cases (*II* - 2.00 ex.), *A. lucii* in 2.78 ± 0.11% of cases (*II* - 1.67 ex.), and with the species *S. teres,* larva in 1.85% of cases (*II* - 1.00 ex.).

In the juveniles of *P. esculentus*, the presence of 15 helminth species was identified, of which 11 species of trematodes, 4 species of nematodes and one species of acanthocephalans. According to the analysis of helminthological indices, infestation with *O. ranae* was established in 31.03 ± 0.66% of cases (*II* - 9.94 ex.), *H. variegatus* in 18.97 ± 0.12% of cases (*II* - 1.82 ex.), *C. retusus* in 15.52% of cases (*II* - 1.00 ex.), *P. confusus* in 10.34 ± 0.16% of cases (*II* - 2.33 ex.), *P. confusus* in 10.34 ± 0.16% of cases (*II* - 2.33 ex.), *D. subclavatus* in 25.86 ± 0.10% of cases (*II* - 2.33 ex.), *C. urniger,* mtc. in 18.97 ± 0.07% of cases (*II* - 1.36 ex.), *H. cylindracea,* mtc. in 22.41% of cases (*II* - 1.00 ex.), *P. brumpti,* mtc. in 17.24 ± 0.33% of cases (*II* - 6.50 ex.), *P. brumpti,* mtc. in 17.24 ± 0.33% of cases (*II* - 6.50 ex.), *T. excavata,* mtc. in 20.69 ± 0.47% of cases (*II* - 13.33 ex.), *M. gracillimus,* mtc. in 17.24% of cases (*II* - 1.00 ex.). Infestation of juveniles *of P. esculentus* with the nematode species *C. ornata* was recorded in 18.97 ± 0.08% of cases (*II* - 1.82 ex.).), *O. filiformis* in 29.31 ± 0.17% of cases (*II* - 4.24 ex.), *I. neglecta* in 8.62%

of cases (*II* - 1.00 ex.), *T. canis,* larva in 25.86 ± 0.56% of cases (*II* - 17.07 ex.), and *A. rane* in 10.34 ± 0.07% of cases (*II* - 1.33 ex.).

Juveniles of *P. lessonae* are infested with 14 helminth species, including 12 species of trematodes and 2 nematode species. As a result of the evaluation of the main helminthological indices, infestation with the trematode species *O. ranae* was identified in 14.71 ± 1.84% of cases (*II* - 14.33 ex.), *H. variegatus* in 1.96 ± 0.07% of cases (*II* - 3.50 ex.), *C. retusus* in 9.80 ± 0.12% of cases (*II* – 2.20 ex.), *P. confusus* in 0.98% of cases (*II* – 1.00 ex.), *D. subclavatus* in 19.61 ± 0.06% of cases (*II* - 1,50 ex.), *S. sphaerula,* mtc. in 7.84 ± 4.00% of cases (*II* – 95.25 ex.), *S. faclconis,* mtc. in 8.82 ± 2.82% of cases (*II* – 53.56 ex.), *T. excavata,* mtc. in 1.96 ± 0.35% of cases (*II* – 9.50 ex.), *I. melis,* mtc. in 9.80 ± 0.19% of cases (*II* – 4.00 ex.), *M. gracillimus,* mtc. in 0.98% of cases (*II* - 9.00 ex.), and with nematode species *C. ornata* in 10.78 ± 0.19% of cases (*II* - 3.45 ex.) and *T. canis,* larva in 0.98% of cases (*II* - 2.00 ex.).

In contrast to green frog juveniles, agile and common frog juveniles are characterized by a poorer diversity of their helminth fauna. Thus, *R. dalmatina* juveniles are infested with 9 helminth species, including 5 trematode and 2 nematode species, one species of acanthocephalans and one species of monogeneans. As a result of the evaluation of the main helminthological indices, infestation with the species *O. ranae* was established in 15.85 ± 0.20% of cases (*II* - 2.62 ex.), *D. subclavatus* in 23.17 ± 0.11% of cases (*II* - 3.50 ex.), *S. sphaerula,* mtc. in 13.41 ± 1.30% of cases (*II* - 14.15 ex.), *H. culindracea*. in 26.83 ± 0.99% of cases (*II* - 20.23 ex.), H. *culindracea*. in 26.83 ± 0.99% of cases (*II* - 20.23 ex.), *T. excavata,* mtc. in 13.41 ± 0.61% of cases (*II* - 18.18 ex.), *C. ornata* in 19.51 ± 0.18% of cases (*II* - 2.94 ex.), *R bufonis* in 14.63 ± 0.06% of cases (*II* - 1.58 ex.), *R bufonis* in 14.63 ± 0.06% of cases (*II* - 1.58 ex.), *A. ranae* in 12.20 ± 0.05% of the cases (*II* - 1.20 ex.), *P. integerrimum* was recorded in 10.98 ± 0.04% of cases (*II* - 1.11 ex.).

In the juveniles of the species *R. temporaria*, the presence of 8 species of helminths was determined, of which 5 species of trematodes, 2 species of nematodes and one species of monogeneans, and according to the analysis of helminthological indices, infestation with *O. ranae* was detected in 25.23 ± 0.20% of cases (*II* - 3.54 ex.), *G. vitelliloba* in 13.51 ± 0.05% of cases (*II* - 3.50 ex.), *D. subclavatus* in 22.52 ± 0.11% of cases (*II* - 2.40 ex.), *H. culindracea* in 22.52 ± 0.87% of cases (*II* - 19.84 ex.), *H. cylindracea* in 22.52 ± 0.87% of cases (*II* - 19.84 ex.), *P. elegans* in 15.32 ± 0.19% of cases (*II* – 3.82 ex.), *C. ornata* in 25.23 ± 0.17% of cases (*II* – 4.57 ex.), *Agmospirura sp. II,* larvae in 17.12 ± 0.88% of cases (*II* - 22.16 ex.), and infestation with *Polystoma integerrimum* species was recorded in 17.12% of cases (*II* - 1.00 ex.).

The juveniles of the amphibian species *H. arborea* and *B. bombina* were infested with 7 species of parasites each.

Of the 7 helminth species detected in *H. arborea* juveniles, 4 species are trematodes and 3 are nematodes. According to the evaluation of the degree of invasion of the juveniles, it was established that they were infested with the trematode species *O. ranae* in 24.39 ± 0.08% of cases (*II* - 1.30 ex.), *D. subclavatus* in 19.51% of cases (*II* - 1.00 ex.), *S. sphaerula, larva* in 21.95 ± 0.11% of cases (*II* - 2.00 ex.), *S. sphaerula, larva* in 21.95 ± 0.11% of cases (*II* - 2.00 ex.), *S. falconis,* larva in 31.71% of cases (*II* - 1.00 ex.), *C. ornata* in 34.15 ± 0.13% of cases (*II* - 2.21 ex.), *O. filiformis* in 31.71% of cases

(*II* - 1.00 ex.), and infestation with *I. neglecta* was determined in 9.76% of cases (*II* - 1.00 ex.).

The structure of the helminth fauna of *B. bombina* juveniles is represented by 5 species of trematodes, one species of nematodes and one species of acanthocephalans, Thus, according to the evaluation of helminthological indices, it was established that they were infested with *O. ranae* in 36.47 ± 0.12% of cases (*II* - 2.26 ex.), *P. medians* in 25.88 ± 0.05% of cases (*II* - 1.23 ex.), *P. medians* in 25.88 ± 0.05% of cases (*II* - 1.23 ex.), *P. confusus* in 49.41 ± 0.08% of cases (*II* – 1.50 ex.), *C urniger*, larva in 9.41 ± 0.09% of cases (*II* – 2.88 ex.), *S.sphaerula*, larva in 37.65 ± 0.60% of cases (*II* – 3.28 ex.), *S. sphaerula*, larva in 37.65 ± 0.60% of cases (*II* – 3.28 ex.), *C. ornata* in 70.59 ± 0.10% of the cases (*II* - 2.27 ex.) and *A. ranae* infestation was recorded in 5.88% of cases (*II* - 1.00 ex.).

Of the 12 amphibian species helminthologically assessed according to host ontogeny, 3 (*B. viridis, P. fuscus, L. vulgaris*) of these in the juvenile, or post-metamorphic stage were found to be infested with 6 helminth species each.

Therefore, helminthologic evaluation of *B. viridis* juveniles demonstrated their infestation with 4 trematode species, one nematode species and one monogenean species. Therefore, their infestation with the species O. ranae was detected in 45.92 ± 0.07% of cases (*II* – 1.49 ex.), *P. robusta*, mtc in 24.49% of cases (*II* – 1.00 ex.), *S. sphaerula*, larva in 34.69 ± 0.09% of cases (*II* – 1.88 ex.), *H. cylindracea* in 74.79 ± 0.08% of the cases (*II* - 1.71 ex.), *C. ornata* in 1.02% of the cases (*II* - 3.00 ex.) and *P. integerrimum* was established in 80.61% of cases (*II* - 1.00 ex.).

Helminthologic analysis of juveniles of *P. fuscus* species showed their infestation with 5 species of trematodes and one species of nematodes. Thus, their infestation with *O. ranae* was established in 12.50% of cases (*II* - 1.00 ex.), *D. subclavatus* in 25.00% of cases (*II* - 1.00 ex.), *S. sphaerula*, larva in 28.13 ± 0.06% of cases (*II* - 1.11 ex.), *N. spathoides*, mtc. in 31.25 ± 1.10% of cases (*II* - 1.10 ex.), *P. brumpti*, mtc. in 21.88% of cases (*II* - 1.00 ex.) and *C. ornata* in 25.00% of cases (*II* - 1.75 ex.).

Helminthological estimation of juveniles of *L. vulgaris* species showed their infestation with 3 species of trematodes and 3 species of nematodes, so that the infestation with *O. ranae* species was detected in 9,76 ± 0,10% of cases (*II* - 1,75 ex.), *S. sphaerula*, larvae in 48.78 ± 0.11% of cases (*II* - 2.5 ex.), *H. volgensis*, mtc. in 30.49 ± 0.50% of cases (*II* - 3.80 ex.), *C. ornata* in 19.51 ± 0.10% of cases (*II* - 2.00 ex.), *S. contorta*, larvae in 19.51 ± 0.28% of cases (*II* - 7.73 ex.), and infestation with *Agamospirura sp. II larvae* occurred in 9.76% of cases (*II* - 1.00 ex.).

Helminthologic assessment of *B. bufo* juveniles showed their infestation with 5 species of helminths, including 3 species of trematodes and 2 nematodes. Therefore, their infestation with *O. ranae* species was identified in 87.50 ± 0.10% of cases (*II* - 1.75 ex.), *D. subclavatus* in 33.93% of cases (*II* - 1.00 ex.), *P. robusta*, mtc. in 62.50 ± 0.76% of cases (*II* - 4.57 ex.). *C. ornata* in 32.14 ± 0.10% of cases (*II* - 1.44 ex.), and *O. filiformis* infestation was recorded in 57.14 ± 0.47% of cases (*II* - 2.31 ex.).

The helminthologic investigations, carried out on juveniles of the species *T. cristatus* revealed the lowest diversity of helminths which consists of 2 species of nematodes and 2 species of trematodes. According to the obtained data, infestation with *O. ranae* species was established in 36.49 ± 0.06% of cases (*II* - 1.41 ex.), *S. sphaerula*, larva in 48.78 ± 0.11% of cases (*II* - 2.5 ex.), *D. subclavatus* in 27.03 ± 0.06% of the cases (*II* -

1.50 ex.), *C. ornata* in 51.35 ± 0.41% of cases (*II* - 2.32 ex.), and *S. contorta*, larvae in 43.24 ± 0.11% of cases (*II* - 2.69 ex.).

