

HELMINTH FAUNA OF *Rana dalmatina* (Bonaparte, 1840) IN THE REPUBLIC OF MOLDOVA

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Abstract

The paper presents data on the identification for the first time in Moldova of the helminth fauna structure of *Rana dalmatina* Bonaparte, 1840, and the determination of its role as bioindicators and as vectors for parasitic agents specific to animals. As result of helminthological investigations, 18 helminths species was established. The predominant group are trematodes with 61.1% of cases, nematodes with 27.7% of cases, acanthocephalan and monogeneans with 5.6% of cases each. According to the assessment of the main helminthological indices, it was established that the species is infested with helminths in 83.3% of cases. When evaluating the data and the composition of helminth species, it was found that adult forms of helminths are predominant over larval forms, so that, when the host species is infected with trematodes, 27.3% are metacercarias, and 72.7% are the adult forms, when the host species is infected with nematodes 20.0% are the larval stages, and 80.0% are the mature forms, when infesting the host species with acanthocephalans and monogeneans, it was established that the helminth species is an adult form, each constituting 5.6%.

Key words: Bioindicators, Moldova, parasitic agents, *Rana dalmatina*, vectors.

INTRODUCTION

One of the indispensable components of the fauna are amphibians, which following the degradation of natural ecosystems, represent a low diversity of species. In the last decades, in the area of Central Forests on the territory of the Republic of Moldova, certain local populations of amphibians have disappeared, or some species such as *Bombina bombina*, *Hyla arborea*, *Triturus cristatus*, *Triturus vulgaris* and *Rana dalmatina* they considerably reduced their numerical effective (Cozari & Gherasim, 2021; Plop, 2011). The multi-year dynamics of the population of amphibian species, especially the *Rana dalmatina* Bonaparte, 1840 species, allowed us to note that this situation was recorded on the entire territory of our country, but as well as on a regional and continental level.

The direct action of the climate changes, global warming, fluctuations in the seasonal thermal regime not specific to a certain area, environmental pollution and the anthropic factor directly contribute to the formation of biocenotic relationships in the ecosystem and its functionality.

As a result of these threats, the *Rana dalmatina* Bonaparte, 1840 species was included in the list of protected species in Annex no II of the Berne Convention, the Habitats Directive of the Convention on the Conservation of Wild Life and Natural Habitats in Europe. Currently, the IUCN has assigned the *Rana dalmatina* species the status of Least Concern (LC) species. In the Red Book of the Republic of Moldova (2015) it is characterized as a vulnerable species (VU). Although, the continuous influence of abiotic and anthropogenic ecological factors reduce the population of the *Rana dalmatina* species on the territory of our country, however, the reduced population of this amphibian species is also determined by the direct influence of biotic ecological factors, which directly contribute to the formation of relationships direct and indirect interspecific (trophic, topical, phoric/vectorization) between the *Rana dalmatina* species and the living organisms in the ecosystems populated by it. According to the adaptability of *Rana dalmatina* species to the conditions of its environment, but also due to the species-specific ecological relationships existing from an evolutionary point of view, it is possible to

form a specific parasitic structure, which certainly has an impact on its population.

In this sense, the amphibian species *Rana dalmatina* through its specific parasitological structure, but also the trophic relations in the ecosystems populated by it, represents a true biological indicator of the ecosystems, but at the same time favors the transmission of its parasitic agents for other groups of vertebrate animals.

According to the helminthological research carried to specimens of the *Pelophylax esculenta* complex (*Pelophylax ridibundus*, *P. lessonae*, *P. esculentus*) on the territory of the Republic of Moldova, it was demonstrated that amphibians have an important role in the formation and maintenance of foci of the parasitic agents specific to domestic, wild, pet and human (Gherasim, 2016; Gherasim, 2023a; Gherasim, 2023b).

On a regional level, in different areas of the world, helminthological research was carried out on various species of amphibians (Düşen et al., 2009; Chikhlyayev et al., 2018), including the *Rana dalmatina* species.