In order to describe and evaluate the interactions and associations between parasitic agents and the different age structures of amphibians, the *Pearson* correlation coefficient (r_{xy}) between the parasite variable and the body mass of the investigated amphibians from different ontogenetic periods was calculated.

According to the *Pearson's* coefficient (r_{xy}) evaluation, for all age structures of amphibians, the *Pearson's* coefficient (r_{xy}) was found to indicate a perfectly positive correlation (-1, +1) between helminth infestation and body mass of amphibian larvae, juveniles and adults (Figure 5.5).

Thanks to these scientific data obtained, we can confirm the existence of a linear relationship between the degree of infestation and the body mass of amphibians, or as the body mass of amphibians increases, the diversity of their helminth fauna increases, and the age of the host affects the susceptibility of amphibians to infection with various parasitic agents

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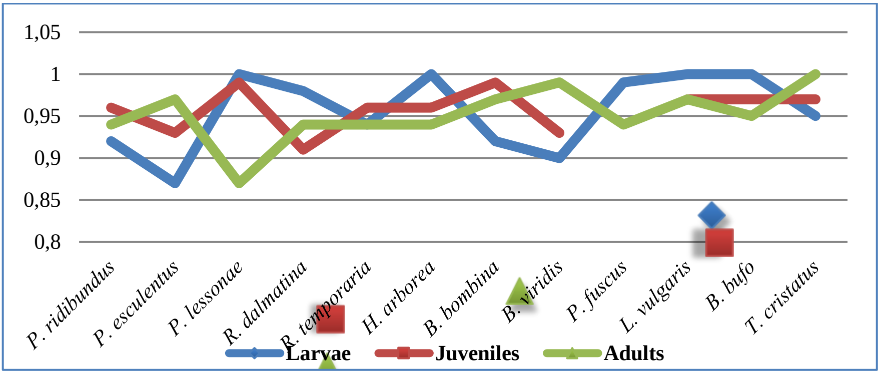

Fig. 5.5. Pearson correlation coefficient in caudate and ecudate amphibians

5.4. Assessment of helminth infestation of amphibians depending on host genus

Helminthological research on host genus-dependent amphibians has demonstrated that the degree of helminth infestation in amphibians depends on both the diversity of invasion and the host genus.

Therefore, the obtained results allowed us to establish that in 9 out of the 12 amphibian species evaluated (*P. ridibundus, P. lessonae, B. bufo, B. viridis, P. fuscus, B. bombina, H. arborea, T. cristatus, L. vulgaris*) there is no significant divergence in the helminth structure, while in 2 amphibian species (*P. esculentus, R. dalmatina*) it was determined that males are characterized by a higher diversity of helminth species than their females, and in *R. temporaria* females are characterized by a higher diversity of helminth species than males (Figure 5.6).

Quantitative analysis of the helminthological indices allowed us to estimate the existence of a highly significant difference in the degree of infestation of females compared to males of *P. ridibundus,* in particular with the trematode species *H. variegatus* with 4.50% (t_d- 13.87; P < 0.001), *C. loossi* with 0.44% (t_d -0.24; P < 0.001), *C. urniger* with 9.55% (t_d -5.01; P < 0.001), with the namtode species *I. neglecta* with 10.91% (t_d -18.93; P < 0.001) and with the acanthocephalan species *A. ranae* with 3.64% (td- 21.59; P < 0.001), *A. lucii* with 2.04% (t_d - 11.10; P < 0.001), and with *P. bufonis* with -0.71% (t_d - 4.44; P < 0.001). In this context, a significant difference in invasiveness of *P. ridibundus* females was recorded with the trematode *P. medias* with 4.11% (t_d -2.03; P < 0.05) compared to males. Assessment of the difference in the degree of infestation of *P. ridibundus* males compared to its females allowed to identify a highly significant probability of the degree of invasion with the trematodes *G. varsoviensis* with 2.42% (t_d -14.51; P < 0.001), *C. complanatum* with 2.60% (t_d -4.12; P < 0.001) and *O. filiformis* with 1.41% (t_d -3.61; P < 0.001). A significant difference in infestation was recorded in males with the species *D. subclavatus* with 2.45% (t_d -2.14; P < 0.05) and *C. ornata* with 3.37% (t_d -3.26; P < 0.01) compared to females.

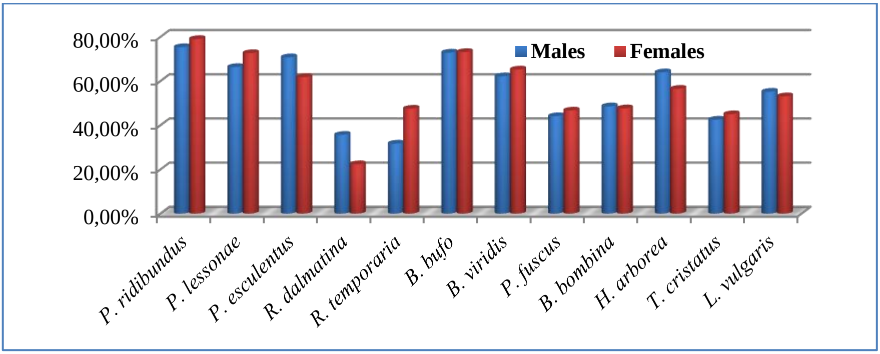

Fig. 5.6. Degree of helminth infestation in amphibians depending on host gender

Quantitative analysis of helminthological indices in the amphibian species *P. lessonae* depending on the host genus, allowed us to register a highly significant difference in the degree of infestation of females compared to the degree of infestation of males, with *H. variegatus* - by 3.02% (t_d- 17.55; P < 0.001), *C. urniger* - by 5.75% (t_d- 16.99; P < 0.001), *S. sphaerula* with - 5.02% (t_d- 12.30; P < 0.001), *P. elegans* - with 8.98% (t_d -9.59; P < 0.001), *C. compolanatum* - with 2.47% (t_d - 4.34; P < 0.001), with nematode species *C. ornata* - by 11.84% (t_d -6.44; P < 0.001), *O. filiformis* - by 2.81% (t_d -5.37; P < 0.001) and *R. bufonis* - by 2.78% (t_d - 6.12; P < 0.001). A highly significant difference in the degree of helminth infestation of males compared to females *of P.*

lessonae species was determined with *P. lessonae* species. *medians* - by 2.94% (t_d -3.32; $P < 0.001$), *D. subclavatus* - by 19.93% (t_d- 26.03; $P < 0.001$), *N. corvinum* - by 2.21% (t_d - 8.82; $P < 0.001$), nematode species *I. neglecta* - by 3.75% (t_d - 6.20; $P < 0.001$), and with the acanthocephalan species *A. rane* - by 7.05% (t_d- 54.23; $P < 0.001$). A significant probability of infestation of males of the species *P. lessonae* compared to their females was established with *G. varsoviensis* - by 0.52% (t_d -2.26; $P < 0.05$) and *M. longicolis,* mtc. - by 0.67% (t_d -2.11; $P < 0.05$). A significant difference in infestation was recorded in females with *O. diplodiscoides* species with 3.86% (t_d -2.25; $P < 0.05$) and *Agamospirura sp. II,* larva with 0.14% (t_d - 0.33; $P < 0.05$) compared to females.

Quantitative analysis of helminthological indices in the amphibian species *P. esculentus,* depending on the host genus, allowed us to detect a highly significant difference in the degree of infestation of females compared to the degree of infestation of host males, especially with the species *O. ranae* - by 3.91% (t_d -6.74; $P < 0.001$), *P. confusus* - by 2.70% (t_d -4.34; $P < 0.001$), *C. urniger* - by 2.85% (t_d -5.74; $P < 0.001$), *H. cylindracea* - with 2.77% (t_d - 92.33; $P < 0.001$), *C. ornata* - with 4.68% (t_d -3.94; $P <0.01$) and with the acanthocephalan species *A. ranae* - with 2.00% (t_d -6.85; $P < 0.001$). At the same time, the quantitative evaluation of the helminthological indices allowed us to determine a highly significant difference in the degree of helminth infestation of males compared to females of the species *P. esculentus*, with the trematode species *H. variegatus* - by 4.21% (t_d - 9.87; $P < 0.001$), *C. retusus* - by 1.83% (t_d - 4.21; $P < 0.001$), *G. varsoviensis* - 2.14% (t_d - 24.88; $P < 0.001$), *D. subclavatus* - 2.38% (t_d - 4.14; $P < 0.001$), *P. robusta* - with 1.48% (t_d - 4.44; $P < 0.001$), as well as the nematode species *O. duboisi* - with 2.43% (t_d - 11.56; $P < 0.001$) and *I. neglecta* - with 2.30% (t_d - 6.35; $P < 0.001$).

Quantitative evaluation of helminthological indices in the amphibian species *R. dalmatina* allowed us to determine the existence of a highly significant difference in the degree of infestation of females compared to that of host males, in particular with the trematode species *O. ranae* - with 33.91% of cases (t_d - 31.20; $P < 0.001$), *H. variegatus* - with 12.71% (t_d - 94.47; $P < 0.001$), *C. retusus* - by 31,50% (t_d - 66,42; $P < 0,001$), *G. viteliloba* - by 10,63% (t_d – 20.74; $P < 0,001$), *P. claviger* - by 19.73% (t_d – 35.85; $P < 0,001$), *P. medians* - by 13.85% (t_d - 8.48; $P < 0.001$), *P. robusta* - by 16.51% (t_d -8.26; $P < 0.001$), *T. excavata* - by 5.31% (t_d - 12.36; $P < 0.001$), *T. stosichi,* mtc. - with 10.63% (t_d - 6.42; $P < 0.001$), nematode species *C. ornata* - with 18.03% (t_d -9.38; $P < 0.001$), *O. duboisi* - with 19.73% (t_d - 78.17; $P < 0.001$), *R. bufonis* - with 4.18% (t_d - 19.73; $P < 0.001$) and with the acanthocephalan species *A. ranae* - with 4.75% of cases (t_d - 14.13; $P < 0.001$). At the same time, the helminthological researches carried out in dependence on the host genus allowed us to establish a highly significant probability of the degree of helminth infestation of males compared to females of the species *R. dalmatina* with the following parasitic agents: *G. varsoviensis* with 1.14% (t_d - 6.33; $P < 0.001$), *D. subclavatus* - with 1.14% of cases (t_d - 12.67; $P < 0.001$) and *A. strongylina* - with 4.37% (t_d - 13.24; $P < 0.001$) of cases. A significant divergent probability of the degree of helminth infestation of male *R. dalmatina* species compared to their females was observed for the nematode species *O. filiformis* - with 2.85% of cases (t_d -2.78; $P < 0.01$) and *S. contorta*, larva with 1.14% (t_d -2.71; $P < 0.01$) of cases.

The helminthological research carried out on the amphibian species *R. temporaria*, depending on the host genus, allowed us to determine a highly significant probability between the degree of infestation with helminths of males compared to females, especially with the trematode species *C. retusus* - with 1.07% of cases (t_d -5.39; $P < 0.001$), *G. vitelliloba* - with 3.07% (t_d – 33.30; $P < 0.001$), *P. confusus* - with 1.07% (t_d -3.88; $P < 0.001$), *D. subclavatus* - with 6.64% (t_d – 20.41; P < 0,001), *P. elegans* - by 5.93% (t_d - 8.91; $P < 0.001$) and with the nematode species *O. filiformis* - by 31.50% (t_d - 98.97; $P < 0.001$) and with the species *Agamospirura sp.* II. - with 17.07% (t_d -4.98; $P < 0.001$) of the cases. At the same time, the research allowed us to identify a highly significant probability of helminth infestation of females compared to males of *R. temporaria* species with *H. cylindracea* - with 5.21% of cases (t_d - 4.95; $P < 0.001$), *C. ornata* - with 4.50% (t_d – 31.27; $P < 0.001$), *O. duboisi* - with 6,07% (t_d – 6.40; $P < 0.001$) and the acanthocephalan species *A. ranae* - with 11.21% (t_d – 21.98; P < 0,001) of the cases. At the same time, according to the evaluation, it was found that there was a semi-significant difference between the infestation of males with *P. medians* - with 2.64% of cases (t_d -2.44; $P < 0.05$), compared to its females.

Quantitative analysis of helminthological indices in the species of ecaudata amphibians *P. fuscus*, allowed us to deduce the existence of a highly significant difference in the degree of infestation of males compared to the degree of infestation of host females, especially with the species *O. ranae -with* 5.37% (t_d - 28.11; $P < 0.001$), *S. sphaerula* with 8.54% (t_d - 12.63; $P < 0.001$), and with the nematode species *C. ornata* the males of the species are characterized by a significant degree of infestation compared to females, and this is with 0.50% (t_d -3.05; $P < 0.01$). A highly significant probability between the degree of invasiveness of females compared to males of the species *P. fuscus* was established with the trematode *H. variegatus* with 3.02% of cases (t_d - 151.00; $P < 0.001$), *P. claviger* - with 5.32% (t_d - 66.50; $P < 0.001$), *P. medians* - with 2.71% (t_d -6.59; $P < 0.001$), *D. subclavatus* - by 4.45% (t_d - 24.99; $P < 0.001$), *P. brumpti*, mtc. - by 3.38% (t_d - 4.01; $P < 0.001$), and the nematode species *O. filiformis* - with 3.61% (t_d - 29.98; $P < 0.001$), *A. strongylina* - with 1.90% (t_d - 15.78; $P < 0.001$) and *R. bufonis* - with 7.33% (t_d - 19.47; $P < 0.001$) of the cases.

The investigations of the species *H. arborea*, depending on the host genus, allowed us to determine a highly significant probability between the degree of helminth infestation of males compared to females, in particular with the trematode species *O. ranae* with 1,77% of cases (t_d -5.90; $P < 0.001$), *P. medians* - with 7.78% (t_d- 39.29; $P < 0.001$), *C. ornata* - with 9.80% (t_d – 18.32; $P < 0.001$), *O. duboisi* - with 4.89% (t_d – 40.61; $P < 0.001$), *Agamospirura sp. II* - by 5.82% (t_d - 15.85; $P < 0.001$), *A. ranae* - by 3.03% (t_d - 67.75; $P < 0.001$) and *Agamospirura sp.* II. - with 17.07% (t_d - 4.98; $P < 0.001$) of the cases. A highly significant probability was established between the degree of helminth infestation of females compared to males, with *D. subclavatus* species with 3.85% (t_d - 30.06; $P < 0.001$), *S. sphaerula*, mtc. with 3.52% (t_d -9.57; $P < 0.001$), *O. filiformis* with 5.63% (t_d - 10.08; $P < 0.001$) and *I. neglecta* with 2.49% (t_d - 11.35; $P < 0.001$).

Investigations of the species *B. bombina* allowed us to determine a significant high threshold of infestation of males with *H. variegatus* - with 4.74% of cases (t_d - 25.48; $P < 0.001$), *G. vitelliloba* - with 0.46% (t_d -7.18; $P < 0.001$), *P. claviger* - with 5.95% (t_d -

31.14; P < 0.001), *H. cylindracea* - by 1.83% (t_d -3.64; P < 0.001), *P. cloacicola* - by 2.73% (t_d - 22.67; P < 0.001), *S. falconis* mtc. - by 1.68% (t_d -4.48; P < 0.001), *R. bufonis* - by 0.76% (t_d - 17.91; P < 0.001) and *A. ranae - by* 8.26% (t_d - 105.76; P < 0.001) of cases compared to the phelemmas. Similarly, a significant high infestation threshold of females with *O. ranae* pecia was detected with 7.37% of cases (t_d - 43.28; P < 0.001), *P. medians* - with 3.62% (t_d - 16.51; P < 0.001), *P. confusus* - with 2.57% (t_d -7.87; P < 0.001) and *C. ornata* - with 2.06% (t_d - 11.20; P < 0.001) of cases compared to males.

In contrast to the above-mentioned species of ecaudata amphibians, the helminthologic research carried out on bufonids allowed the identification of a highly significant degree of infestation of *B. bufo* males with parasitic agents such as *O. filiformis* with 9.39% of cases (t_d - 16.59; P < 0.001), *O. duboisi* - with 9.78% (t_d - 18.66; P < 0.001) and a significant degree with *R. bufonis* - with 2.79% (t_d -2.47; P < 0.05) of cases compared to females. However, in females *of B. bufo* species there was a highly significant degree of infestation with the monogenean species *P. integerrimum* with 2.06% (t_d - 36.42; P < 0.001) of cases compared to males.

In the second amphibian species of the Bufonidae family - *B. viridis*, according to the researches carried out depending on the host genus, a highly significant degree of infestation of the females of the species was determined only with *O. ranae* in 0.65% (t_d - 8.32; P < 0.001) of cases, compared to males, and in males a highly significant degree of infestation with *S. sphaerula* with 5.17% (t_d - 45.70; P < 0.001) and with *P. integerrimum* with 0.30% (t_d -3.54; P < 0.001) compared to females, and with the trematode species *H. cylindracea* with 5.30% (t_d -1.98; P < 0.05) and *O. filiformis* with 2.76% (t_d -2.98; P < 0.01) of cases a significant infestation of males compared to females.

The helmintological research carried out on the species of caudata amphibians depending on their genus, allowed us to observe a significant high degree of infestation of females of the species *T. cristatus* with *median P.* of 13.78% of cases (t_d - 56.92; P < 0.001), *P. confusus* - with 1.72% (t_d - 17.37; P < 0.001), *D. subclavatus* - with 5.32% (t_d - 27.69; P < 0.001), *C. ornata* - with 3.64% (t_d -4.30; P < 0.001) and *O. filiformis* - with 77.27% of cases (t_d - 340.82; P < 0.001), compared to males. At the same time, a significant threshold of infestation with *P. medians* was established in males of *T. cristatus* species 3.91% of cases (t_d - 16.26; P < 0.001), *P. brumpti* mtc. - with 6.40% (t_d - 15.86; P < 0.001) and *O. duboisi* - by 5.37% (t_d - 74.47; P < 0.001), and *O. ranae* were found to have a significant threshold of infestation with *O. ranae* with 2.33% of cases (t_d - 2.78; P < 0.01) compared to females.

Quantitative analysis of the helminthological indices of the species *L. vulgaris*, depending on the host genus, allowed us to find a significant high threshold of infestation of females with *O. ranae* with 25.89% of cases (t_d - 58.46; P < 0.001), *S. sphaerula* mtc. - with 12.78% (t_d - 91.99; P < 0.001) and *C. ornata* - with 3.74% of cases (t_d - 10.37; P < 0.001) compared to males, and with the trematode species *H. volgensis* a significant degree of infestation was established with 0.41% of cases (t_d -3.01; P < 0.01) compared to males. At the same time, a significant high degree of infestation was established in males of *L. vulgaris* species with *P. claviger* with 4.55% of cases (t_d - 16.08; P < 0.001), *P. medians* - with 5.78% (t_d - 15.32; P < 0.001), *D. subclavatus* - with 23.66% (t_d - 34.35; P < 0.001), *O. filiformis* - with 3.62% (t_d - 18.10; P < 0.001), *O. duboisi* - with 6.70% (t_d -

31.31; P < 0.001) and *Agamospirura sp. II* with 2.59% (t_d - 15.21; P < 0.001) of cases compared to females.

Therefore, according to the results obtained, it was determined that out of the 48 species of helminths detected in the amphibians surveyed, 18.75% of them had a significant high threshold of infestation of females compared to males, 8.33% - a significant high threshold of infestation of males compared to females, 12.5% - a significant high degree of infestation in both males and females, 10,42% of - a significant high threshold of infestation in amphibian males only, 10.42% - a significant high threshold of infestation in females only, 20.83% - a significant high threshold of infestation in males compared to females, 8.33% - a significant high threshold of infestation in females compared to males, and 10.42% did not show a difference between the infestation of females compared to males.

5.5. Assessment of helminth infestation of amphibians depending on the season

Seasonal changes in parasitic fauna are complex phenomena, driven by a variety of abiotic and biotic environmental factors, closely linked to changes in host nutrition and lifestyle, which affect susceptibility to parasitic infestation [8, 13].

The knowledge of the spread of parasitosis by amphibians on the territory of our country is of great importance in the zoning of invasions and allows to specify the seasonal periods in which to act by differentiated application of complex biological measures for prophylaxis and control of the most aggressive parasites.

The helminthological surveys, carried out in caudata and ecaudata amphibians, depending on the sequence of seasonal factors, allowed to determine a variation in the structure of the helminth fauna in amphibians and in the main helminthological indices (Figure 5.7).

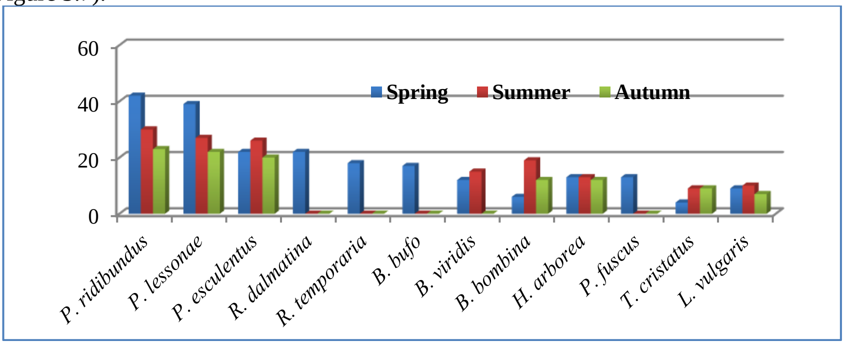

Fig. 5.7. Diversity of the helminthic fauna of caudate and ecaudate amphibians depending on the season

According to the helmintological analysis on the determination of the structure of the helminthic fauna of amphibians, it was found that in 50,00% of the amphibian species evaluated (*P. ridibundus, P. lessonae, R. dalmatina, R. temporaria, B. bufo, P. fuscus*), the diversity of their helminthic fauna is predominant during the spring season, in 33,33% of the cases in amphibians (*P. esculentus, B. viridis, B. bombina, L. vulgaris*) the infestation predominates in the summer season, in 8,33% of the species (*H. arborea*) the infestation with helminths was found to be predominant in the spring and summer seasonal periods, and in 8,33% of the cases (*T. cristatus*) the infestation with parasitic agents was predominantly recorded in the summer and fall seasonal periods.