On the territory of the Republic of Moldova, this species is especially distributed in the natural ecosystems of the Central and Northern areas of the country, and helminthological research on this species of ecaudate amphibians was carried for the first time.

Therefore, carrying of helminthological research on the *Rana dalmatina* species is an effective necessity both for the functioning of ecosystems and for the well-being of society.

MATERIALS AND METHODS

In order to determine the structure of the helminthic fauna of *Rana dalmatina* species, complex research was carried out with reference to the phenology of this, its population structure (age structure, spatial structure) as well as helminthological research in the area of distribution of the species - the area of the Central Forests of the Republic of Moldova.

The helminthological analysis of biological samples was performed according to the standard method proposed by K.I. Skriabin, which involves the examination of all the internal organs of the animal (Skriabin, 1928).

Helminthological research of the parenchymal organs was performed with the help of compressors, and the digestive tract - by successive washes.

The collection, fixing, determination and processing of the helminthological material was carried after the methods proposed by various authors (Gashev et al., 2006, Kuzmin, 2012, Ryzhikov, 1980).

In order to quantify the characteristics of helminthes contamination, the intensity indexes (II, specimens) were calculated - the minimum and maximum number of parasites of a species and the extent of invasion (EI, %) - the percentage of host contamination by a parasite species.

The helminthological research of the *Rana dalmatina* Bonaparte, 1840 species regarding the determination of the presence of helminthic agents depending on the age structure of the host includes the helminthological evaluation of the different stages of ontogenetic development of the host: egg - larvae - juvenile - adult, which will contribute to the knowledge of the importance of the species in the formation and maintenance of foci of parasitic agents common to wild, domestic animals, pets and human, but also to the development of biological methods to effectively combat parasitic zoonosis.

RESULTS AND DISCUSSIONS

The agile frog is the species of ecaudate amphibians with the earliest breeding period recorded not only on the territory of the Republic of Moldova, but also in Romania and other European countries.

In order to decipher and understand the interaction or relationships in the host-parasite system on the example of the amphibian species *Rana dalmatina*, but also to approach the common effects of the host and parasites on the increased risk of vectorization by specific parasitic agents, the multi-year phenology of this species throughout the country was evaluated.

Thus, according to the multi-year evaluations, in the area of the Central Forests of the Republic of Moldova, the first breeding specimens appeared on the soil surface in the site forestry were registered in the 3rd decade of March - the 1st

decade of April (Cozari & Gherasim, 2021), but in Romania, depending on the climatic conditions, it takes place out between February and April months (Fuhn, 1960).

As a result of the data analysis, the active period of the annual life cycle of amphibians was determined and it was established that their phenology is in full accord with the establishment of certain favorable climatic conditions of the temperature and humidity, and all together have an important role in the formation of intra- and interrelationships species-specific ecology of amphibians, with living environments and biological diversity.

In order to determine the structure of the helminthic fauna of the *Rana dalmatina* species, helminthological investigations were carried out, and according to the data obtained, it was established that the agile frog is infected with 18 species of helminthes, which from a taxonomic point of view fits into three phylums (Platyhelminthes, Nematoda, Acanthocephala), four classes (Trematoda, Monogenea, Secernentea, Palaeacanthocephala), eight orders (Plagiorchiida, Echinostomida, Diplostomida, Ascaridida, Strongylida, Rhabditida, Echinorhynchida, Polystomatida), fifteen families (Omphalometridae, Haematoloecidae, Gorgoderidae, Lecithodendriidae, Pleurogenidae, Diplodiscidae, Diplostomatidae, Strigeidae, Macroderoididae, Telorchidae, Cosmocercidae, Molineidae, Rhabdiasidae, Echinorhynchida, Polystomatidae) and seventeen genera (*Opisthioglyphe*, *Haematoloechus*, *Gorgodera*, *Gorgoderina*, *Pleurogenes*, *Pleurogenoides*, *Diplodiscus*, *Strigea*, *Haplometra*, *Tylodelphys*, *Telorchis*, *Cosmocerca*, *Oswalcocruzia*, *Agamospirura*, *Rhabdias*, *Acanthocephalus*, *Polystoma*).