Therefore, almost all the parasitic agents detected in caudate and ecaudate amphibians, evaluated under the direct influence of the sequence of environmental factors, demonstrated clear evidence of their seasonal dynamics. Thus, data analysis allowed us to determine that out of the total number of helminth species (n=48) detected in amphibians, 95.8% of species (n=46) infested amphibians in the spring season, 91.7% of species (n=44) infested amphibians in the summer season, and 87.5% of species (n=42) infested amphibians in the fall season. However, 81.25% of the species (n=39) infested caudata and ecaudata amphibians during the three spring-summer-fall seasons, and 85.5% of the species (n=39) infested caudata and ecaudata amphibians during the two spring- summer seasons, 85.42% of the species (n=41) infested captive and ecaudata amphibians during two spring-fall seasons, and 85.42% of the species (n=41) infested captive and ecaudata amphibians during two spring-spring seasons.

Whereas the only characteristic in the dominance of amphibian-specific parasitic agents is reflected in the literature by the presence of intermediate hosts, which amplify parasite transmission rates [18], our data suggest that, along with these and intrinsic factors that contribute to the formation of amphibian-specific helminthic fauna, this variable dynamic of amphibian parasitic agent infestation is valid with changing seasonal factors.

5.6. Assessment of helminth infestation of amphibians by area

In order to analyze the distribution of parasitic agents specific to caudate and ecaudate amphibians, as well as the increased risk of infection of fish, birds, wild animals, domestic animals, pets and humans with parasitic agents, helminthologically investigated amphibians from various natural and anthropized ecosystems of the Central, Northern and Southern parts of the republic.

Area-dependent helminth surveys of caudata and ecaudata amphibian species have allowed us to detect a diversity of helminth fauna that varies from area to area and from host to host (Figure 5.8).

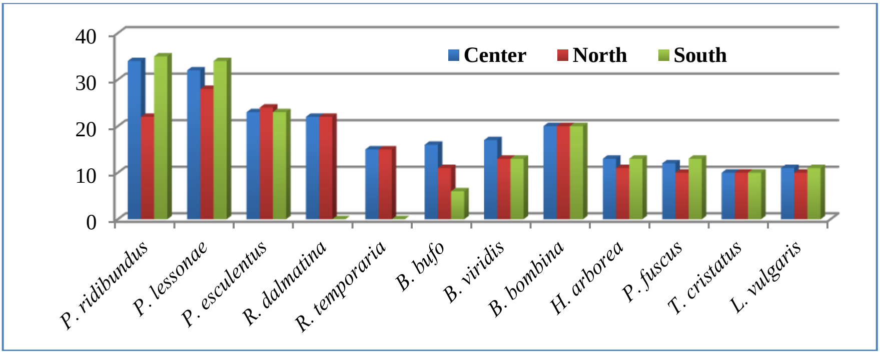

Fig. 5.8. Diversity of helminthic fauna in amphibians depending on the area

According to the obtained data, it was found that 25% of the assessed amphibian species (*P. ridibundus, P. lessonae, P. fuscus*) have a higher diversity of helminth fauna in the southern part of the republic, 8.3% of the amphibian species (*P. esculentus*) have a higher diversity of helminth fauna in the northern part of the republic, 16.7% of the amphibian species (*B. bufo, B. viridis*) have a higher diversity of the helminth fauna in the central part of the republic, 33.3% of amphibian species (*R. dalmatina, R. temporaria, B. bombina, T. cristatus*) have a higher diversity of the helminth fauna in the central and northern part of the country, and 16.7% of amphibian species (*H. arborea, L. vulgaris*) have a higher diversity of the helminth fauna in the central and southern part of the country.

The scientific data, obtained as a result of the investigations in the Central part of the republic, allowed us to find that 41.7% of the amphibians evaluated (*P. ridibundus, P. lessonae, B. viridis, B. bombina, P. fuscus*) have a higher parasite load in anthropized areas, 25% of the amphibians evaluated (*R. dalmatina, R. temporaria, B. bufo*) have a higher parasite load in natural areas, and 33.3% of the amphibians assessed (*P. esculentus, H. arborea, T. cristatus, L. vulgaris*) have a constant parasite load in both natural and anthropized biotopes.

The results obtained in the northern part of the republic allowed us to identify that 58.3% of the evaluated amphibians (*P. ridibundus, R. dalmatina, B. bufo, B. bombina, H. arborea, T. cristatus, L. vulgaris*) have a higher parasite load in anthropized areas, 8.33% of the evaluated amphibians (*P. lessonae*) have a higher parasite load in natural areas, and 33.3% of the assessed amphibians (*P. esculentus, R. temporaria, B. viridis, P. fuscus*) have a constant parasite load in both natural and anthropized biotopes.

The results of the heminthological evaluation of caudata and ecaudata amphibians in the southern part of the republic allowed us to note that 40% of the amphibians evaluated (n=10) (*P. ridibundus, P. lessonae, B. viridis, B. bombina*) have a higher parasite load in natural areas, while 60% of the evaluated amphibians (*P. esculentus, P. fuscus, B. bufo, H. arborea, T. cristatus, L. vulgaris*) show a constant parasite load in both natural and anthropized biotopes.

The evaluation of the helminthological indices of amphibians, depending on the type of biotope and geographical areaallowed to determine certain comparisons of the structure of the helminthic fauna of amphibians depending on the type of biotope, which changed depending on the area and host species, with a decreasing trend in the parasite load of synanthropic host species (*P. ridibundus, P. lessonae, B. viridis, B. bombina*) in natural ecosystems in the central part of the republic and an increasing trend in parasite load in ecosystems in highly anthropized areas. However, in amphibian species such as *P. esculentus, R. dalmatina, R, temporaria, B. bufo, T. cristatus* and *L. vulgaris,* according to our investigations, a tendency of maintenance of parasite load in both natural and anthropized biotopes, or a tendency of increasing parasite load only in natural biotopes was illustrated.

According to the helmintological investigations carried out on amphibians in the northern part of the republic, a tendency of slightly significant increase in the parasite load of the species *P. ridibundus, R. dalmatina, B. bufo, B. bombina, H. arborea, T. cristatus* and *L. vulgaris* in anthropized aquatic and terrestrial ecosystems, and in the southern part of the republic there was an increasing trend of parasite load for all amphibian species in natural aquatic and terrestrial ecosystems.

The assessed species of synanthropic amphibian species, thanks to their amphibiont mode of life (terrestrial, aquatic), show a high degree of adaptability to their living environment with increased anthropogenic impact, inhabit areas populated by humans and their livestock or pets, and as a result of these ecological interrelationships there is a change in the ecological balance specific to the given ecosystem. Such a change leads to the formation of the relationship in the parasite - amphibian (host) system and to

the formation of outbreaks of dangerous parasitic agents among wild animals (*Agamospirura sp.II, A. strongylina, C. aluconis, C. aluconis,T. excavata, N. major, N. spathoides, N. corvinum, I. melis, P. cordatum, C. complanatum, P. elegans, S. lupi, P. sexalatus, S. teres, S. sphaerula, S. falconis, C. urniger, P. robusta*), domestic (*A. strongylina, P. elegans, S. lupi, P. sexalatus*), pet (*T. canis*) and human (*T. canis, C. complanatum, P. elegans, P. bufonis*).

High intensity record of green frog species (*P. ridibundus, P. lessonae, P. esculentus*) with helminth species *S. sphaerula* (*II* - 87,75 ex.), *S. falconis,* mtc. (*II - 134,33* ex.), *Agamospirura sp. II* (*II* – 184,0 ex.), *T. canis L. III* (*II* – 136,5 ex.), *A. lucii* (*II* – 209.14 ex.), *N. major* mtc. (*II* – 114.0 ex.), *T. excavata* mtc. (*II* – 143.0 ex.), *S. lupi (II* – 96.0 ex.), *A. strongylina* (*II* – 86.0 ex.), in the natural and anthropized ecosystems of the central, northern and southern parts of the republic represent reliable relations of formation and maintenance of stationary outbreaks of helminths dangerous for wild animals, domestic pets and humans.

Establishment of high intensity of brown ranid species (*R. dalmatina, R. temporaria*) with helminth species *P. sexalatus* (*II* – 147.0 ex.) in natural and anthropized ecosystems of the Central and Northern part of the republic represent reliable relations of formation and maintenance of stationary outbreaks of dangerous parasitic agents.

Determination of high intensity of bufonids (*B. bufo, B. viridis*) with helminth species *P. sexalatus* (*II* – 60.50 ex.), *S. lupi* (*II* – 77.0 ex.), *A. strongylina* (*II* – 258.0 ex.), in their specific natural and anthropized ecosystems in the Central, Northern and Southern parts of the republic represent reliable relations of formation and maintenance of stationary outbreaks of dangerous parasitic agents.

The high intensity record of *H. arborea* with the helminth species *S. falconis,* mtc. (*II – 134.33* ex.), of the amphibian species *P. fuscus* with the helminth species *N. spathoides* mtc. (*II* – 139.75 ex.) and of *B. bombina* with the helminth species *T. excavata* mtc. (*II* – 206.75 ex.) in their specific natural and anthropized ecosystems in the central, northern and southern parts of the country represent reliable relations for the formation and maintenance of stationary outbreaks of dangerous parasitic agents.

The finding of high intensity of caudaceous amphibians (*T. cristatus, L. vulgaris*) with the helminth species *S. contorta* (*II* – 68.54 ex.) in their specific natural and anthropized ecosystems in the Central, Northern and Southern parts of the country represents reliable relations of formation and maintenance of stationary outbreaks of dangerous parasitic agents.

Thus, the formation of these outbreaks of parasitic agents dangerous to different species of animals and humans has occurred as a result of a sharp increase in environmental contamination with parasitic agents, their accumulation in aquatic biotopes populated by amphibians and their adjacent areas and consequently the presence of intermediate hosts, obligatory for helminth species detected in amphibians, which serve as a trophic base for the species of caudata and ecaudata amphibians.

6. THE ROLE OF AMPHIBIANS AS BIOINDICATORS AND VECTORS IN THE FORMATION AND MAINTENANCE OF PARASITIC ZOONOSES OUTBREAKS

6.1. The role of caudata and ecaudata amphibians as bioindicators of ecosystems and as hosts of parasitic agents

Thanks to the fact that amphibians are organisms extremely sensitive to the action of various environmental factors, they are also species that, due to their biological, morpho-physiological, ecological, ethological and helminthological peculiarities, allow characterization of the state of the ecosystems populated by them and highlight, as early as possible, natural or anthropogenic changes in it [15, 31].

Helminthological research on amphibians has revealed the presence of 48 species of helminths, in whose evolutionary cycle several types of hosts necessarily participate, reflecting the ecological status of amphibian-populated ecosystems. By identifying the diversity of the helminth fauna of amphibians, but also by deciphering the evolutionary cycle of each helminth species, the presence of different groups of vertebrates and invertebrates was established (Figure 6.1).

In addition to the faunistic importance of the research, amphibians are definitive hosts for several classes of helminths, including Cestoda, Monogenea, Trematoda, Secernentea and Palaeacanthocephala [10, 12]. Furthermore, amphibians serve as intermediate hosts [10, 16, 20] or paratenic hosts [10, 17, 23,] for a wide variety of vertebrate-specific helminths.

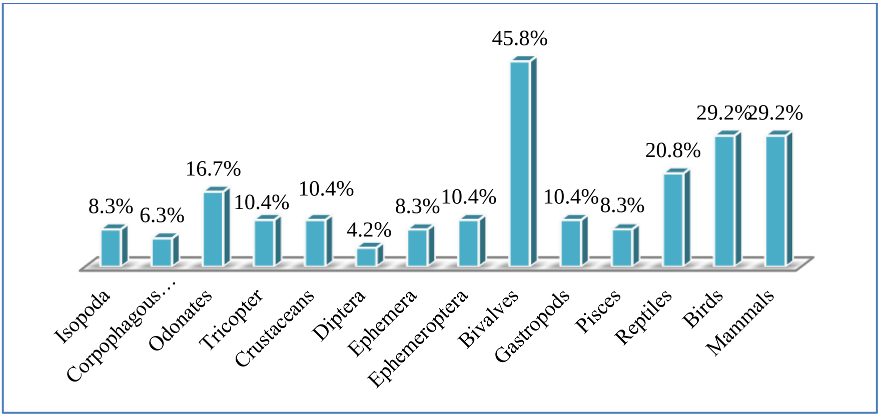

Fig. 6.1. The role of caudate and ecaudate amphibians as bioindicators

According to our data, it was identified that for the 47 species of established parasitic agents, adult forms of amphibians serve as definitive hosts in 44.7% of cases (n=21 species), as intermediate hosts in 36.2% of cases (n=17 species), and as paratenic hosts in 19.1% of cases (n=9 species) (Figure 6.2).

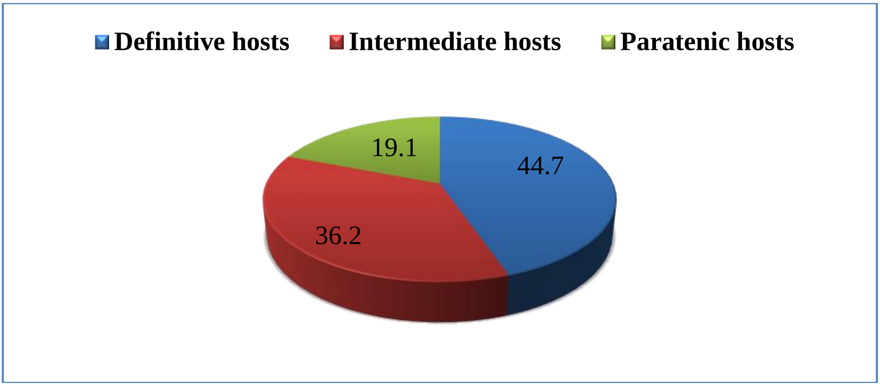

Fig. 6.2. The role of adult amphibians as hosts of helminths

Thanks to the different ethological peculiarities of amphibians in their living environment, as well as the differentiated structure of the helminth fauna depending on the host species, the role of amphibians in dependence on the host species was assessed (Figure 6.3).

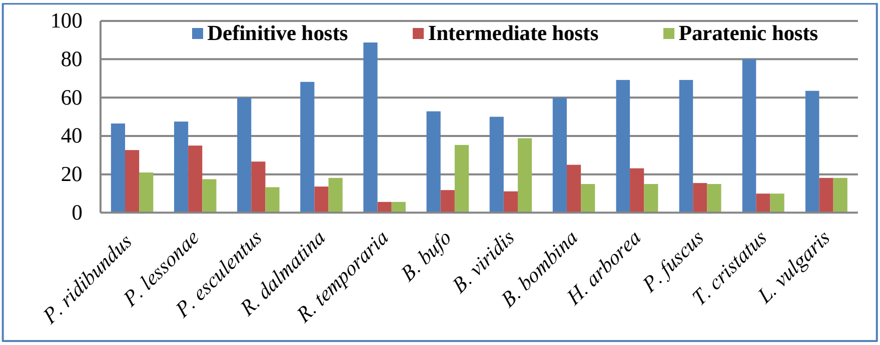

Fig. 6.3. Specific host types

According to the data obtained, all the 12 amphibian species evaluated were found to fulfill the role of definitive, intermediate and paratenic hosts for the 47 helminths species. However, 41.7% of these (*P. ridibundus, P. lessonae, P. esculentus, B. bombina, H. arborea*) serve predominantly as intermediate hosts compared to their role as paratenic hosts, 25% of the species (*R. dalmatina, B. bufo, B. viridis*) serve predominantly as paratenic hosts compared to their role as intermediate hosts, and 33.3% of amphibians (*R. temporaria, P. fuscus, T. cristatus, L. vulgaris*) show at the same level their role as both intermediate and paratenic hosts.

6.2. The role of amphibians as vectors in the formation and maintenance of outbreaks of dangerous parasitic agents

Ecosystem biodiversity plays an important role in supporting human well-being, including regulating the transmission of helminths and infectious diseases. Many of these services are not fully appreciated due to complex environmental dynamics and lack of baseline data. This previously unrecognized impact of biodiversity loss illustrates the often-hidden costs of failures to conserve human well-being [14].

Amphibians, as definitive, intermediate and complimentary hosts for different species of parasitic agents, are "vector" organisms which, being obligatory in the development of parasites, constitute the favorable environment for the penetration, development and conservation of the evolutionary forms of parasitic agents common to wild, domestic, pet and human animals.

According to helminthological researches, carried out in the Republic of Moldova, it was found that out of 178 parasitic agents established in wild animals, 20 species were recorded in humans and domestic animals [7, 27]. According to the helminthological investigations, carried out on equalized and caudatidae amphibians, the presence of 28 species of helminths was established, which cause outbreaks of parasitic agents dangerous for wild, domestic, pet and human animals.

Among the parasitic diseases of domestic and wild vertebrates, in which amphibians play an important role, are **spirocercosis** caused by the nematode *Spirocerca lupi* Rudolphi, 1809, **ascaropsosis** - by *Ascarops strongylina* larva Rudolphi 1819, **histriciosis** - by *Hystrichis tricolor* Dujardin 1845, **phisocephalosis** - by *Physocephalus sexalatus*, larva Molin 1860, **spiroxosis** - by *Spiroxys contorta* larva Rudolphi 1819, **codonocephalosis** - by *Codonocephalus urniger* Rudolphi 1819, **parastrigeoysis** - by *Parastrigea robusta* Szidat 1928, **strigeoysis** - by the species *Strigea falconis* Szidat 1928 and *Strigea sphaerula* Rudolphi 1803, **tylodelphiosis** - by *Tylodelphys excavata* etc., **plagiorchiosis** - by *Plagiorchis elegans* Rudolphi 1802 and **toxocariasis** in humans caused by *Toxocara canis* Werner, 1782.

In order to determine the risk of infestation of definitive hosts (reptiles, birds, mammals, humans) by amphibians, the presence of dangerous parasitic agents in amphibians was determined depending on the area, the season favorable to infestation, the host species and the age of the host. According to the results obtained, concerning the degree of vectorization of parasitic agents, depending on the age structure of the host, it was established that amphibians throughout their entire life cycle (larvae, juveniles, adults) contribute directly to the vectorization of dangerous parasitic agents. Thus, for 17.9% of the dangerous helminth species detected (*I. melis, P. robusta, S. falconis, S. sphaerula*) amphibians vector them from the larval, juvenile and adult stages of development, for 42.8% of the dangerous helminth species detected (*A. lucii, A. strongylina, Agamospirura sp II., C. urniger. H. volgensis, N. spathoides, P. brumpti, P. elegans, S. lupi. S. teres, T. canis, T. excavata*) amphibians vector them in the juvenile as well as in the adult stage, for 32.1% of the dangerous helminth species detected (*C. aluconis, C. complanatum, H. tricolor, M. longicolis, N. corvinum, N. major, P. cloacicola. P. cordatum, P. sexalatus*) amphibians vector them only at the adult stage, for 3.6% of the dangerous helminth species detected (*M. gracillimus*) amphibians vector them only at the juvenile stage, and for 3.6% of the dangerous helminth species detected (*T. stossichi*) amphibians vector them at both the larval and adult stages of development

(Figure 6.4).

Taking into account the phenology of amphibians, their role as vectors was assessed according to the length of the active period of the annual cycle. Thus, the amphibian species with early reproduction and higher annual cycle activity (*R. dalmatina, R. temporaria, B. bufo, B. viridis*) for 16.7% of the detected dangerous helminth species (*P. robusta, S. sphaerula*) vector them both in the larval, juvenile and adult stages, for 8.3% of the dangerous helminth species detected (*T. stossichi*) amphibians vector them both in the larval and adult stages, for 25% of the dangerous helminth species detected (*Agamospirura sp II, P. elegans, T. excavata*) amphibians vector them at the juvenile and adult stage, and for 50% of the dangerous helminth species detected (*A. strongylina, C. aluconis, P. sexalatus, S. contorta, S. lupi, S. teres*) amphibians vector them only at the adult stage (Figure 6.5a).

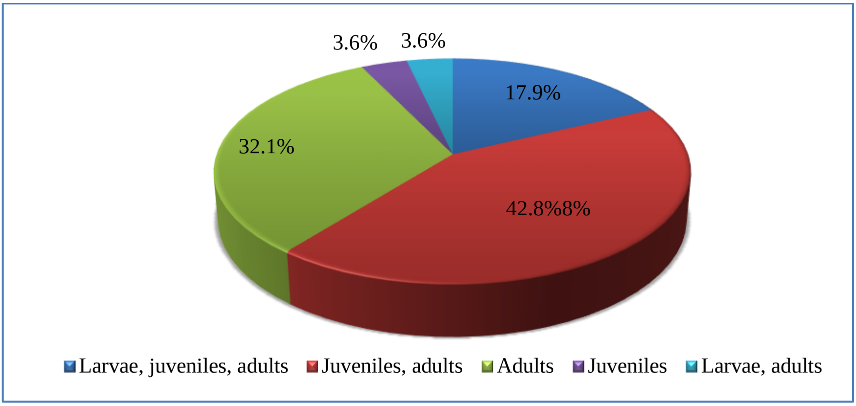

Fig. 6.4. The degree of vectorization of parasitic agents depending on the age structure of the host

The amphibian species with late reproduction and lower annual cycle activity (*P. ridibundus, P. lessonae, P. esculentus, B. bombina, H. arborea, P. fuscus, T. cristatus, L. vulgaris*) for 14.8% of the detected dangerous helminth species (*I. melis, S. contorta, S. falconis, S. sphaerula*) vector them in the larval, juvenile and adult stages, for 3.7% of the dangerous helminth species detected (*P. robusta*) amphibians vector them in both larval and adult stages, for 37% of the dangerous helminth species detected (*A. lucii, A. strongylina, C. urniger, H. volgensis, N. spathoides, P. brumpti, S. lupi, S. teres, T. canis, T. excavata*) amphibians vector them at both juvenile and adult stages, for 3.7% of the dangerous helminth species detected (*M. gracillimus*) amphibians vector them only at the juvenile stage, and for 40.8% of the dangerous helminth species detected (*C. aluconis, C. complanatum, H. tricolor, M. longicollis, N. corvinum, N. major, P. cloacicola, P. cordatum, P. elegans, P. sexalatus, T. stossichi*) amphibians vector them only at the adult stage (Figure 6.5b).

According to the obtained data we can conclude that amphibians belonging to the category of species with a late reproductive period and a shorter contact period with their living environment are vectors for a higher diversity of dangerous helminth species, and this reflects their role in the formation and maintenance of outbreaks of parasitic zoonotic diseases.