According to the evaluation of the helminthological multiannual data obtained, we will mention that the host species *Rana dalmatina* is characterized by a high degree of infestation, which constitutes 83.3% of the cases, for which the main helminthological indices are very variable, and the extensivity of the invasion (E.I) - oscillates from 4.2% of cases, to infected by the trematode species *Gorgodera varsoviensis* up to - 74.0% of cases to infected with the *Haplometra cylindracea* species. At the same time, the intensivity of

invasion (I.I) with helminthes of the host is enough variable, and its values oscillate from - 1 ex. when the host is infected by the monogenean species *Polistoma integerrimum* up to 99 ex., when it is infected by the nematode of species *Tylodelphys excavata*.

The divergence of invasive values of the host species can be explained by the fact that the natural ecological factors are different during an annual cycle, but also its vital cycle. These factors as a whole favor the activity of the faunal diversity (intermediate, complementary, reservoir, definitive hosts), in a certain area and in a certain period of time participating in the development life cycle of specific helminthes species to *Rana dalmatina*.

The older the host, the more time it has had for the formation of biocenotic relationships in their specific ecosystems but and the possibility of infection with certain parasitic agents. Therefore, the extensivity and intensivity of the invasion of the agile frog, as well as the other species of amphibians investigated helminthologically on the territory of our country, changes so that the diversity of their helminthes species becomes richer, and the helminthological indices more amplified.

In addition, the changes of the helminthological indices and the structure of the helminth fauna related to age host are often proportional to their morpho-physiological changes, resulting in the behavior or the nutritional spectrum, which also contributes to a certain change in infection with various parasitic agents.

In order to appreciation the distribution of parasitic agents detected in amphibians, as well as to determine a possible infection with these species of helminthes of other groups of vertebrate animals, the structure of the helminthic fauna in four age categories of the population of *Rana dalmatina* egg - larvae - juveniles - adult forms were evaluated.

According to the results obtained, during the helminthological investigation of the *Rana dalmatina* eggs, species of helminthes they weren't established, while the presence of parasitic agents from 2 taxonomic classes (Secernentea, Trematoda) was established in its larvae and juveniles, but and in the adult forms of amphibians, the presence of four taxonomic classes (Secernentea, Trematoda, Palaeacanthocephala, Monogenea) was

established, with a different number of species (Figure 1).

Therefore, in the larvae of the *Rana dalmatina* species, four species of helminths was established, of which three species of trematodes (*Opisthioglyphe ranae*, *Haplometra cylindracea*, *Strigea sphaerula*) and one species of nematode (*Agamospirura* sp.), which constitutes 22.2% of the total species of detected helminthes.

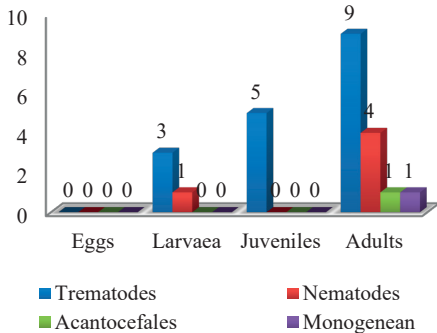


Figure 1. The structure of the helminthic fauna in agile frog species depending on its ontogenesis

In the juveniles, the presence of five helminth species was established, all of which are trematodes (*Opisthioglyphe ranae*, *Strigea sphaerula*, *Haplometra cylindracea*, *Telorchis stossichi*) which constitutes 27.7% of the total helminth species detected, and in the adult forms of amphibians the presence of nine trematode species (*O. ranae*, *H. variegatus*, *G. varsoviensis*, *G. vitelliloba*, *P. medians*, *D. subclavatus*, *S. sphaerula*, *H. cylindracea*, *T. stossichi*), four nematode species (*C. ornate*, *O. filiformis*, *O. duboisi*, *Rh. bufonis*), and a species of acanthocephalus (*A. ranae*) and a species of monogeneans (*P. integerrimum*) was established which constitutes 83.3% of the total species of helminthes detected in the host species.