Therefore, ***spirocercosis*** can be established in carnivores (dog, fox, wolf), and incidentally also in goats, horses, cattle, pigs, etc., throughout the republic, ***ascariasis*** - in domestic pigs, wild boars, rodents, birds and reptiles throughout the republic, ***histriciosis*** and ***proventriculitis*** with large visible nodular lesions - in geese throughout the republic, ***phisocephalitis*** - in domestic pigs and wild boars, occasionally also in field crows throughout the republic, ***toxocariasis*** - in cats, dogs and humans in the southern part of the republic, ***codonocephalosis, strigeollosis*** and ***parastrigeosis*** - in birds throughout the republic, ***tylodelphtholosis*** - in fish and birds in the southern part of the republic, ***clinostomosis*** - in fish, birds and occasionally in humans in the central and northern part of the republic, ***holostephanosis*** - in domestic and wild birds, ***isthmiophthorosis*** and ***pharyngostomosis*** - in fish, birds and mammals*:* dogs (*Canis familiaris*), cats (*Felis catus, Felis sylvestris*), wolves (*Canis lupus*), foxes (*Vulpes vulpes*) and raccoon dogs (*Genetta tigrina aequatorialis*) in the Central part of the republic, ***neodiplostomosis*** - in reptiles and birds of prey in the Central and Southern part of the republic**,** ***plagiorchioosis*** - in birds and humans throughout the country and ***acanthocephalosis*** in fish in the Central and Southern part of the republic.

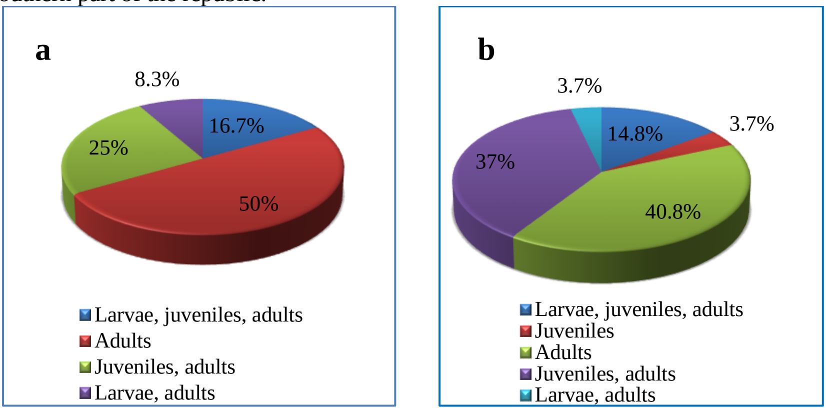

Fig. 6.5. Degree of vectorization of dangerous helminths by early (a) and late (b) reproductive amphibians

Therefore, the evaluation of the obtained data allowed us to determine the age of the host, the period and areas of infection of definitive hosts (fish, reptiles, birds, mammals) by amphibians, which is particularly important for determining their role as vectors in the formation and maintenance of outbreaks of parasitic zoonoses, and the distribution and dynamics of the occurrence of parasitic agents in a given environment, time and in different hosts, as well as the factors regulating the host-parasite relationship at the individual, or population level, represent a rather complex study in terms of addressing in depth the various biological, ecological and helminthological aspects of both amphibians as host organisms and the parasite.

Therefore, according to the evaluation of the obtained results, it was established that amphibians are reliable sources of vectorization of common helminths for fish with 12.5% of helminth species (*A. lucii, C. complanatum, G. varsoviensis, H. tricolor, H. volgensis, I. melis*), for reptiles - with 27.1% of helminth species (*A. strongylina, C. complanatum, M. gracillimuls, M. longicolis, N. major, N. spathoides, N. corvinum, P. brumpti, P. cloacicola, P. elegans, P. sexalatus, S. contorta, T. stossichi*), for birds - with 37.5% of helminth species (*A. strongylina, C. complanatum, C. aluconis, C. urniger, H. tricolor, H. volgensis, I. melis, N. major, N. corvinum, N. spathoides, P. elegans, P. robusta, P. sexalatus, S. falconis, S. sphaerula, S. lupi. S. teres, T. excavata*), for mammals - with 18.8% of helminth species (*A. strongylina, Agamospirura sp. II, C. aluconis, I. melis, P. cordatum, P. elegans, P. sexalatus, S. lupi, T. canis*), and for humans - with 6.3% of the determined helminth species (*P. elegans, I. melis, C. complanatum, T. canis*) (Figure 6.6).

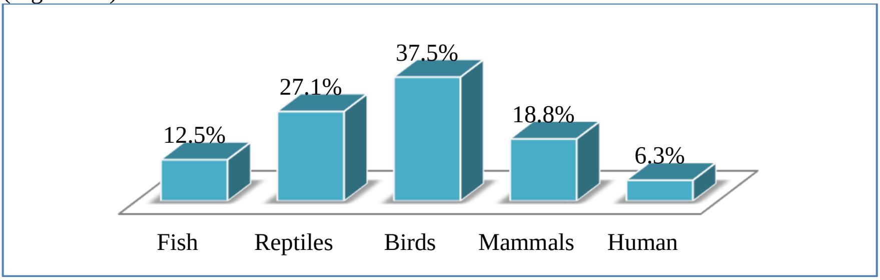

Fig. 6.6. The degree of vectorization of parasitic agents by amphibians of various animal groups

6.3. The role of amphibians in the biological control of parasitic zoonoses in livestock

The most common diseases recorded in livestock are endo-parasitic diseases, which cause major economic damage. One of the most common parasitic diseases in livestock is fasciolosis, caused by the trematode *Fasciola hepatica* [7, 9]. Considerable changes occur in the host's body, both in the liver and in the muscle tissue, which lead to a considerable reduction in the quality of these products [7]. At the same time, the multiple actions aimed at increasing the qualitative and quantitative productivity of ruminants will not be effective until concrete measures to control fasciolosis are taken.

The infestation of ruminants is achieved by the consumption of metacercariae of *Fasciola hepatica* (the infesting form), either by grazing or drinking water from biotopes favorable for the development of fasciolosis. The development cycle of *Fasciola hepatica* involves the mollusc species *Lymnaea truncatula*, which is an important food source for amphibians, and these in turn can be definitive and facultative hosts in the development cycle of various helminth species such as *Haplometra cylindracea* [5, 7, 11].

Both the trematode species *Haplometra cylindracea* and *Fasciola hepatica*, in their development cycle, parasitize the snail species *Lymnaea truncatula* as intermediate host in the cercarial stage, and the trematodes, meeting in the same host (*Lymnaea truncatula*), *Haplometra cylindracea* antagonizes *Fasciola hepatica*, thus causing its death [5, 14, 26].

In the snail *the miracidium-mold* transforms into *sporocyst 1*. This divides into *sporocyst 2,* then into *redia, the daughter redii,* migrate into the snail's hepatopancreas, where they transform into *cercaria*. *The cercaria* has a digestive tract and a tail. Several hundred cercariae are usually shed from a snail. After emerging from the snail, the cercariae attach themselves to the grass with the help of chistiogenic glands, which secrete a coat and develop into the larvae known as *adolescarids.* The entire life cycle lasts for 2 2.5 months. Metacercariae are very resistant to environmental conditions (they are the cystic form), they persist on grass until fall (the disease is more frequent in the fall). Cattle become infected *with Fasciola hepatica*, either when grazing grass with metacercariae in wet biotopes or by eating hay already infested. Once in the liver, they pass through the Glisson capsule, which is cyoresitized where the young Fasciolae pass through due to their mechanical (cell breaking) and toxic action. From the host organism (cattle) egg shedding starts after 2-3 months. The total life cycle is 5-6 months [26].

According to the helmintological investigations carried out in amphibians, it was determined that amphibian species are infested with the trematode species *H. cylindracea* throughout the republic, thus in *P. ridibundus* infestation was recorded in 7.49% of cases (*II* - 26.21 ex.), *P. lessonae* is infested with the trematode species *H. cylindracea* in 1,26% of the cases (*II* - 22,0 ex.), *P. esculentus* - in 6,33% of the cases (*II* - 1,05 ex.), *R. dalmatina* - in 7,93% of the cases (*II* - 10,60 ex.), *R. tamporaria* - in 20,75% of the cases (*II* - 20,0 ex.), *B. viridis* - in 24,55% of the cases (*II* - 25,04 ex.), and *B. bombina* is infested with the trematode species *H. cylindracea* in 7,03% of the cases (*II* - 17,00 ex.).

In order to determine the antagonistic relationships between the trematode species *H. cylindracea* and *F. hepatica*, both field and laboratory studies were carried out. Initially, miracidia of *Fasciola hepatica* and *Haplometra cylindracea* were obtained. To accomplish the proposed aim, adult fasciolae were collected from animals slaughtered at the slaughterhouse, from which eggs were obtained. In a thermostat, in the dark and at a temperature of 24-26^{o}C, *Fasciola hepatica* miracidia were obtained from the eggs over 10 days. Similarly, miracidia of *Haplometra cylindracea* were obtained from amphibians (*P. ridibundus, P. lessonae, P. esculentus, R. temporaria, R. temporaria, B. bombina, B. viridis*). Specimens *of Haplometra cylindracea* were kept alive in a thermostat at a constant temperature of 37^{0} C in physiologic solution.

For the realization of the fascioliasis control process under laboratory conditions, the experiments were carried out in 3 glass vessels of identical size and following the same scheme (Table 6.1).

In the first glass jar, with a volume of 0.5 liter of water, 50 miracidia *of Fasciola hepatica* and 50 miracidia *of Haplometra cylindracea* were placed in a ratio of 1:1. In the second glass vessel, also with a volume of 0.5 liters of water, 50 *Fasciola hepatica* and 25 *Haplometra cylindracea* miracidia were placed in a ratio of 2:1. In the third glass vessel, with a volume of 0.5 liters of water, only *Fasciola hepatica* miracidia were placed (control batch).

Within 14 hours, at regular time intervals (T=24h), the number of miracidia *of Fasciola hepatica* and *Haplometra cylindracea* was calculated.

Significant results were obtained during the experiments in pots No. 1 and No. 2 from the first hour of contact between *Fasciola hepatica* and *Haplometra cylindracea*. As a result in pot No. 1, in which the species were introduced in a 1:1 ratio, *Fasciola hepatica* miracidia were absent from the 8th hour of the survey. In pot No. 2, in which the species were in a 2:1 ratio, *Fasciola hepatica* miracidia were absent from the 9th hour of the survey. In pot No. 3 (control), there was virtually no numerical change in *Fasciola hepatica* miracidia, which shows its presence in the absence of the antagonistic species *Haplometra cylindracea* [26].

Table 6.1. Result of antagonistic action of *Haplometra cylindracea* miracidia on *Fasciola hepatica* miracidia

Nr. hours	Vessel No. 1		Vessel no. 2		Vessel no. 3	
	No. of *miracidium F.hepatica*	No. of *H.cylindracea*	No. of *F.hepatica*	No. of *H.cylindracea*	No. of *F.hepatica*	No. of *miracidium H.cylindracea*
1	50	50	50	25	50	-
2	49	50	48	25	50	-
3	32	50	40	25	50	-
4	24	50	35	25	50	-
5	16	50	22	25	50	-
6	7	50	14	25	50	-
7	3	50	8	25	50	-
8	0	50	2	25	50	-
9	0	50	0	25	50	-
10	0	49	0	25	50	-
11	0	49	0	25	50	-
12	0	49	0	25	49	-
13	0	49	0	24	49	-
14	0	49	0	24	49	-

In order to achieve the proposed goal, 100 head of cattle, aged 3-5 years, were helminthologically investigated by the coprological - Darling method. As a result of these investigations, *Fasciola hepatica* infestation of cattle was identified in 35.0% of cases.

In the spring, parasitological investigations were carried out in the fasciolosis-favorable farm, where cattle were to graze, and the presence of *Lymnaea Lymnaea* snails was identified.

Fasciolosis is also a major public health problem and the risk of infestation is estimated at around 180 million people. Every year, more than 600 million animals are infected with fascioliasis [7].

In the Republic of Moldova, grazing areas for animals are limited, therefore on these lands graze different species of animals of different age classes, which contribute to environmental contamination with various parasitic agents. For this reason, the main profilactic measures consist in freeing these lands from the causative agent of fasciolosis in the intermediate host (*Lymnaea truncatula*) by increasing the contact with amphibians infected with the trematode species *Haplometra cylindracea*.

Thus, according to the results obtained, amphibians play a particularly important role in the biological control of fasciolosis in livestock, and this effect ensures the interruption of the biological cycle of development of the trematode *Fasciola hepaticus* and, subsequently, the interruption of its chain of transportation in biotopes, without the administration of chemical, toxic and immunosuppressive antiparasitic remedies.

GENERAL CONCLUSIONS AND RECOMMENDATIONS

1. During the evolutionary process, amphibians have developed certain survival strategies in two living environments: aquatic and terrestrial, which are manifested in different ways during the annual and life cycle characterized by: *hibernation, pre-reproductive migrations, post-reproductive* and *reproduction*.
2. Amphibian species, which utilize constant aquatic/perennial habitats for breeding are *Bufo bufo, Rana temporaria, Rana ridibunda* and partially: *Hyla arborea, Triturus cristatus* and *Rana dalmatina*, which belong to the category of ***k-strategi*** species with relatively constant and quite numerous population density, while the species *Pelobates fuscus, Lissotriton vulgaris, Bombina bombina, Bufotes viridis* use temporary aquatic pools and belong to the ***r - strateg -*** type species with high but fluctuating population numbers from year to year.
3. Depending on the specifics of oviposition, the amphibian species evaluated are divided into species with simultaneous oviposition - characteristic of ***k-strategic*** amphibians, and species with gradual and prolonged oviposition - characteristic of ***r-strategic*** amphibians. Embryonic and larval development in both ecological categories of amphibians, according to reproductive strategies (***k-strategists, r-strategists***), is similar due to similar climatic conditions.
4. For the first time, the complex helminth fauna of caudata (*Triturus cristatus, L. vulgaris*) and ecaudata (*Rana dalmatina, R. temporaria, Hyla arborea, Pelobates fuscus, Bufo bufo, Bufotes viridis, Bombina bombina*) from the Center, North and South of the Republic of Moldova, as a result of which the presence of 48 helminth species was identified, 32 of which are new species for the fauna of the Republic of Moldova, and taxonomically, they are classified into 3 families, 6 classes, 11 orders, 25 families and 42 genera.
5. It was found that the species of helminths detected in amphibians, according to their evolutionary cycle, are monogenean species in 14.58% of cases, digenean - in 16.67%, trigenean - in 60.4% and tetragenean - in 8.3% of cases.
6. A study of the structure of the helminth fauna of amphibians depending on the host species was carried out, as a result of which it was determined that *Pelophylax ridibundus* is infested with 43 species of helminths, *P. lessonae* - with 42 species, *P. esculentus* - with 30 species, *Rana dalmatina* - with 22 species, *R. temporaria* - with 18 species, *Bufo bufo* - with 17 species, *Bufotes viridis* - with 18 species, *Pelobates fuscus* - with 13 species, *Bombina bombina* - with 20 species, *Hyla arborea* - with 13 species, *Triturus cristatus* - with 10 species, and *Lissotriton vulgaris* is infested with 11 helminth species.
7. The degree of helminth infestation in amphibians was assessed, as a result of which it was determined that the host species *P. ridibundus* is infested in 76.33% of cases, *P. lessonae* - in 69.19% of cases, *P. esculentus* - in 66.67% of cases, *R. dalmatina* - in 28,14%, *R. temporaria* - in 38,41%, *B. bufo* - in 72,99%, *B. viridis* - in 63,46%, *P. fuscus* - in 45,1%, *B. bombina* - in 48,03%, *H. arborea* - in 60.17%, *T. cristatus* - in 43.66% of cases and *L. vulgaris* - in 53.99% of cases, which demonstrates that the formation of the helminth fauna, the divergent degree of infestation from one host species to another reflects the level of their adaptability to environmental factors in relation to biotic factors, which is the mechanism of formation of relationships in the

parasite-host system in amphibians.

8. The extent of helminth spread in the host population at the pre-reproductive (egg/outgut, larvae, juveniles) and reproductive (adults) age classes was studied, which allowed to deduce that in the life cycle of amphibians the probability of helminth infestation starts from the larval stage of development in the aquatic environment. The *Pearson* correlation coefficient (r_{xy}) between the parasite variable and the body mass of the pre-reproductive and reproductive age classes of amphibians was evaluated, which indicated a perfectly positive correlation between the degree of invasion and the body mass of larval, juvenile and adult amphibian larvae.
9. For the first time the effect of the metamorphic and post-metamorphic age of amphibians on their helminth infestation has been described, which contributes to predicting the impact of parasitic agents and managing populations of endangered amphibians both for reducing the host population itself and as potential vectors for various species of dangerous helminths in the larval stage (*O. ranae, P. robusta, S. sphaerula, S. falconis, I. melis, T. stossichi, S. contorta*) and juveniles (*O. ranae,C. urniger, T. excavata, P. elegans, P. robusta, S. sphaerula, S. falconis, I. melis, T. stossichi, S. lupi, S. contorta, T. canis, S. teres*).
10. It was determined that helminths established in amphibians, under the direct influence of the succession of environmental factors, are a clear evidence of their seasonal dynamics, so, data analysis allowed us to detect that, out of the total number of helminth species (n=48), detected in amphibians, 95.8% of species (n=46) infested amphibians in the spring season, 91.7% of species (n=44) infested amphibians in the summer season, and 87.5% of species (n=42) infested amphibians in the fall season. However, 81.25% of the species (n=39) infested caudata and ecaudata amphibians during the three seasons: spring - summer - fall, 85.5% of the species (n=39) infested caudata and ecaudata amphibians during two seasons: spring - summer, 85.42% of the species (n=41) infested caudata and ecaudata amphibians during two seasons: spring - fall and 85.42% of the species (n=41) infested caudata and ecaudata amphibians during two seasons: spring - summer.
11. In order to quantify the seasonal fluctuations in relation to the average annual parasite infestation of amphibians, their seasonality index was evaluated, which allowed to establish the cyclical dynamics of parasite invasion, as well as to identify the critical periods of transmission of parasites from amphibians to other vertebrate groups, including humans.
12. The diversity of the helminth fauna and the main helminthological indices of amphibian invasion were assessed in dependence on the geographical area and the type of populated ecosystem, according to which the degree of invasion was found to depend on the area, the host species and the presence of hosts specific to the helminth life cycle.
13. For the first time it was determined that 41.7% of amphibian species serve predominantly as intermediate hosts compared to their role as paratenic hosts, 25% of species serve predominantly as paratenic hosts compared to their role as intermediate hosts, and 33.3% of species manifest their role as both intermediate and paratenic hosts. For this reason, amphibians play an important role in the epidemiology and epizootiology of parasitic diseases.
14. For the first time, a complex study was carried out, with an in-depth approach to the

various biological, ecological and helminthological aspects of amphibians, which allowed to determine the evolutionary mechanisms in the parasite-host system and to identify the increased risk of formation of parasitic zoonoses by amphibians.

15. For the first time, the role of amphibians as vectors in the formation and maintenance of outbreaks of parasitic zoonoses common to fish (in 12.50% of cases), reptiles (in 27.10% of cases), birds (in 37.50% of cases), mammals (in 18.80% of cases) and humans (in 6.30% of cases) was determined.
16. For the first time, the biological method of control of fasciolosis in ruminants by using amphibians was developed and implemented in practice, which allowed to increase the qualitative and quantitative productivity and viability of ruminants.

PRACTICAL RECOMMENDATIONS

1. In order to perpetuate the species of caudate and ecaudate amphibians, it is recommended to protect their specific ecosystems by reducing pollution of aquatic basins, increasing the number of protected areas, reducing the population of breeding basins, reducing the use of pesticides, trying captive breeding, strict control of harvesting and marketing, conservation of natural habitats, ecological restoration of affected habitats, introduction of more effective measures to halt environmental degradation, which, in their complexity, will contribute to the recovery of the ecological status of amphibians and increase their population numbers.
2. In the context of maintaining the ecological balance, ensuring the preservation of biodiversity through rational, sustainable utilization and its conservation, in the Republic of Moldova it is necessary to carry out parasitological studies on animals and their inclusion in the category of limiting factors in the next editions of the Red Book of the Republic of Moldova, because the parasite factor is an important regulator of the fauna, which considerably reduces the population of certain species, or even their extinction.
3. Theoretical and practical scientific results obtained on the main biological and helminthological characteristics of amphibians will serve as theoretical and practical support in substantiating their role as bioindicators of the ecological status of populated ecosystems.
4. In order to reduce the risk of the formation and maintenance of outbreaks of parasitic agents dangerous to wild, domestic and pet animals and humans, as well as to preserve ecosystem biodiversity, it is recommended to carry out cyclic helminthological investigations in amphibians, which will contribute to early detection of the formation and maintenance of parasitic zoonoses.
5. The obtained scientific data represent an important methodological support for specialists in the fields of biology, ecology and helminthology, human and veterinary medicine, university teachers, conservationists, students, as well as in environmental education of the younger generation.
6. In order to protect the wild and domestic fauna, to minimize the risk of formation of parasitic zoonoses, especially in ruminants, it is recommended to widely apply in practice the invention patent "Method of prophylaxis of fasciolosis in ruminants No MD 1231 Z 2018.09.30, which ensures the interruption of the evolutionary cycle of the species *Fasciola hepatica,* which causes fasciolosis in animals (co-authors: Toderaș I., Erhan D., Rusu Șt.).

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18. **GHERASIM, E.,** COZARI, T. The effective of amphibians populations in the Orhei National Park. Life Sciences In Dialogue of Generations: Connections Between Universities, Academia And Business. National Conference with international participation, 29 – 30 September 2022, Chisinau, Republic Of Moldova. P. 154

9. Patents and other intellectual property objects, materials at invention fairs

1. TODERAȘ, Ion; ERHAN, Dumitru; **GHERASIM, Elena**; RUSU, Ștefan. Metodă de profilaxie a fasciolozei la rumegătoare. Brevet de invenție. MD 1231 Y 2018.02.28 https://agepi.gov.md/sites/default/files/bopi/BOPI_02_2018.pdf

ANNOTATION (ROMANIAN)

GHERASIM Elena „Fauna helmintică a amfibienilor (Amphibia), importanța acestora ca vectori în formarea și menținerea zoonozelor parazitare”, teză de doctor habilitat în științe biologice, Chișinău, 2025.

Structura tezei: introducere, șase capitole, concluzii generale și recomandări practice, bibliografia din 404 titluri, 168 de anexe, 257 de pagini text de bază, 48 tabele și 63 figuri. Rezultatele obținute sunt publiate în 115 lucrări științifice.

Cuvinte-cheie: amfibieni, gazde, ecologie, fauna helmintică, taxonomie, vector, zoonoze parazitare, importanță, combatere biologică.

Scopul lucrării constă în fundamentarea unei concepții integre și detaliate cu privire la descifrarea particularităților eco-evolutive în sistemul parazit-gazdă, pe exemplul amfibienilor, evaluarea rolului acestora ca vectori în formarea și menținerea focarelor de zoonoze parazitare, precum și elaborarea măsurilor de combatere biologică a acestora.