According to the evaluation of the obtained data, it was determined that some detected helminth species are specific only for a certain ontogenetic period, such as the helminth species (*H. variegatus*, *G. varsoviensis*, *G. vitelliloba*, *P. claviger*, *P. medians*, *D. subclavatus*, *C. ornata*, *O. filiformis*, *O. duboisi*, *Rh. Bufonis*, *A. rane*,

P. integerrimum) detected only in the adult forms of agile frog.

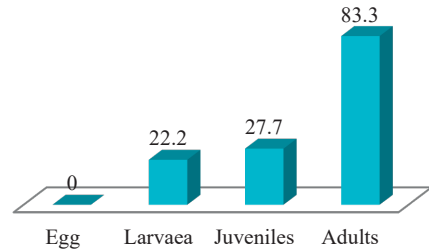


Figure 2. The structure of the helminthic fauna depending of the ontogeny of host

At the same time, the presence of helminth species that are specific to only one period of the host's ontogeny was determined, for example *T. stossichi* species in juveniles and *Agamospirura* sp. species in the stage of larval development. One and the same helminth species was established to be specific of the host in different ontogenetic periods of it. Thus 11.0% of the total helminth species detected are specific to both larval, juvenile as well as adult forms, 5.6 % of cases are specific to juveniles and adult forms of amphibians, larvae and juveniles, respectively juveniles, but the species specific only to adult forms of amphibians constitute 66.65% of the total species of helminthes detected in the host species (Figure 3).

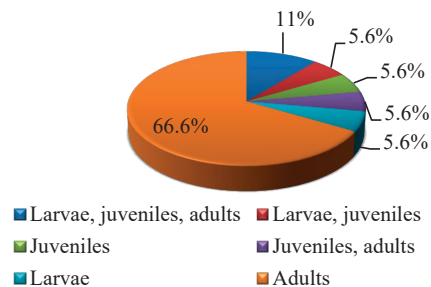


Figure 3. The degree of specificity of helminths depending on the ontogeny of host

To determine the diversity of the helminthic fauna in amphibians depending on their main phenological phases, these were investigated over the entire annual life cycle during the spring, summer, autumn seasonal successions.

As a result of the investigations, an evolution of the parasitic fauna of the *Rana dalmatina* species was recorded in its different phenological phases: the exit phase from the hibernation period, the movement towards the summer sites, the achievement of metamorphosis, the leaving of the summer sites and the initiation of their hibernation phase (Figure 4).

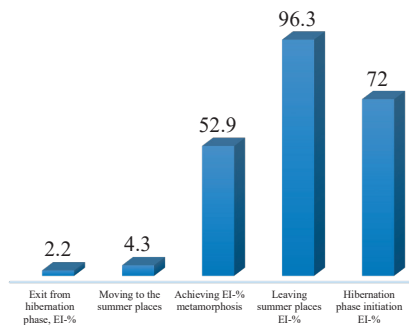


Figure 4. Degree of helminth infestation of *Rana dalmatina* species according to specific phenological phases

The recording of a poorer parasitic fauna in this amphibian species during the spring period (exit from the hibernation phase, orientation towards the breeding pools, breeding period) it is characterized by the absence of the obligatory intermediate hosts in the development cycle of helminth species specific for amphibian due to their unfavorable ecological optimum, as well as very low amount of food for the hosts.

In order to evaluate the degree of infestation with helminthes in the aspect of mono- and polyinvasions during an annual and vital cycle of amphibians, it was established that in the *Rana dalmatina* species the infestation in the aspect of polyinvasion are predominates (Figure 5).

In order to establish the role of the *Rana dalmatina* species in the formation and maintenance of foci of parasitic agents common to synanthropic, domestic, wild, companion animals and humans, complex research was carried out that allowed the determination of different stages of development (larval forms, adult forms) of all elements invasiveness detected in this species of ecaudate amphibians.

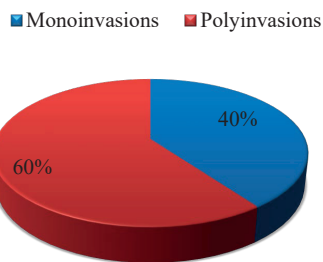


Figure 5. The structure of the helminthic fauna in *Rana dalmatina* species in terms of mono and polyinvasions

Thus, according to our helminthological investigations, as a result of which the presence of 18 species of helminthes was established, it was found that the predominant group is represented by trematodes (n = 11) which constitute 61.1% of cases, nematodes - 27.7% of cases (n = 5) and acanthocephals and monogeneans with 5.6% of cases (n = 1) each (Figure 6).

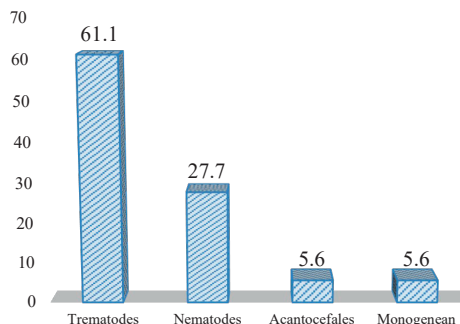


Figure 6. Indices of extensivity of taxonomic classes of helminths in amphibians

When evaluating the data and the invasive composition, it was found that the adult forms of helminthes are predominant for the larval forms, so that, when the host species is infested with trematodes 27.3% of cases are metacercariae and 72.7% of cases are the adult forms, when the host species is infested with nematodes 20.0% of cases are the larval stages, and 80.8% of cases are the adult forms, when infesting the host species with acanthocephales and monogeneans, it was established that the helminth species are an adult form, each constituting 5.6% of cases (Figure 7).

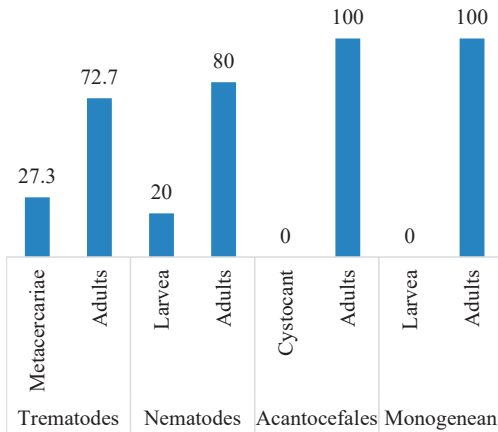


Figure 7. Helminthological indices of *Rana dalmatina* species depending on the ontogeny of the parasitic agents

Therefore, during the helminthological investigation of *Rana dalmatina* species, it was found that this host is predominantly infested with invasive elements of adult form.

According to specialized scientific works, the role of amphibians as vectors are mentioned (Gherasim, 2023a; Herczeg et al., 2021; Frolov et al., 2015; Buga et al., 2013). Analyzing our data referring to the diversity of helminth species in amphibians, determining the ontogenetic stages of the infected host, but also of the established parasitic agents, we can mention that the *Rana dalmatina*, host species, shows an increased degree of vectorization of her helminthic agents for wild, domestic, company animals and the human. This process is explained not only by the evolutionary trophic relations in the ecosystem (prey-predator), but also by the possibility of its simultaneous infestation with several species of parasitic agents.

Rana dalmatina species was characterized by an infestation with parasitic agents with up to 5 species of helminthes simultaneously. According to the assessments, it was determined that the specimens in 40.0% of cases were infested with a single species of helminthes, 10% of cases - with 2 species of helminthes, 20.0% of cases - with 3 species of helminthes, 20.0 % of cases - with 4 species of helminthes and 10.0% of cases - with 5 species of helminthes (Figure 8).