Obiectivele cercetării: Identificarea particularităților eco-etologice ale ciclului anual și vital al amfibienilor ca gazde a helminților; determinarea diversității faunei helmintice la amfibieni; evaluarea gradului de infestare cu helminți a amfibienilor în dependență de factorii intrinseci și extrinseci; evaluarea rolului amfibienilor în calitate de bioindicatori ai ecosistemelor și ca gazde; stabilirea importanței amfibienilor în formarea și menținerea focarelor de agenți parazitari; elaborarea și implementarea măsurilor biologice de combatere a helmintozelor specifice animalelor de rentă.

Noutatea și originalitatea științifică. Pentru prima dată în R. Moldova a fost realizată o abordare sistemică și complexă a amfibienilor finalizată cu: determinarea diversității faunei helmintice în aspect eco-evolutiv; stabilirea a 32 de specii noi de helminți pentru fauna R. Moldova; evaluarea gradului de răspândire a helminților în funcție de factorii intrinseci și extrinseci; identificarea perioadelor critice de transmitere a agenților parazitari de la amfibieni altor grupe de vertebrate; identificarea particularităților parazitologice ale amfibienilor ca vectori în profunzime și importanța acestora în formarea și menținerea focarelor de agenți parazitari periculoși în calitate de gazde; elaborarea unui procedeu biologic de combatere a fasciolozei prin intermediul amfibienilor.

Rezultate principale:A fost determinată diversitatea faunei helmintice a amfibienilor în aspect eco-evolutiv, s-au stabilit 32 de specii noi de helminți pentru fauna R. Moldova; s-a evaluat gradul de răspândire a helminților în dependență de factorii ecologici, au fost identificate particularitățile parazitologice ale amfibienilor ca vectori; s-a stabilit rolul amfibienilor în formarea și menținerea focarelor de agenți parazitari periculoși în calitate de gazde; s-a elaborat și implementat un procedeu biologic de combatere a fasciolozei prin intermediul amfibienilor.

Semnificația teoretică. Rezultatele științifice obținute contribuie semnificativ la completarea cu noi date multiplele aspecte de ordin interdisciplinar în domeniul științelor biologice în R. Moldova, care, prin determinarea faunei helmintice la amfibieni (48 de specii dintre care 32 - specii noi), extind nivelul de cunoaștere a faunei naționale - ca reprezentanți ai lumii animale. Pentru prima dată au fost elaborate fișele biologice ale speciilor de helminți depistați la amfibieni și determinat rolul acestora ca potențiale gazde. Rezultatele științifice permit determinarea legităților de formare a relațiilor în sistemul parazit-gazdă pe exemplul amfibienilor, precum și stabilirea strategiilor de funcționare a helmintocenozelor în condiții de instabilitate a factorilor climatici.

Valoarea aplicativă a lucrării constă în aportul esențial în evaluarea epizootică a biotopurilor populate de amfibieni și ca bază la elaborarea și implementarea măsurilor biologice de combatere și profilaxie a helmintozelor specifice animalelor de rentă cu impact asupra dezvoltării economiei naționale prin intermediul amfibienilor. De asemenea, aceste rezultate vor servi drept bază instituțiilor de stat, din domeniu, pentru consolidarea legislației R.Moldova în domeniul protecției și valorificării sustenabile a lumii animale.

Implimentarea rezultatelor obținute. S-a elaborat și aplicat în practică o nouă metodă biologică de combatere a fasciolozei prin intermediul amfibienilor. Rezultatele științifice sunt utilizate și implementate în procesul didactic al instituțiilor de învățământ, precum și în managementul subdiviziunilor Ministerului Mediului al Republicii Moldova.

ANNOTATION (RUSSIAN)

ГЕРАСИМ Елена «Гельминтофауна земноводных (Amphibia) и их значение как переносчиков в формировании и поддержании паразитарных зоонозов», диссертация на соискание ученой степени доктора биологических наук, Кишинев, 2025.

Структура диссертации: введение, шесть глав, общие выводы и практические рекомендации, библиография из 404 наименований, 168 приложения, 257 страниц основного текста, 48 таблиц и 63 рисунков. Полученные результаты опубликованы в 115 научных работах.

Ключевые слова: амфибии, хозяева, экология, гельминтофауна, таксономия, переносчик, паразитарные зоонозы, значение, биологический контроль.

Цель работы: состоит **в** обоснование целостной и детальной концепции относительно расшифровки эколого-эволюционных особенностей в системе паразит-хозяина на примере амфибий, оценка их роли как переносчиков в формировании и поддержании очагов паразитарных зоонозов, а также разработка биологическими методами борьбы с ними.

Задачи исследования: Выявление эколого-этологических особенностей годового и жизненного цикла земноводных как хозяев гельминтов; определение разнообразия гельминтофауны земноводных; оценка степени зараженности земноводных гельминтами в зависимости от внутренних и внешних факторов; оценка роли амфибий как биоиндикаторов экосистем и как хозяев; установление значения земноводных в формировании и поддержании очагов паразитарных заболеваний; разработка и внедрение биологических мер борьбы с гельминтозами сельскохозяйственных животных.

Научная новизна и оригинальность. Впервые в Республике Молдова осуществлен системный и комплексный подход к земноводным, включающий: определение разнообразия гельминтофауны в эколого-эволюционном аспекте; установление 32 новых видов гельминтов для фауны Республики Молдова; оценка степени распространения гельминтов в зависимости от внутренних и внешних факторов; выявление критических периодов передачи паразитарных агентов от земноводных к другим группам позвоночных; выявление паразитологических особенностей земноводных как переносчиков и их значения в формировании и поддержании очагов опасных паразитарных агентов в качестве хозяев; разработка биологического метода борьбы с фасциолезом с использованием амфибий.

Основные результаты: Определено разнообразие гельминтофауны земноводных в эколого-эволюционном аспекте, установлено 32 новых вида гельминтов для фауны Республики Молдова; оценена степень распространения гельминтов в зависимости от экологических факторов, выявлены паразитологические особенности амфибий как переносчиков; установлена роль земноводных в формировании и поддержании очагов опасных паразитарных возбудителей как хозяев; разработан и внедрен биологический метод борьбы с фасциолезом с использованием амфибий.

Теоретическая значимость. Полученные научные результаты вносят значительный вклад в дополнение новыми данными многочисленных междисциплинарных аспектов в области биологических наук в Республике Молдова, которые, определяя гельминтофауну земноводных (48 видов, из которых 32 – новые виды), расширяют уровень знаний о национальной фауне – как представителях животного мира. Впервые составлены биологические летописи видов гельминтов, обнаруженных у земноводных, и определена их роль как потенциальных хозяев. Полученные научные результаты позволяют определить закономерности формирования взаимоотношений в системе паразит-хозяин на примере амфибий, а также установить стратегии функционирования гельминтных сообществ в условиях нестабильности климатических факторов.

Прикладное значение работы заключается в существенном вкладе в эпизоотологическую оценку биотопов, заселенных амфибиями, и как основы для разработки и внедрения биологических мероприятий по борьбе и профилактике гельминтозов сельскохозяйственных животных, оказывающих влияние на развитие народного хозяйства через амфибий. Также, эти результаты послужат основой для государственных учреждений в данной области по укреплению законодательства Республики Молдова в области охраны и устойчивой валоризации животного мира.

Внедрение полученных результатов. Разработан и внедрен на практике новый биологический метод борьбы с фасциолезом с использованием земноводных. Научные результаты используются и внедряются в учебный процесс учебных заведений, а также в деятельность подразделений Министерства окружающей среды Республики Молдова.

ANNOTATION (ENGLISH)

GHERASIM Elena "Helminthic fauna of amphibians (Amphibia), their importance as vectors in the formation and maintenance of parasitic zoonoses", thesis of doctor habilitated in biological sciences, Chisinau, 2025.

Thesis structure: introduction, six chapters, general conclusions and practical recommendations, bibliography of 404 titles, 168 annexes, 257 pages of basic text, 48 tables and 63 figures. The results obtained are published in 115 scientific works.

Keywords: amphibians, hosts, ecology, helminthic fauna, taxonomy, vector, parasitic zoonoses, importance, biological control.

Aim: The purpose of the work is to substantiate an integral and detailed concept regarding the deciphering of eco-evolutionary peculiarities in the parasite-host system, using the example of amphibians, evaluating their role as vectors in the formation and maintenance of outbreaks of parasitic zoonoses, as well as developing measures for their biological control.

Research target interests: Identification of eco-ethological peculiarities of the annual and vital cycle of amphibians as hosts of helminths; determination of the diversity of helminthic fauna in amphibians; evaluation of the degree of helminth infestation of amphibians depending on intrinsic and extrinsic factors; evaluation of the role of amphibians as bioindicators of ecosystems and as hosts; establishment of the importance of amphibians in the formation and maintenance of outbreaks of parasitic agents; development and implementation of biological measures to combat helminthosis specific to livestock.

Scientific novelty and originality. For the first time in the Republic of Moldova, a systemic and complex approach to amphibians was carried out, completed with: determining the diversity of helminth fauna in an eco-evolutionary aspect; establishing 32 new species of helminths for the fauna of the Republic of Moldova; assessing the degree of spread of helminths depending on intrinsic and extrinsic factors; identifying critical periods of transmission of parasitic agents from amphibians to other groups of vertebrates; identifying the parasitological peculiarities of amphibians as vectors in depth and their importance in the formation and maintenance of outbreaks of dangerous parasitic agents as hosts; developing a biological process for combating fasciolosis through amphibians.

Main results: The diversity of the helminthic fauna of amphibians was determined in eco-evolutionary terms, 32 new species of helminths were established for the fauna of the Republic of Moldova; the degree of spread of helminths was assessed depending on ecological factors, the parasitological peculiarities of amphibians as vectors were identified; the role of amphibians in the formation and maintenance of foci of dangerous parasitic agents as hosts was established; a biological procedure for combating fasciolosis using amphibians was developed and implemented.

Theoretical significance. The obtained scientific results significantly contribute to the completion of new data on multiple interdisciplinary aspects in the field of biological sciences in the Republic of Moldova, which, by determining the helminthic fauna of amphibians (48 species, of which 32 are new species), expand the level of knowledge of the national fauna - as representatives of the animal world. For the first time, biological records of helminth species detected in amphibians were developed and their role as potential hosts was determined. The scientific results allow determining the regularities of the formation of relations in the parasite-host system using the example of amphibians, as well as establishing strategies for the functioning of helminthic communities in conditions of instability of climatic factors.

The applied value of the work consists in the essential contribution to the epizootic assessment of biotopes populated by amphibians and as a basis for the development and implementation of biological measures for the control and prevention of helminthiasis specific to livestock with an impact on the development of the national economy through amphibians. Also, these results will serve as a basis for state institutions in the field to strengthen the legislation of the Republic of Moldova in the field of protection and sustainable utilization of the animal world.

Implementation of the obtained results. A new biological method for combating fasciolosis using amphibians was developed and applied in practice. Scientific results are used and implemented in the teaching process of educational institutions, as well as in the management of subdivisions of the Ministry of Environment of the Republic of Moldova.

GHERASIM ELENA

HELMINTH FAUNA OF AMPHIBIANS (AMPHIBIA), THEIR IMPORTANCE AS VECTORS IN THE FORMATION AND MAINTENANCE OF PARASITIC ZOONOSES

Abstract of the Thesis of the Doctor Habilitatus in Biological Science

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