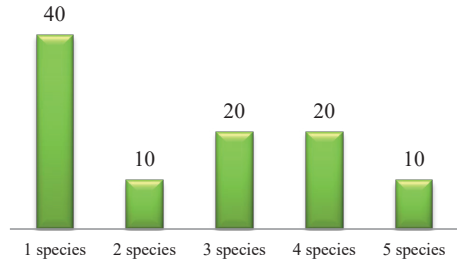


Figure 8. The degree of co-infection of the *Rana dalmatina* species

To evaluating the degree of vectorization of parasitic agents by the host species *Rana dalmatina* in the ecosystems of the Republic of Moldova, it was determined that of the total helminth species detected, 5.6% of the cases are specific to fish, 5.6% of the cases are specific to reptiles, 11.1 % of the cases are specific to birds and 77.7 are specific only to amphibians (Figure 9).

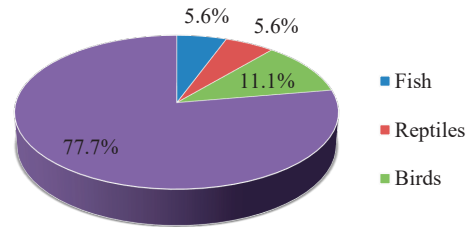


Figure 9. The degree of vectorization of parasitic agents by the *Rana dalmatina* species

So, the distribution and dynamics of the appearance of parasites in a special environment and time in the host species *Rana dalmatina*, as well as the factors that regulate the relationship between the host with parasite at the individual level, or at the population level, represent a complex study with a importance approach to various aspects biological, ecological and helminthological both of the host organism and of the parasite.

CONCLUSIONS

For the first time in the Republic of Moldova, helminthological investigations were carried

out on the species of ecaudate amphibians *Rana dalmatina* Bonaparte, 1840.

It has been established the presence of 18 species of helminths, which from a taxonomic point of view fall into three phylums (Platyhelminthes, Nematoda, Acanthocephala), four classes (Trematoda, Monogenea, Secernentea, Palaeacanthocephala), 8 ordine (Plagiorchiida, Echinostomida, Diplostomida, Ascaridida, Strongylida, Rhabditida, Echinorhynchida, Polystomatida), fifteen families (Omphalometridae, Haematoloechidae, Gorgoderidae, Lecithodendriidae, Pleurogenidae, Diplodiscidae, Diplostomatidae, Strigeidae, Macroderoididae, Telorchiiidae, Cosmocercidae, Molineidae, Rhabdiasidae, Echinorhynchida, Polystomatidae) and seventeen genres (*Opisthioglyphe*, *Haematoloechus*, *Gorgodera*, *Gorgoderina*, *Pleurogenes*, *Pleurogenoides*, *Dipodiscus*, *Strigea*, *Haplometra*, *Tylodelphys*, *Telorchis*, *Cosmocerca*, *Oswalocruzia*, *Agamospirura*, *Rhabdias*, *Acanthocephalus*, *Polystoma*)

The main helminthological indices were studied depending on the host age, but the obtained results allowed us to conclude that with the increase in the size and age of amphibians, the parasitic fauna increases as well as the degree of helminthes infestation. This appearance demonstrates the intensification of the feeding of adult amphibians and the accumulation of parasitic agents in their body from previous periods, as well as the increase in the size and diversity of food favoring the simultaneous breakthrough of a greater number of parasitic agents into the host's body, which leads to the increase in the diversity of the helminthic fauna.

The structure of the helminthic fauna was studied depending on the phenology of the host, which allowed the appreciation of the degree of knowledge of the period, stations and habitats where it is possible to vectorize of parasitic agents by the *Rana dalmatina* species to the definitive hosts of these helminthes, which is particularly important for the agile frog, which uses aquatic ecosystems only during the breeding season.

ACKNOWLEDGEMENTS

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